UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE
Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias

-TESIS DOCTORAL-

ESTRATEGIAS INNOVADORAS CON ELICITORES PARA REDUCIR LAS PÉRDIDAS EN LA COSECHA Y PRESERVAR LA CALIDAD Y VIDA ÚTIL DE PRODUCTOS VEGETALES

-2025-

María Celeste Ruiz Aracil

Director: Dr. Antonio Fabián Guillén Arco

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UNIVERSITAS Miguel Hernández

El **Dr. D. Antonio Fabián Guillén Arco**, director, y el **Dr. D. Juan Miguel Valverde Veracruz**, codirector de la tesis doctoral titulada "**Estrategias innovadoras con elicitores para reducir las pérdidas en la cosecha y preservar la calidad y vida útil de productos vegetales**",

INFORMAN:

Que **Dña. María Celeste Ruiz Aracil** ha realizado bajo nuestra supervisión el trabajo titulado "**Estrategias innovadoras con elicitores para reducir las pérdidas en la cosecha y preservar la calidad y vida útil de productos vegetales**" conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo con el 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 firmamos para los efectos oportunos, en Orihuela a 19 de febrero de 2025.

Director de la tesis	Codirector de la tesis
Dr. D. Antonio Fabián Guillén Arco	***Dr. D. Juan Miguel Valverde Veracruz***

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

INFORMA:

Que **Dña. María Celeste Ruiz Aracil** ha realizado bajo la supervisión de nuestro Programa de Doctorado el trabajo titulado "**Estrategias innovadoras con elicitores para reducir las pérdidas en la cosecha y preservar la calidad y vida útil de productos vegetales**" conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo con el 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 19 de febrero de 2025.

Prof.ª Dra. Dña. Juana Fernández López

Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias,

Agroambientales y Alimentarias (ReTos-AAA)

"Un país sin investigación es un país sin desarrollo"

Margarita Salas

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*Todas las imágenes incluidas en la Tesis Doctoral no relacionadas con la experimentación son de uso libre y se han obtenido a través del programa de inteligencia artificial generativa Midjourn*ey (https://www.midjourney.com).

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La presente Tesis Doctoral, titulada “Estrategias innovadoras con elicitores para reducir las pérdidas en la cosecha y preservar la calidad y vida útil de productos vegetales”, se presenta bajo la modalidad de **tesis por compendio** de las siguientes publicaciones:

I. **Ruiz-Aracil, M. C.**, Valverde, J. M., Beltrà, A., Carrión-Antolí, A., Lorente-Mento, J. M., Nicolás-Almansa, M., & Guillén, F. (2023). Putrescine increases frost tolerance and effectively mitigates sweet cherry (*Prunus avium* L.) cracking: a study of four different growing cycles. *Agronomy*, 14(1), 23. doi: 10.3390/agronomy14010023

II. **Ruiz-Aracil, M. C.**, Valverde, J. M., Beltrà, A., Lorente-Mento, J. M., Carrión-Antolí, A., Valero, D., & Guillén, F. (2024). Enhancing sweet cherry resilience to spring frost and rain-induced cracking with pre-harvest melatonin treatments. *Current Plant Biology*, 40, 100388. doi. 10.1016/j.cpb.2024.100388

III. **Ruiz-Aracil, M. C.**, Valverde, J. M., Lorente-Mento, J. M., Carrión-Antolí, A., Castillo, S., Martínez-Romero, D., & Guillén, F. (2023). Sweet cherry (*Prunus avium* L.) cracking during development on the tree and at harvest: the impact of methyl jasmonate on four different growing seasons. *Agriculture*, 13(6), 1244. doi: 10.3390/agriculture13061244

IV. Lezoul, N. E. H., Serrano, M., **Ruiz-Aracil, M. C.**, Belkadi, M., Castillo, S., Valero, D., & Guillén, F. (2022). Melatonin as a new postharvest treatment for increasing cut carnation (*Dianthus caryophyllus* L.) vase life. *Postharvest Biology and Technology*, 184, 111759. doi: 10.1016/j.postharvbio.2021.111759

V. **Ruiz-Aracil, M. C.**, Guillén, F., Ilea, M. I. M., Martínez-Romero, D., Lorente-Mento, J. M., & Valverde, J. M. (2023). Comparative effect of melatonin and 1-methylcyclopropene postharvest applications for extending ‘Hayward’ kiwifruit storage life. *Agriculture*, *13*(4), 806. doi: 10.3390/agriculture13040806

VI. **Ruiz-Aracil, M. C.**, Valverde, J. M., Ilea, M. I. M., Valero, D., Castillo, S., & Guillén, F. (2024). Innovative postharvest management for Hass avocado at the preclimacteric stage: a combined technology with GABA and 1-MCP. *Foods*, 13(16), 2485. doi: 10.3390/foods13162485

VII. **Ruiz-Aracil, M. C.**, Guillén, F., Castillo, S., Martínez-Romero, D. & Valverde, J. M. The application of 1-MCP in combination with GABA reduces chilling injury and extends the shelf life in tomato (cv. Conquista). *Agriculture*. doi: 10.3390/agriculture14112040

ÍNDICE DE CALIDAD DE LAS PUBLICACIONES

I

Putrescine increases frost tolerance and effectively mitigates sweet cherry (*Prunus avium* L.) cracking: a study of four different growing cycles

Autores

Ruiz-Aracil, M. C., Valverde, J. M., Beltrà, A., Carrión-Antolí, A., Lorente-Mento, J. M., Nicolás-Almansa, M., & Guillén, F.

Revista

Agronomy

DOI

10.3390/agronomy14010023

Publicación

2023

Quartil	Categoría JCR®
Q1	Agronomy
Rango	**ISNN**
19/126	2073-4395
Factor de impacto (2023)	**Factor de impacto (5 años)**
3,3	3,7

II

Enhancing sweet cherry resilience to spring frost and rain-induced cracking with pre-harvest melatonin treatments

Autores

Ruiz-Aracil, M. C., Valverde, J. M., Beltrà, A., Lorente-Mento, J. M., Carrión-Antolí, A., Valero, D., & Guillén, F.

Revista

Current Plant Biology

DOI

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Publicación

2024

Quartil	Categoría JCR®
Q1	Plant Sciences
Rango	**ISNN**
33/265	2214-6628
Factor de impacto (2023)	**Factor de impacto (5 años)**
5,4	4,7

III

Sweet cherry (*Prunus avium* L.) cracking during development on the tree and at harvest: the impact of methyl jasmonate on four different growing seasons

Autores

Ruiz-Aracil, M. C., Valverde, J. M., Lorente-Mento, J. M., Carrión-Antolí, A., Castillo, S., Martínez-Romero, D., & Guillén, F.

Revista

Agriculture

DOI

10.3390/agriculture13061244

Publicación

2023

Quartil	Categoría JCR®
Q1	Agronomy
Rango	**ISNN**
16/126	2077-0472
Factor de impacto (2023)	**Factor de impacto (5 años)**
3,3	3,5

IV

Melatonin as a new postharvest treatment for increasing cut carnation (*Dianthus caryophyllus* L.) vase life

Autores

Lezoul, N. E. H., Serrano, M., **Ruiz-Aracil, M. C.**, Belkadi, M., Castillo, S., Valero, D., & Guillén, F.

Revista

Postharvest Biology and Technology

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Publicación

2022

Quartil	**Categoría JCR®**
Q1	Agronomy
Rango	**ISNN**
1/126	1873-2356
Factor de impacto (2023)	**Factor de impacto (5 años)**
6,4	6,9

V

Comparative effect of melatonin and 1-methylcyclopropene postharvest applications for extending 'Hayward' kiwifruit storage life

Autores

Ruiz-Aracil, M. C., Guillén, F., Ilea, M. I. M., Martínez-Romero, D., Lorente-Mento, J. M., & Valverde, J. M.

Revista

Agriculture

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Publicación

2023

Quartil

Q1

Categoría JCR®

Agronomy

Rango

16/126

ISNN

2077-0472

Factor de impacto (2023)

3,3

Factor de impacto (5 años)

3,5

VI

Innovative postharvest management for Hass avocado at the preclimacteric stage: a combined technology with GABA and 1-MCP

Autores

Ruiz-Aracil, M. C., Valverde, J. M., Ilea, M. I. M., Valero, D., Castillo, S., & Guillén, F.

Revista

Foods

DOI

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Publicación

2024

Quartil	Categoría JCR®
Q1	Food Science and Technology
Rango	**ISNN**
40/173	2304-8158
Factor de impacto (2023)	**Factor de impacto (5 años)**
4,7	5,1

VII

The application of 1-MCP in combination with GABA reduces chilling injury and extends the shelf life in tomato (cv. Conquista)

Autores

Ruiz-Aracil, M. C., Guillén, F., Castillo, S., Martínez-Romero, D. & Valverde, J. M.

Revista

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Publicación

2024

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Rango

16/126

ISNN

2077-0472

Factor de impacto (2023)

3,3

Factor de impacto (5 años)

3,5

ÍNDICE

ABREVIACIONES Y SÍMBOLOS

JaMe: Jasmonato de metilo

PUT: Putrescina

MT: Melatonina

GABA: Gamma-aminobutyric acid; ácido gamma-aminobutírico

1-MCP: 1-metilciclopropeno

IGP: Indicación Geográfica Protegida

MDA: Malondialdehido

ABA: Ácido abscísico

AG: Ácido giberélico

AS: Ácido salicílico

ROS: Reactive oxygen species; especies reactivas de oxígeno

PAs: Poliaminas

Enzimas:

SAM: S-adenosil metionina

PPO: Polifenol oxidasa

POD: Peroxidasa

SOD: Superóxido dismutasa

CAT: Catalasa

APX: Ascorbato peroxidasa

GABA-T: Ácido gamma-aminobutírico transamina

GAD: Glutamato descarboxilasa

PAL: fenilalanina amonio liasa

LOX: Lipoxigenasa

PLD: Fosfolipasa D

ACC: 1-aminociclopropano-1-carboxilato sintasa

HCAA: Hydroxycinnamic acida mides; amidas derivadas del ácido hidroxicinámico

BBCH: Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie

E1: Estadio 1 (botón floral)

E2: Estadio 2 (plena floración)

E3: Estadio 3 (endurecimiento del hueso)

E4: Estadio 4 (inicio de los cambios de color)

PF: Peso fresco

ASF: Absorción de solución en florero

IEM: Índice de estabilidad de membrana

CTP: Contenido Total de Polifenoles

CTC: Contenido Total de Clorofila

AAT: Actividad antioxidante total

AAT-H: Actividad antioxidante total hidrosoluble

AAT-L: Actividad antioxidante total liposoluble

SST: Sólidos solubles totales

AT: Acidez titulable

FE: Fuga de electrolitos

ANOVA: Analysis of variance; análisis de varianza

SPSS: Statistical Package for the Social Sciences; Paquete estadístico para ciencias sociales

LSD: Least significant difference; diferencia menos significative

HSD: Honestly significant difference; test de comparaciones múltiples

EE: Error estándar

FID: Flame ionization detector; detector de ionización de llama

HPLC: High Performance Liquid Chromatography; Cromatografía líquida de alta resolución

Trolox: Ácido 6-Hidroxi-2,5,7,8-tetrametilchroman-2-carboxílico

ABTS: Ácido 2,2-azinobis (3-etilbenzotazolina-6-sulfónico)

TCA: Trichloroacetic acid; ácido tricloroacético

TBA: Thiobarbituric acid; ácido tiobarbitúrico

NAF: Floruro de sodio

Na_2CO_3: Carbonato de sodio

FC: Folin-Ciocalteu

rpm: revoluciones por minuto

mM: milimolar

µM: micromolar

L: litros

mL: mililitros

nL: nanolitros

µL: microlitros

kg: kilogramos

g: gramos

mg: miligramos

mm: milímetros

N: Newton

nm: nanometros

% v/v: Porcentaje volumen-volumen

h: hora

min: minutos

s: segundos

L*: Luminosidad

a*: Valor rojo/verde

b*: Valor azul/amarillo

CO_2: Dióxido de carbono

H_2O_2: Peróxido de hidrógeno

ESTRUCTURA DE LA TESIS DOCTORAL

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

- **Resumen**: descripción de los resultados y conclusiones más relevantes (castellano e inglés).

- **Introducción**: descripción de las características generales y la importancia de los cultivos de la cereza, clavel, kiwi, aguacate y tomate, incluyendo los desafíos en su producción y manejo poscosecha. Así como el uso de los elicitores y el 1-MCP como herramienta precosecha o poscosecha.

- **Objetivos**: desarrollo del objetivo principal y de los objetivos específicos de la investigación.

- **Metodología**: breve descripción de la metodología empleada para la consecución de los objetivos, incluyendo el material vegetal utilizado, el diseño experimental empleado y los métodos analíticos aplicados para la realización de esta Tesis Doctoral.

- **Publicaciones científicas**: transcripción literal de las publicaciones científicas que componen esta Tesis:

    I. «Putrescine increases frost tolerance and effectively mitigates sweet cherry (*Prunus avium* L.) cracking: a study of four different growing cycles». Publicado en la revista *Agronomy*.

    II. «Enhancing sweet cherry resilience to spring frost and rain-induced cracking using melatonin». Publicado en la revista *Current Plant Biology*.

    III. «Sweet cherry (*Prunus avium* L.) cracking during development on the tree and at harvest: the impact of methyl jasmonate on four different growing seasons». Publicado en la revista *Agriculture*.

    IV. «Melatonin as a new postharvest treatment for increasing cut carnation (*Dianthus caryophyllus* L.) vase life». Publicado en la revista *Postharvest Biology and Technology*.

V. «Comparative effect of melatonin and 1-methylcyclopropene postharvest applications for extending ‘Hayward’ kiwifruit storage life». Publicado en la revista *Agriculture*.

VI. «Innovative postharvest management for Hass avocado at the preclimacteric stage: a combined technology with GABA and 1-MCP». Publicado en la revista *Foods*.

VII. «The application of 1-MCP in combination with GABA reduces chilling injury and extends the shelf life in tomato (cv. Conquista)». Publicado en la revista *Agriculture*.

- ❖ **Resultados, discusión y conclusión:** exposición de los principales resultados y discusiones obtenidos en cada uno de los artículos, así como sus conclusiones.

- ❖ **Conclusiones generales:** redacción de las conclusiones obtenidas en la presente Tesis Doctoral.

- ❖ **Futuras líneas de investigación:** determinación breve de las posibles futuras investigaciones que se pueden desarrollar a partir de los resultados obtenidos.

- ❖ **Referencias bibliográficas**: indicación de las referencias bibliográficas empleadas para la redacción y justificación de esta Tesis Doctoral.

RESUMEN/ABSTRACT

RESUMEN

En la presente Tesis se han estudiado los efectos de la aplicación de elicitores sobre distintas especies vegetales, con el objetivo de reducir fisiopatías que se producen en la precosecha, así como disminuir la pérdida de calidad y los daños por frío originados durante el almacenamiento de los frutos en la poscosecha incrementado su vida útil. Otro de los objetivos principales ha sido evaluar el efecto sinérgico al combinar los elicitores como tratamientos de origen natural junto con sustancias de origen artificial ampliamente utilizadas en la poscosecha de frutos como es el 1-metilciclopropeno (1-MCP).

Las cerezas son frutas de gran relevancia a nivel nacional, y su producción se destina principalmente al consumo en fresco. No obstante, los cultivos de esta fruta experimentan pérdidas considerables debido a su vulnerabilidad frente a las heladas y el agrietado, especialmente en las variedades de cosecha temprana. Las heladas ocurridas durante la floración y las lluvias acaecidas en el estado de fructificación, comprometen la integridad de los órganos florales y del fruto, ocasionando importantes pérdidas económicas. Las excesivas precipitaciones, los cambios de temperatura y la alta humedad provocan el agrietado de las cerezas que son frutos con la piel fina. Esto facilita que sean más sensibles al agrietado a diferencia de otras especies vegetales. Este cultivo también sufre heladas primaverales que afectan a su rendimiento y al número de frutos cosechados. Las fisiopatías del fruto debido a las condiciones climáticas generan una cantidad importante de pérdidas económicas para los agricultores, y en especial, para aquellos dónde la topografía y la localización del terreno implica la incidencia de lluvias de manera constante como es el caso de la cereza evaluada en esta Tesis acogida a la Indicación Geográfica Protegida (IGP) “Cerezas de la Montaña de Alicante”. Por ello, para poder mitigar los efectos negativos que se están produciendo en el cultivo de la cereza debido a la variabilidad de las condiciones climáticas, en la presente Tesis se han desarrollado diferentes estrategias precosecha.

Además, la influencia de los distintos estados de madurez del fruto en el árbol no ha sido evaluado todavía en el agrietado de las cerezas. El estudio de este factor podría aportar el conocimiento suficiente para optimizar herramientas eficaces en la reducción del agrietado de las cerezas y por esta razón se ha decidido que sea materia de estudio para la Tesis. Este trabajo se enfoca en aplicar tratamientos de origen natural en etapas clave del desarrollo de las cerezas de las variedades 'Prime Giant', 'Sweetheart', 'Early Lory' y 'Staccato', con el propósito de ofrecer soluciones para mitigar los problemas de heladas y agrietado de los frutos y evaluar su efecto en la calidad al momento de la cosecha. Para poder paliar esta

problemática se han utilizado elicitores como el jasmonato de metilo (JaMe) a 0,5 mM, la putrescina (PUT) a concentraciones de 1 mM y 10 mM y la melatonina (MT) (0,01, 0,05 y 0,1 mM) mediante pulverización foliar durante diferentes ciclos productivos (2019, 2020, 2021, 2022, 2023). El JaMe, la PUT y la MT son hormonas vegetales que poseen los tejidos vegetales de forma endógena y se encargan del crecimiento y el desarrollo de las plantas, así como la inducción de respuesta al estrés. Con la utilización de PUT y MT es posible minimizar el impacto de las heladas primaverales sobre las cerezas al reducir el contenido de malondialdehído (MDA) en los botones florales y aumentar el cuajado de los frutos. Las cerezas tratadas con JaMe, PUT y MT aumentaron de forma eficaz la tolerancia del fruto al agrietado inducido por la lluvia. Se ha podido comprobar que existe diferente variabilidad en la sensibilidad del fruto al agrietado, siendo dependiente del cultivo y de la etapa de maduración. Los tratamientos de JaMe y PUT en cereza han mostrado eficacia en la reducción de la maduración de la fruta; aumentando la firmeza, la acumulación de los sólidos solubles y retrasando la evolución del color en el momento de la cosecha. Además, los tratamientos consiguieron mantener la calidad general del fruto, con valores incrementados de firmeza y un retraso en el desarrollo del color, los sólidos solubles y la acidez total en comparación con la fruta no tratada en el momento de la cosecha. Los resultados sugieren que los tratamientos con elicitores en precosecha de la cereza pueden servir como una herramienta eficaz para mitigar las pérdidas de producción, proporcionando así una estrategia prometedora para adaptarse al cambio climático y mitigar el estrés.

Además, se han estudiado los efectos poscosecha de los elicitores en distintas especies vegetales y entre ellos, se ha comprobado el efecto del uso de la MT a distintas concentraciones sobre el clavel cortado. Aunque es una flor comestible, su uso está más extendido como flor ornamental y especialmente el clavel de color blanco, que es el utilizado en esta Tesis. La vida útil en florero es el número de días que la flor mantiene sus cualidades decorativas una vez cortada. Por ello, es de gran interés retrasar los procesos de senescencia de la flor para ampliar el tiempo de comercialización. Este hecho es un importante factor económico para el sector, ya que alargando los días en los que la flor conserva un estado óptimo, se consigue mejorar la eficiencia en el transporte y se aumentan las posibilidades de exportación. En el momento de su publicación, fue el primer artículo en el que se describe el impacto de la MT en la vida útil de las flores cortadas. Los claveles se colocaron individualmente en tubos falcon con 10 mL de los diferentes tratamientos, usando MT a concentraciones de 0, 0,01, 0,1 y 1 mM. El tratamiento con MT 0,1 mM consiguió retrasar la senescencia de los claveles aumentando el doble la vida útil respecto a las flores controles. Esto podría atribuirse a una reducción en el metabolismo, con un aumento en la estabilidad de las membranas y una mejora en la relación hídrica. La energía adicional proporcionada por el tratamiento con MT podría estar actuando en la reducción de la respiración y de los requerimientos energéticos de los tejidos celulares, aumentando así su vida útil. Además, se ha comprobado que la MT incrementó la actividad antioxidante y el contenido fenólico, reduciendo el daño oxidativo en los tejidos del clavel.

Estos elicitores han sido descritos como sustancias que además de regular el balance energético, estimulan las defensas de las plantas. Por ello, de forma paralela se han realizado estudios sobre el efecto de compuestos elicitores como la MT y el ácido γ-aminobutírico (GABA) aplicados solos o en combinación con otros tratamientos convencionales de origen artificial sobre la calidad poscosecha y la reducción de fisiopatías en diferentes especies vegetales. El control de la temperatura es un método de conservación ampliamente utilizado para retrasar el proceso de maduración del fruto y aumentar su vida útil. Sin embargo, el almacenamiento prolongado a bajas temperaturas provoca daños por frío en especies vegetales sensibles, produciendo un desorden fisiológico acumulativo y aumentando la susceptibilidad a podredumbres. Por lo general, los síntomas de las lesiones por frío aumentan después de que la fruta se traslada a temperatura ambiente. El kiwi, el aguacate y el tomate son frutos susceptibles a sufrir desórdenes fisiológicos por daños por frío y la tolerancia depende de factores como la especie, la variedad, el momento de la cosecha, la temperatura y el tiempo de exposición a las temperaturas subóptimas. Para paliar esta problemática, en esta Tesis se ha evaluado el efecto de los tratamientos con elicitores como la MT y el GABA de forma individual o en combinación con el 1-MCP en la poscosecha del kiwi, aguacate y tomate. Los tratamientos han demostrado ser efectivos en la reducción de estas fisiopatías en las distintas especies vegetales de forma individualizada. Sin embargo, mientras que el 1-MCP es una sustancia de origen artificial, el GABA y la MT son elicitores presentes de forma natural en las plantas con características más respetuosas y sostenibles con el medio ambiente.

Los resultados obtenidos en esta Tesis Doctoral tras aplicar MT en kiwis a concentraciones bajas (0,1 mM) mostraron una mejora de la calidad general y de los daños por frío que se producen durante el almacenamiento de este fruto, con resultados similares a los mostrados con aplicaciones a concentraciones comerciales del 1-MCP (0,5 $\mu L\ L^{-1}$). Ambos tratamientos retrasaron la maduración del kiwi, minimizaron las pérdidas de peso y la respiración del fruto y mantuvieron valores más altos de firmeza y acidez. Por tanto, se podría concluir que la MT tuvo un impacto similar al observado con el tratamiento convencional con 1-MCP.

Los tratamientos combinados de GABA (1 mM) + 1-MCP (0,3 $\mu L\ L^{-1}$) en aguacate y GABA (10 mM) + 1-MCP (0,5 $\mu L\ L^{-1}$) en tomate mostraron cómo la sinergia entre ambos compuestos condujo a una mayor eficacia que la observada cuando se aplicaron estas sustancias de forma individual en la mejora de la vida útil de los frutos. El aumento de la calidad general y la mayor protección contra los daños por frío en los frutos podría ser debido a una mayor firmeza e integridad de las membranas celulares. De hecho, se observaron valores más bajos de los daños por frío en los frutos tratados con la combinación de los tratamientos, reduciendo la fuga de electrolitos y las lesiones provocadas por el frío. De esta forma se minimizaron los desórdenes fisiológicos y la pérdida de calidad que se produce durante el almacenamiento. La aplicación sinérgica del GABA y 1-MCP redujo de forma eficaz el metabolismo respiratorio, las pérdidas de peso y retrasó la evolución del color y de los compuestos bioactivos del fruto. Cuando se aplicaron los tratamientos combinados entre sí se obtuvieron los valores más bajos de los daños causados por el frío.

Estos hallazgos suponen un avance del conocimiento en el uso de estos compuestos de origen natural, lo que permitiría en el futuro, desarrollar tecnologías para reducir el uso de compuestos artificiales como el 1-MCP dadas las características sinérgicas entre ambas sustancias. De este modo, en un futuro se podría optimizar las concentraciones aplicadas para obtener una reducción en las fisiopatías con la utilización de la menor cantidad posible de compuestos artificiales y mejorando la sostenibilidad de los tratamientos poscosecha.

ABSTRACT

In this Doctoral Thesis, the effects of applying elicitors on different plant species have been studied, aiming to reduce physiological disorders occurring during preharvest, as well as minimizing quality loss and chilling injuries the postharvest storage of fruits, thereby extending their shelf life. Another primary objective has been to evaluate the synergistic effect of combining elicitors as natural-origin treatments with artificial-origin substances widely used in postharvest fruit preservation, such as 1-methylcyclopropene (1-MCP).

Sweet cherries are fruits of high national importance, primarily produced for fresh consumption. However, their production often suffers significant losses due to susceptibility to frost and cracking, especially in early-harvest varieties. The frost during flowering and rainfall during the fruiting stage can compromise the integrity of floral organs and the fruit itself, leading to substantial economic losses. Excessive rainfall, temperature fluctuations, and high humidity levels cause cracking in sweet cherry, which have thinner skins, making them more prone to cracking compared to other plant species. This crop is also affected by spring frosts, impacting yield and the number of harvested fruits. The physiological disorders caused by climatic conditions result in significant economic losses for growers, particularly those in areas where the topography and location result in consistent rainfall, as is the case for the sweet cherry evaluated in this Doctoral Thesis under the Protected Geographical Indication (PGI) "Cerezas de la Montaña de Alicante." Therefore, to mitigate the negative impacts on sweet cherry due to the variability of climatic conditions, this Doctoral Thesis has developed various preharvest strategies.

Moreover, the influence of different maturity stages on sweet cherry cracking has not yet been evaluated. Studying this factor could provide sufficient knowledge to optimize effective tools for reducing sweet cherry cracking, which is why it has been chosen as a subject of study for this Doctoral Thesis. The contribution of this work focuses on the use of natural-origin treatments applied at key development stages for the 'Prime Giant', 'Sweetheart', 'Early Lory' and 'Staccato' cultivars, aiming to address the challenges of frost and cracking in cherries and evaluate their impact on quality at harvest time. To tackle this issue, elicitors such as methyl jasmonate (MeJa) at 0.5 mM, putrescine (PUT) at 1 mM and 10 mM, and melatonin (MT) (0.01, 0.05, and 0.1 mM) were applied through foliar spraying across different growing cycles (2019, 2020, 2021, 2022, 2023). MeJa, PUT, and MT are endogenous plant hormones present in plant tissues that play roles in growth, development, and stress response induction. Using putrescine and MT has been shown to reduce the impact of spring frosts on cherries by lowering the MDA content in flower buds and increasing fruit set. Sweet cherries treated with MeJa, PUT, and MT effectively improved the fruit's tolerance to rain-induced cracking. It has been observed that there is variability in fruit sensitivity to cracking, depending on the cultivar and maturation stage. The MeJa and PUT treatments in sweet cherry have proven effective in slowing down fruit ripening, increasing firmness, enhancing soluble solid accumulation, and delaying colour progression at harvest. Additionally, these treatments helped maintain the overall quality of the fruit, showing increased firmness and delayed development of colour, soluble solids, and total acidity compared to untreated fruit at harvest. The results suggest that preharvest elicitor treatments for cherries can serve as an effective tool for mitigating production losses, offering a promising strategy for adapting to climate change and alleviating stress.

Additionally, the postharvest effects of elicitors on various plant species have been studied, including the evaluation of the effect of using MT at different concentrations on cut carnation. Although this is an edible flower, its primary use is as an ornamental flower, especially white carnations, which were used in this Doctoral Thesis. Vase life is defined as the number of days a flower maintains its decorative qualities after being cut. Therefore, delaying the flower senescence processes to extend the marketing period is of great interest. This aspect is an important economic factor for the floriculture industry, as extending the period during which the flower remains in optimal condition improves transportation efficiency and increases export opportunities. At the time of publication, this was the first article describing the impact of MT on the vase life of cut flowers. The carnations were placed individually in Falcon tubes with 10 mL of different treatments, using MT at concentrations of 0, 0.01, 0.1, and 1 mM. Treatment with 0.1 mM MT delayed senescence in the carnations, effectively doubling vase life compared to control flowers. This effect could be attributed to reduced metabolism, increased membrane stability, and improved water balance. The additional energy provided by the MT treatment could be reducing respiration rates and the energy requirements of cellular tissues, thus extending their shelf life. Additionally, MT has been shown to enhance antioxidant activity and phenolic content, reducing oxidative damage in carnation tissues.

These elicitors have been described as substances that, in addition to regulating energy balance, stimulate plant defenses. Therefore, parallel studies have been conducted on the effects of elicitor compounds such as MT and γ-aminobutyric acid (GABA), applied alone or in combination with other conventional artificial-origin treatments, on postharvest quality and the reduction of physiological disorders in different plant species. Temperature control is a widely used preservation method to delay fruit ripening and extend its shelf life. However, prolonged storage at low temperatures can cause chilling injuries in sensitive plant species, leading to cumulative physiological disorders and increased susceptibility to rots. Generally, symptoms of chilling injury become more pronounced after transferring the fruit to room temperature. Kiwi, avocado, and tomato are susceptible to physiological disorders from chilling injury, and their tolerance depends on factors such as species, cultivar, harvest time, temperature, and duration of exposure to suboptimal temperatures. To address this issue, this Doctoral Thesis has evaluated the effect of treatments with elicitors such as MT and GABA, individually or in combination with 1-MCP, in the postharvest of kiwi, avocado, and tomato. These treatments have proven effective in reducing these physiological disorders in various plant species individually. However, while 1-MCP is an artificial-origin substance, GABA and MT are naturally occurring elicitors in plants, offering more environmentally friendly and sustainable characteristics.

The results obtained in this Doctoral Thesis from applying MT to kiwis at low concentrations (0.1 mM) demonstrated an improvement in overall quality and reduction of chilling injuries during fruit storage, with similar outcomes to those obtained using commercial concentrations of 1-MCP (0.5 μL L^{-1}). Both treatments delayed kiwi ripening, minimized weight loss and fruit respiration, and maintained higher firmness and acidity levels. Therefore, it can be concluded that MT had a similar impact to the conventional 1-MCP treatment.

Combined treatments of GABA (1 mM) + 1-MCP (0.3 μL L^{-1}) in avocado and GABA (10 mM) + 1-MCP (0.5 μL L^{-1}) in tomato demonstrated that the synergy between these compounds resulted in greater efficacy compared to their individual application in extending the shelf life of the fruits. The improvement in overall quality and increased protection against chilling injury may be attributed to enhanced firmness and membrane integrity. In fact, lower values of chilling injury were observed in fruits treated with the combined treatments, reducing electrolyte leakage and chilling injury lesions. This effectively minimized physiological disorders and quality loss during storage. The synergistic application of GABA and 1-MCP effectively reduced respiratory metabolism, weight loss, and delayed the evolution of fruit colour and bioactive compounds. The lowest levels of cold-induced damage were obtained when the treatments were applied in combination.

These findings represent a significant advancement in knowledge, and the results could serve as a basis for expanding the use of natural-origin compounds and facilitating the substitution or reduction of artificial-origin substances, given the synergistic potential between these treatments. This approach could allow for optimizing the applied concentrations to reduce physiological disorders with the lowest possible use of artificial compounds, thereby improving the sustainability of postharvest treatments.

1. INTRODUCCIÓN

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1.1. Características generales e importancia de los cultivos

1.1.1. Cereza (*Prunus avium* L.)

Las cerezas pertenecen a la familia Rosaceae y al género *Prunus*. Su origen geográfico no se conoce con exactitud, pero se cree que proviene de los países que circundan el mar Negro y el mar Caspio, desde donde se expandieron hacia Europa y Asia, y ahora se cultivan ampliamente en todo el mundo (Blando y Oomah, 2019). El desarrollo de este fruto se puede describir mediante una curva sigmoidea doble, donde se pueden identificar tres etapas diferenciadas (Figura 1). La duración de cada etapa varía con el genotipo y las condiciones ambientales del fruto (Zhang y Whiting, 2013).

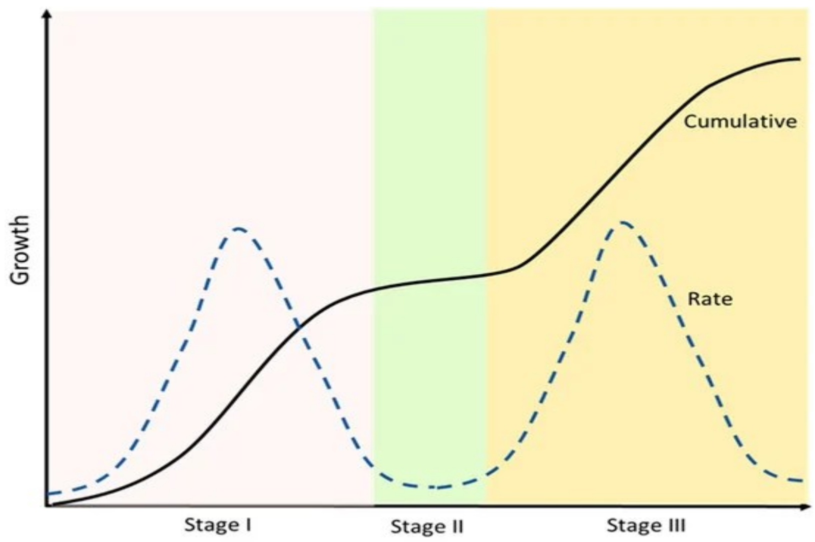


Figura 1. Representación de una curva de crecimiento sigmoidea doble donde se definen el crecimiento acumulativo y la tasa de crecimiento de las cerezas. Fuente: Vignati et al. (2022).

La cereza no es climatérica y la maduración es promovida por el ácido abscísico (ABA). El color de la piel cambia y comienza el ablandamiento; la glucosa y la fructosa se acumulan, junto con el incremento del contenido de vitaminas, ácidos orgánicos y la síntesis de antocianinas (Serrano et al., 2005).

En general, los consumidores se sienten atraídos por la apariencia de las cerezas con una piel brillante y reluciente, intensidad y homogeneidad del color de la piel, ausencia de defectos e imperfecciones, apariencia del pedicelo y el tamaño de la fruta (Díaz-Mula et al., 2009a). Son frutas altamente nutritivas ya que contienen niveles significativos de nutrientes y componentes bioactivos como las antocianinas, los compuestos fenólicos y los flavonoides (Figura 2), que contribuyen a la actividad antioxidante total y están relacionados con numerosos beneficios para la salud (Chezanoglou et al., 2024).

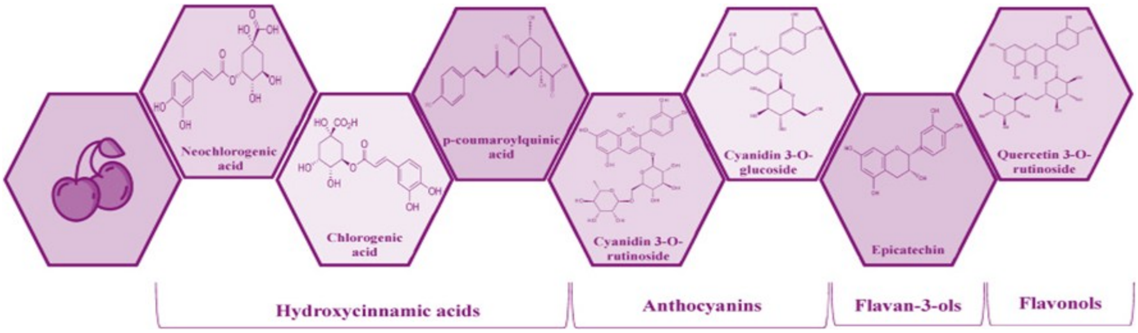


Figura 2. Principales compuestos bioactivos en la cereza. Fuente: Chezanoglou et al. (2024).

También se consideran una excelente fuente de taninos, que son metabolitos secundarios que confieren astringencia y tienen propiedades beneficiosas para la salud. La biosíntesis de compuestos fenólicos se desencadena por la exposición a condiciones de estrés, como un sistema de defensa natural (Kataoka et al., 1996). Las cerezas también son ricas en vitaminas C, A, E y K y carotenoides, especialmente β-caroteno, luteína, zeaxantina y xantofila (Di Matteo et al; 2016).

Debido a la alta demanda de los consumidores a nivel mundial y su cultivo en más de 40 países, la producción de cereza ha aumentado rápidamente. La producción mundial en el año 2022 fue de alrededor de 2,8 millones de toneladas, y los principales productores fueron Turquía, Chile, Uzbekistán y Estados Unidos. España ocupa el quinto lugar en la producción mundial de cereza y el primer puesto como país productor en Europa (Figura 3).

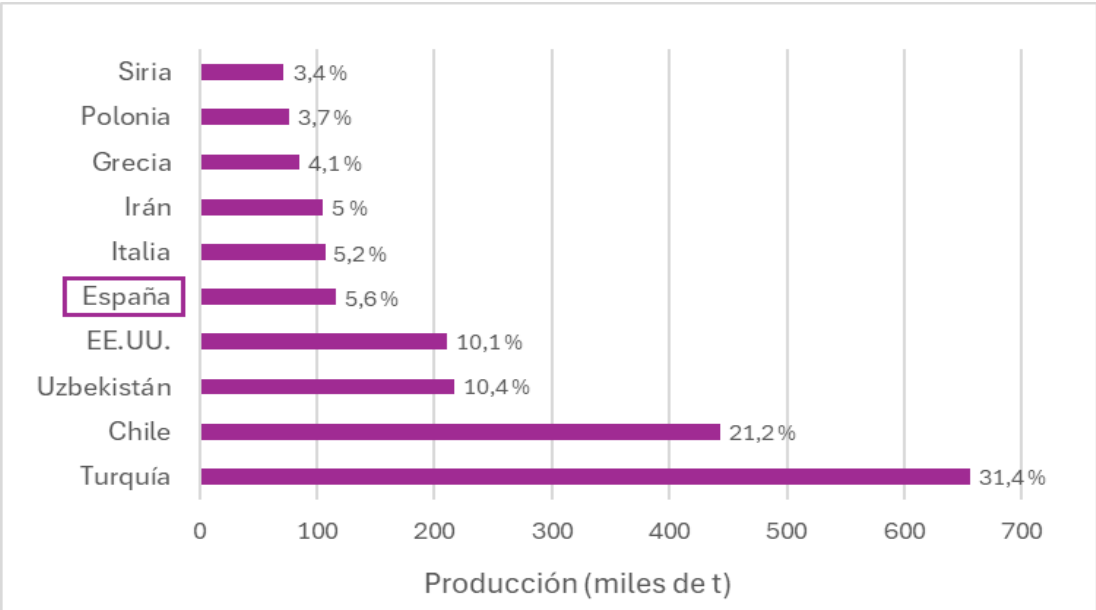


Figura 3. Top 10 países productores de cerezas (miles de toneladas) a nivel mundial en el año 2022. El % representado está calculado en base a la producción total de los 10 principales países. Fuente: FAOSTAT, consultada en agosto de 2024.

En España, la producción y el valor del cultivo de la cereza ha aumentado en los últimos 10 años. Aunque se ha registrado durante varios años (2018 y 2020) una caída en el valor de mercado, en general, el cultivo ha experimentado una revalorización en la última década (Figura 4).

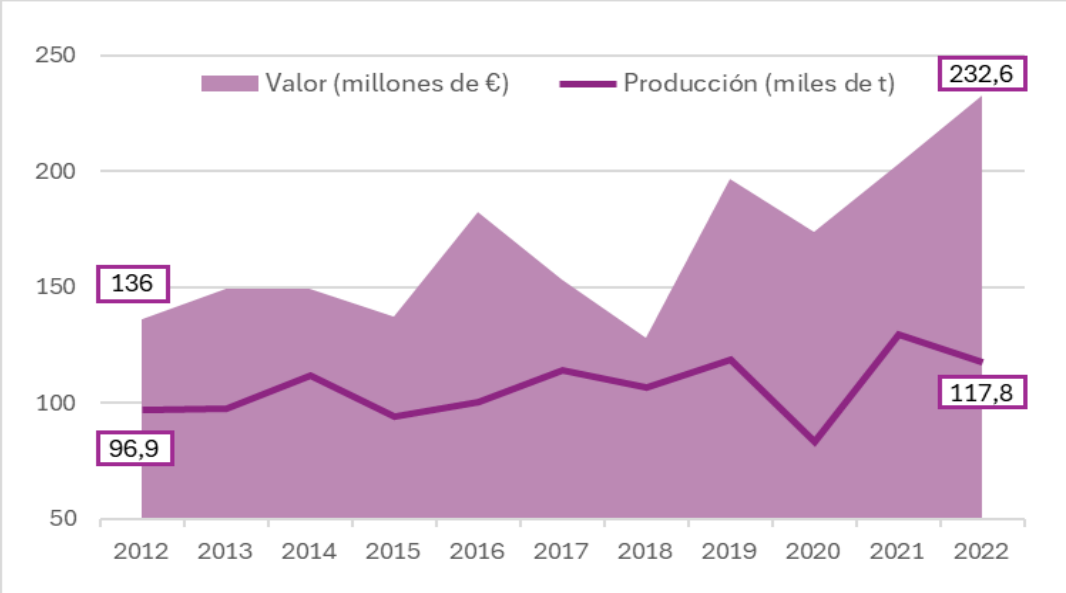


Figura 4. Evolución de la producción (miles de toneladas) y el valor (millones de euros) de cerezo durante los últimos 10 años disponibles (2012-2022) en España. Fuente: MAPA, consultado en agosto de 2024.

El 75 % de la producción de cereza en España está localizada principalmente en Aragón (41 %) y en Extremadura (34 %). Le siguen Cataluña (6 %), Andalucía (5 %), Galicia (4 %) y en sexto lugar se sitúan la Comunidad Valenciana (3 %) y la Región de Murcia (3 %) con alrededor de unas 3.500 toneladas de cerezas producidas en el año 2022 (Figura 5).

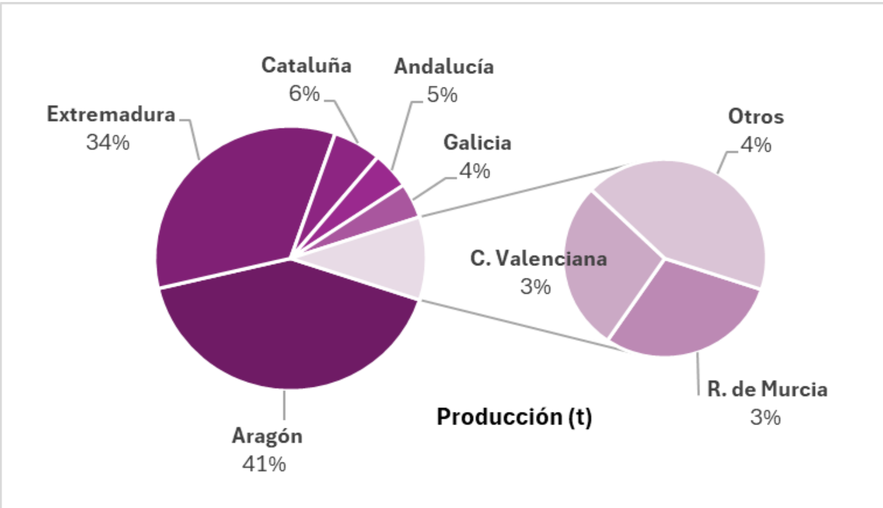


Figura 5. Distribución territorial de la producción total en toneladas de cerezo y guindo en España en el año 2022. Fuente: MAPA, consultado en agosto de 2024.

1.1.2. Clavel (*Dianthus caryophyllus* L.)

El género *Dianthus* pertenece a la familia *Caryophyllaceae* (orden Caryophyllales). Las Caryophyllaceae comprenden más de 80 géneros y 3000 especies, con una distribución mayormente holártica, es decir, en las zonas templadas a árticas de Eurasia y América del Norte. Debido a su excelente calidad de conservación, amplia variedad de formas, aptitud de soportar el transporte a larga distancia y capacidad de rehidratación después del envío, el clavel es la flor ornamental preferida por los productores de países exportadores (Harbaugh et al., 2010).

Entre los diferentes tipos de claveles, los tres más comunes son los anuales, de jardín y de floración continua. Los claveles cultivados y vendidos en la floricultura hoy en día son muy diferentes del *D. caryophyllus* silvestre que crece en las regiones mediterráneas; estos son simples y tienen cinco pétalos (Baris y Uslu, 2009). Existen muchas variedades de claveles utilizados como flores cortadas que han sido seleccionadas por el tamaño, el número de pétalos, la longitud del tallo y la resistencia a enfermedades (Jürgens et al., 2003) (Figura 6).

Figura 6. Tipos de variedades de clavel. Fuente: Jawaharlal et al. (2009).

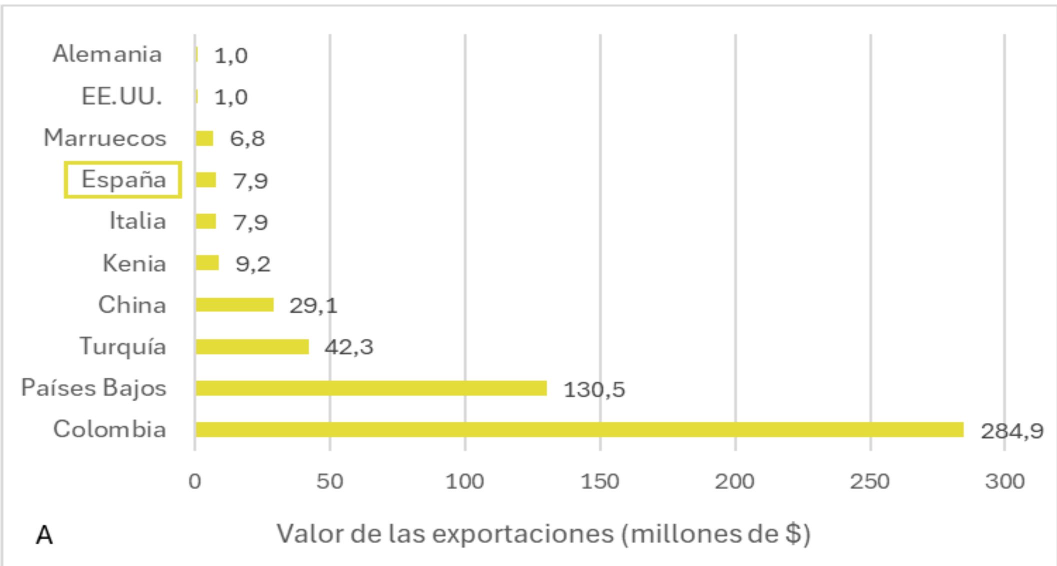
En muchos países, el clavel es una de las flores cortadas más populares y de mayor importancia económica en la industria de la floricultura. Las flores cortadas de clavel se utilizan en dos formas: el tipo estándar o uniflora, en el que los claveles tienen una flor de mayor tamaño por tallo, y el tipo "spray" o multiflora, en el que tienen múltiples flores de tamaño más pequeño por tallo (Figura 7). En los últimos años, el clavel tipo "spray" se ha vuelto popular porque puede cultivarse con menos mano de obra y satisfacen la demanda del consumidor moderno (Satoh et al. 2005).

Figura 7. Variedades de clavel tipo "spray" o multiflora. Fuente: Adaptado de Jawaharlal et al. (2009).

La condición de la flor es el atributo de calidad más importante en lo que respecta a la aceptación del consumidor, seguida de la forma, el color y el tamaño. La ausencia de infección, el precio y la condición del follaje también influyen en la calidad general (Dahal et al., 2018).

El *D. Caryophyllus* silvestre puede tener un aroma a clavo y es una flor comestible que se puede usar en ensaladas o para dar sabor a muchos alimentos, con propiedades antioxidantes y antimicrobianas (Khalid et al., 2019). Sin embargo, los claveles de la floricultura actual han perdido aroma por su mayor vida útil en florero (Chandler y Brugliera, 2011).

La exportación mundial de clavel está concentrada en Colombia (54,7 %) y Países Bajos (25 %) con casi el 80 % del valor, seguidos de Turquía, China, Kenia, Italia y España (Figura 8A). Sin embargo, la importación está más distribuida entre países: Estados Unidos (33,9 %), Japón (17,6 %), Países Bajos (17,4 %), Reino Unido (8,6 %), Polonia (7,4 %), Alemania (5,1 %) y España con un 3,6 % (Figura 8B). España ocupa el séptimo lugar durante el año 2023 tanto en valor de exportaciones como de importaciones de claveles a nivel mundial (Figura 8A, B).



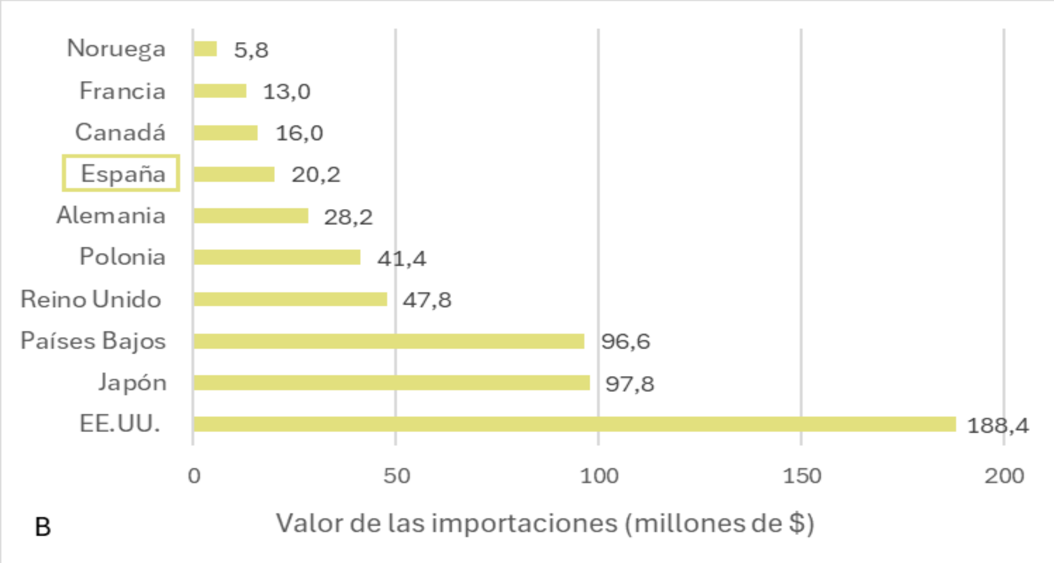


Figura 8. Principales países exportadores (A) e importadores (B) de claveles a nivel mundial en el año 2023. Fuente: UN Comtrade Database, consultada en agosto de 2024.

En el territorio español, la producción de claveles está centrada principalmente en Andalucía con un 63 % del total. A continuación, destaca la Región de Murcia con un 22,9 %, Galicia (9,2 %) y Canarias (4,3 %). La superficie dedicada al cultivo de claveles es proporcional a la distribución de la producción por regiones, con más de la mitad del total del área localizada en Andalucía (58 %) (Figura 9).

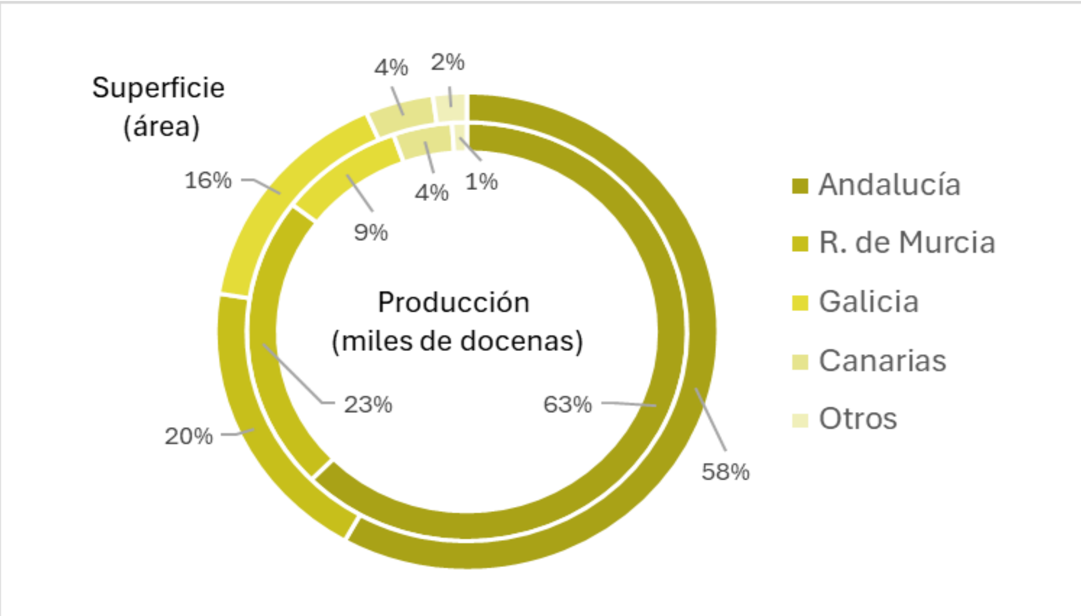


Figura 9. Distribución territorial de la producción en miles de docenas (anillo interior) y de la superficie en área (anillo exterior) de claveles en España en el año 2022. Fuente: MAPA, consultado en agosto de 2024.

1.1.3. Kiwi (*Actinidia deliciosa* (A. Chev.))

El kiwi es una planta trepadora que pertenece a la familia Actinidiaceae, originario de las regiones montañosas de China dónde crecía en forma silvestre, ahora se cultiva comercialmente en muchos países debido a su tolerancia a las condiciones climáticas (Ferguson et al. 2023). Los más consumidos son el kiwi verde (*Actinidia deliciosa*), el kiwi Sungold (*Actinidia chinensis*) y el mini kiwi (*Actinidia arguta*). La diversidad genética del kiwi mejora la disponibilidad de esta fruta en el mercado durante todo el año y sus características influyen en las preferencias de los consumidores (Han et al., 2019; Yuan et al., 2022).

El kiwi ocupa una posición destacada entre las frutas altamente nutritivas, ya que es una fuente de fitonutrientes y compuestos bioactivos (Figura 10) como clorofilas, carotenoides, polifenoles y flavonoides (Lin et al., 2023; Li et al., 2024a). Es una fuente importante de vitamina C, además de tener altos niveles de fibra, potasio, vitamina E y ácido fólico (Vaidya et al., 2022). Además, en el kiwi están presentes otros pigmentos como la luteína, la zeaxantina y el β-caroteno, que contribuyen a la neutralización de radicales libres, la reducción de la degeneración macular, el riesgo de cáncer o el envejecimiento celular (Khutare y Deshmukhs, 2023; Moysidou et al., 2024). La mayor parte de fibra en el kiwi es insoluble y tiene la capacidad de reducir la absorción de glucosa, colesterol, ácidos biliares y carcinógenos dietéticos, mejorando afecciones como diabetes o hipercolesterolemia (Pedrosa y Fabi, 2024).

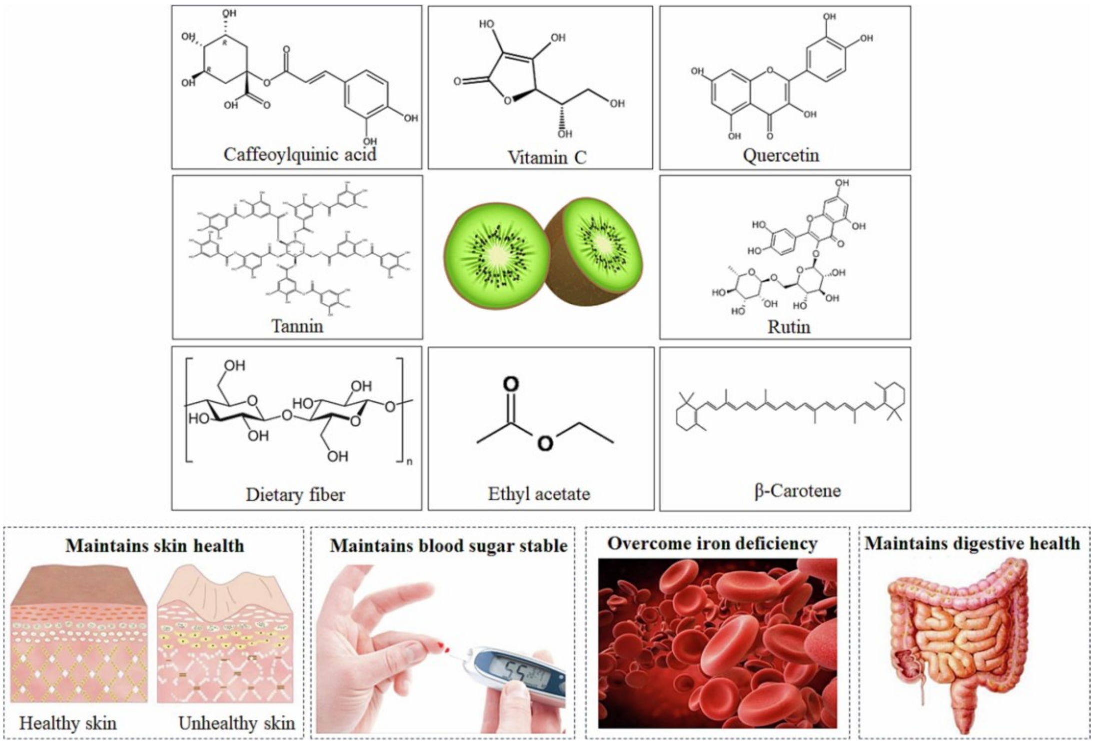


Figura 10. Compuestos beneficiosos del kiwi y sus efectos en la salud. Fuente: Lin et al. (2023).

China es el mayor productor de kiwi con 2,38 millones de toneladas, representando más de la mitad de la producción mundial en el año 2022. Nueva Zelanda ocupa el segundo lugar seguido de Italia, Grecia e Irán. Para otros países, el kiwi no es uno de los principales cultivos, con porcentajes que varían entre el 2,5 % y el 0,6 %. Por ejemplo, la producción española en 2022 fue de sólo 27.380 toneladas, un 0,61 % de la producción mundial (Figura 11).

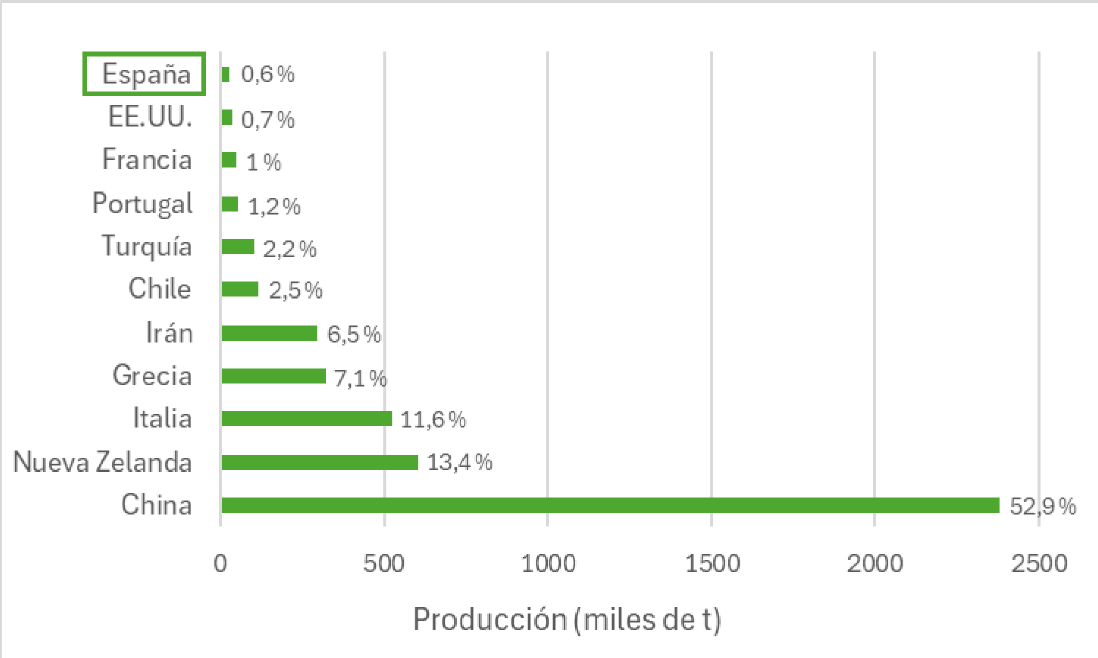


Figura 11. Principales países productores de kiwi (miles de toneladas) a nivel mundial en el año 2022. El % representado está calculado en base a la producción total de los 11 principales países. Fuente: FAOSTAT, consultada en agosto de 2024.

En España, la producción y el valor del kiwi ha incrementado progresivamente durante los últimos 10 años, con una producción de 18.800 27.600 toneladas en el año 2022. Respecto al valor del cultivo, el aumento ha sido sustancial también puesto que se ha multiplicado por dos el valor en 10 años, alcanzando los 29 millones de euros en el año 2022. Debido al incremento de la producción y el valor económico durante los últimos años, se debe tener en cuenta el futuro prometedor que puede tener este cultivo en España (Figura 12).

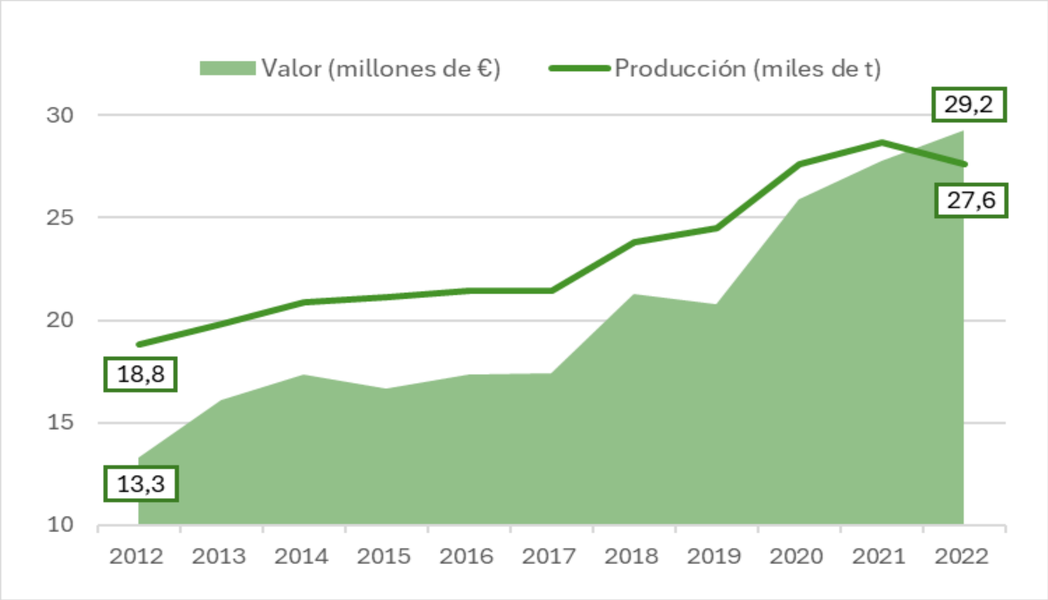


Figura 12. Evolución de la producción (miles de toneladas) y el valor (millones de euros) de kiwi durante los últimos 10 años disponibles (2012-2022) en España. Fuente: MAPA, consultado en agosto de 2024.

En el territorio español, Galicia es el principal productor con más del 50 % del total de toneladas y con un 66 % de la superficie cultivada en el 2022. A continuación, le sigue el Principado de Asturias con un 17 % de la producción y el 14 % de superficie. La Comunidad Valenciana se posiciona en tercer lugar con un 11 % de producción en la mitad de hectárea cultivada (5 %), teniendo un rendimiento mayor de superficie en producción comparado con los principales territorios. En cuarto lugar, se sitúa el País Vasco con un 8 % de la producción total de kiwi del territorio nacional (Figura 13).

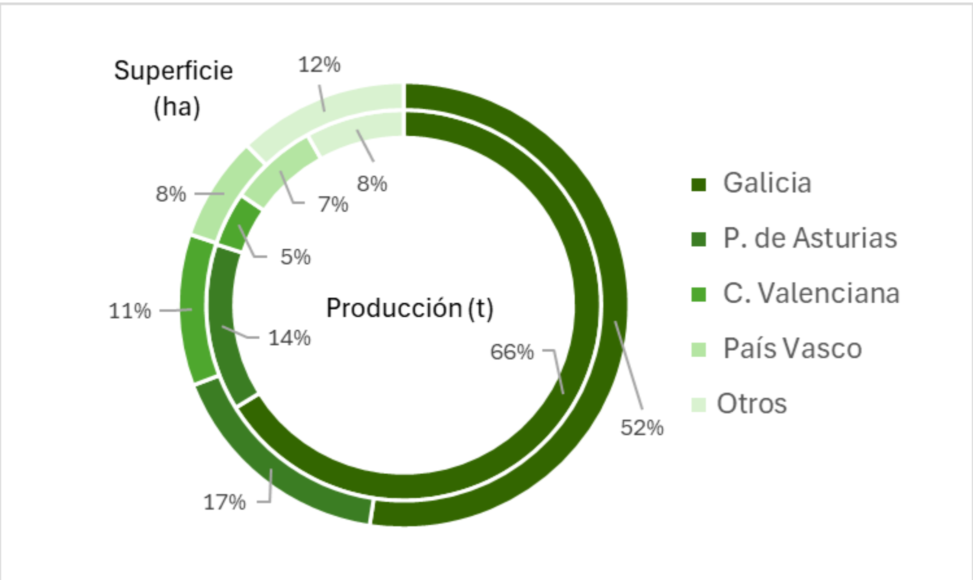


Figura 13. Distribución territorial de la producción (t) y de la superficie (ha) de kiwi en España en el año 2022. Fuente: MAPA, consultado en agosto de 2024.

1.1.4. Aguacate (*Persea americana* Mill.)

El aguacate (*Persea americana* Mill.) es una fruta subtropical/tropical perteneciente a la familia Lauraceae y al género *Persea,* del que se conocen más de 150 especies (Araújo et al., 2018). Los aguacates se originaron en América Central y México, siendo actualmente una fruta ampliamente producida y consumida en todo el mundo (Salazar-López et al., 2020).

Tradicionalmente se han reconocido tres ecotipos de aguacate adaptados a diferentes condiciones climáticas: Antillano (*Persea americana* var. Americana), Mexicano (*Persea americana* var. Drymifolia) y Guatemalteco (*Persea americana* var. Guatemalensis) (Hurtado-Fernández et al., 2018).

Figura 14. Frutos de aguacate 'Hass' colgados del árbol antes de la cosecha. Fuente: Talavera et al. (2023).

No existen barreras de esterilidad entre los tres ecotipos ni entre ninguna categoría taxonómica de *P. americana*. Por ello, la hibridación es frecuente entre árboles de diferentes tipos que crecen en proximidad (Alcaraz y Hormaza, 2021). Entre las variedades comerciales más importantes de aguacate se encuentran 'Hass', 'Fuerte', 'Ettinger' y 'Pinkerton'. 'Hass' es la variedad subtropical más solicitada y constituye la base de la industria de exportación de aguacate subtropical a Europa y Estados Unidos (Figura 14).

La aceptabilidad del consumidor se relaciona principalmente con la textura de la pulpa más que con el sabor o el contenido de aceite (Ballen et al., 2022). Los aguacates maduros con textura cremosa con sabor a nuez son los más valorados por los consumidores (Giuggioli et al., 2023). El aguacate es una matriz muy compleja formada por una gran variedad de compuestos. El contenido de nutrientes de la parte comestible del fruto (pulpa o mesocarpio) varía mucho en función de factores como la variedad, el grado de maduración y las condiciones de cultivo (Kamble et al., 2024).

Los ácidos grasos monoinsaturados son los predominantes en el aguacate y proporcionan los principales beneficios saludables (Lieu et al., 2024). También contiene niveles significativos de fibra dietética, potasio, magnesio, vitaminas C, E, K y del grupo B, carotenoides, clorofilas, antocianinas, compuestos fenólicos, fitoesteroles y terpenoides (Nasri et al., 2023). El consumo del aguacate contribuye al mantenimiento del colesterol, al control de la diabetes, a la prevención del cáncer y a la mejora de la salud cardiovascular (Collignon et al., 2023; Okobi et al., 2023; Probst et al., 2024).

La alta demanda de aguacate a nivel mundial ha contribuido a crear un impulso motivacional para que los agricultores inviertan en la producción de esta fruta. Anualmente se producen más de siete millones de toneladas en todo el mundo, de los cuales el 81,4 % se cosecha en cinco países: México, Colombia, Perú, República Dominicana y Kenia. España es el único país europeo con una producción comercial significativa de aguacate con 105.930 toneladas en 2022, situándose en el puesto número 17 a nivel mundial (Figura 15).

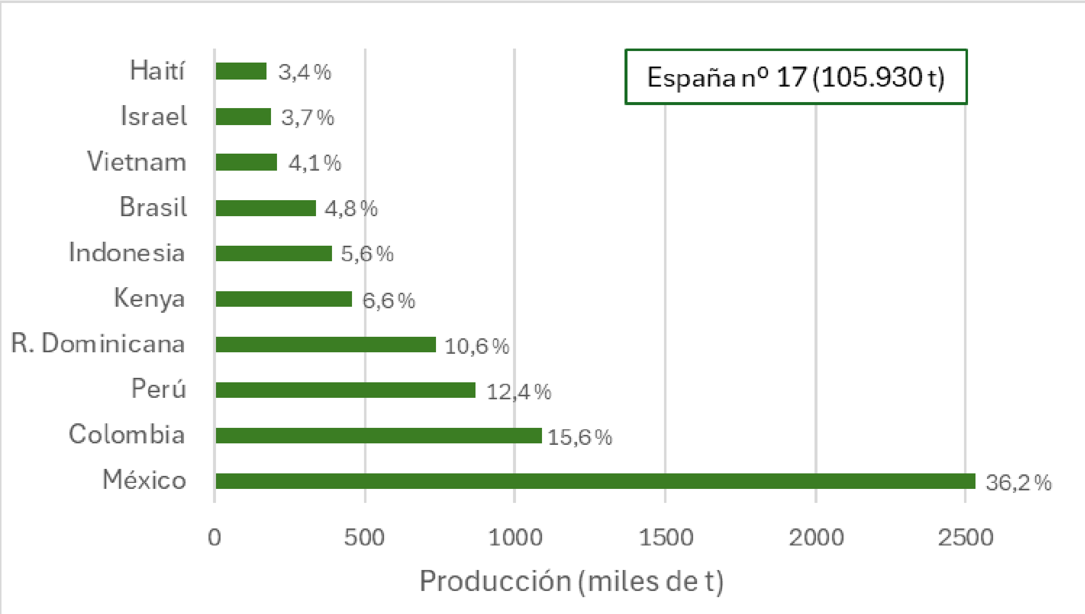


Figura 15. Top 10 países productores de aguacate (miles de toneladas) a nivel mundial en el año 2022 y puesto en el ranking mundial de la producción de España. El % representado está calculado en base a la producción total de los 10 principales países. Fuente: FAOSTAT, consultada en agosto de 2024.

Se ha producido un aumento progresivo en los últimos diez años en la producción de aguacate en España. Sin embargo, la evolución en el valor del aguacate ha sido más notoria alcanzando más del doble con 221,9 millones de euros en el año 2022 (Figura 16). El alto valor de este fruto el mercado y la demanda actual supone un atractivo para que los agricultores inviertan en su producción.

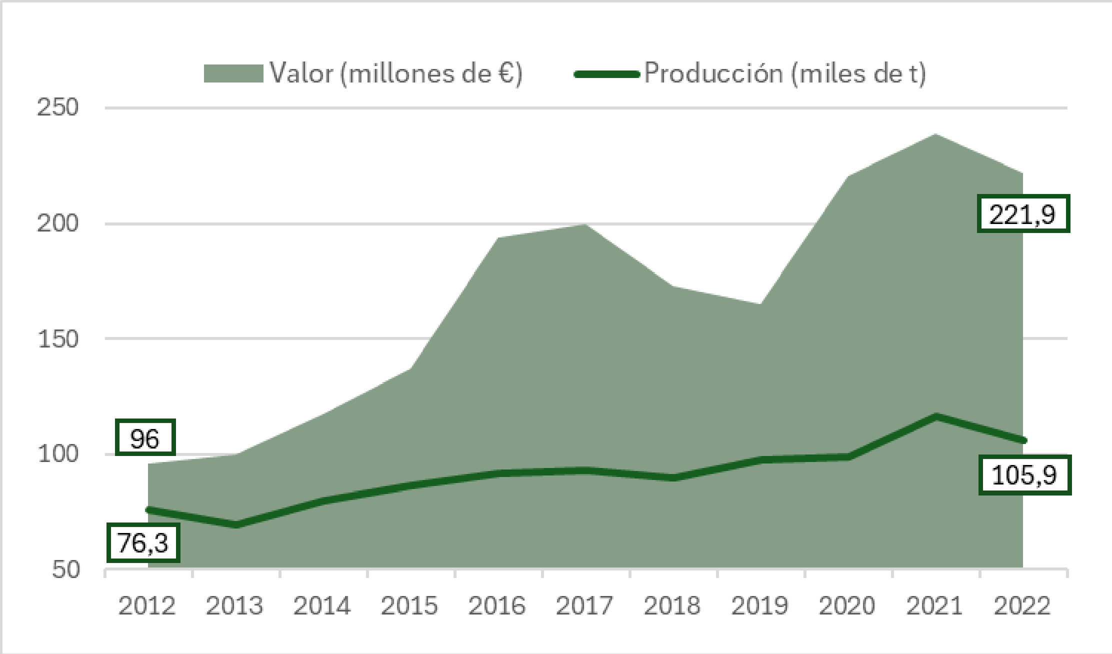


Figura 16. Evolución de la producción (miles de toneladas) y el valor (millones de euros) de aguacate durante los últimos 10 años disponibles (2012-2022) en España. Fuente: MAPA, consultado en agosto de 2024.

La distribución de la superficie y la producción en el territorio nacional está repartida en tres Comunidades Autónomas, siendo Andalucía la mayor productora de España con el 75 % del total y una superficie que representa el 74 % del total del país. A continuación, le sigue Canarias con un 14 % de la producción y la Comunidad Valenciana en tercer lugar, con un 12 % tanto de producción como de superficie cultivada de aguacates a nivel nacional (Figura 17).

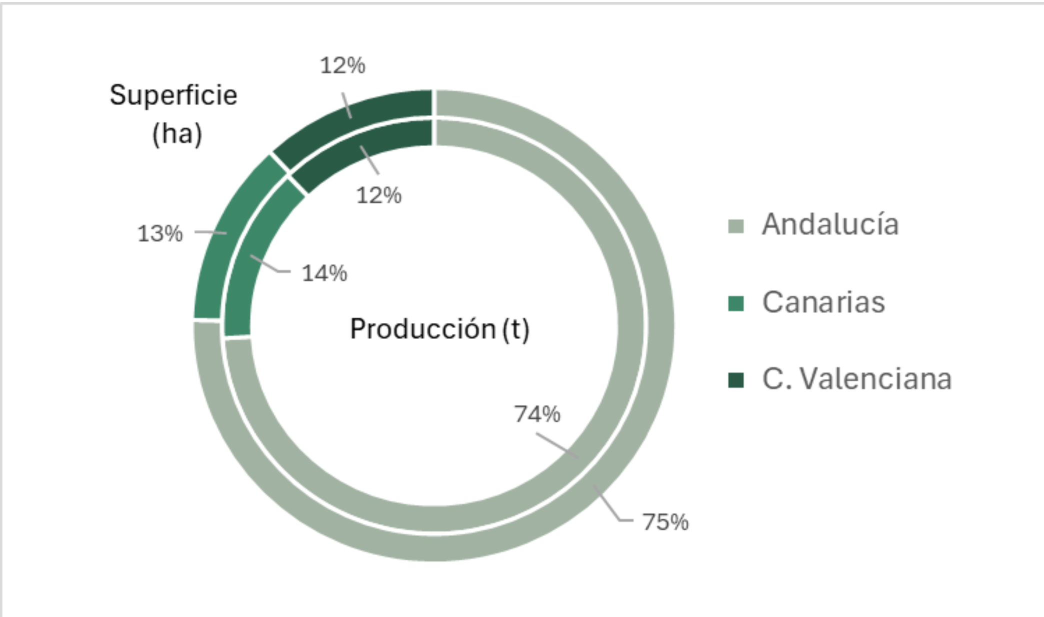


Figura 17. Distribución territorial de la producción total en toneladas de aguacate en España en el año 2022. Fuente: MAPA, consultado en agosto de 2024.

1.2.5. Tomate (*Solanum lycopersicum* L.)

Entre los cultivos comerciales más significativos a nivel mundial se encuentra el tomate, el cual pertenece a la familia Solanaceae con alrededor de 3.000 especies, entre ellas la patata. Se cree que el tomate se originó en los Andes sudamericanos y se extendió por todo el mundo tras la colonización española de América (Razifard et al., 2020).

Los destinatarios y comerciantes del mercado otorgan un gran valor al atractivo estético, la consistencia, la resistencia al agrietado, la calibración uniforme, la capacidad de transporte y la estabilidad del almacenamiento prolongado (Al-Dairi et al., 2021). Desde la perspectiva del consumidor, los componentes de calidad del tomate valorados son principalmente el tamaño, forma y color homogéneo. La firmeza y el sabor también son atributos que desencadenan la decisión de compra del tomate (Umeohia y Olapade, 2024).

Desde el punto de vista nutricional (Figura 18), los tomates son una excelente fuente de fibra, minerales y vitaminas C, E y A en forma de beta-caroteno. También contiene flavonoides y ácido fólico con propiedades antioxidantes (Yong et al., 2023). Sim embargo, el licopeno es el componente más importante del tomate por sus propiedades beneficiosas. Como captador de oxígeno, el licopeno ayuda a reducir la probabilidad de desarrollar diversos tipos de cáncer y enfermedades cardiovasculares e inflamatorias (Qi et al., 2021; Kapała et al., 2022; Landrier et al., 2023; Jiménez-Bolaño et al., 2024).

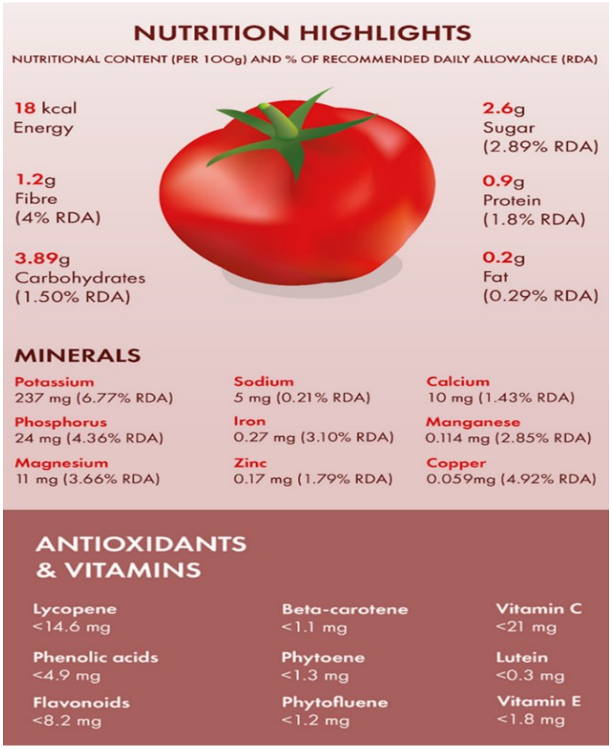


Figura 18. Composición nutricional del tomate. Fuente: Collins et al. (2022).

La producción global de tomate ha aumentado de manera constante en las últimas décadas y en el 2022 se estima que es de aproximadamente 186 millones de toneladas. China es con creces el mayor productor de tomate a nivel mundial seguido de India, Turquía y Estados Unidos. Y el resto de la producción mundial lo abarcan países como Egipto, Italia, México, Brasil, Nigeria y España en el décimo lugar (Figura 19).

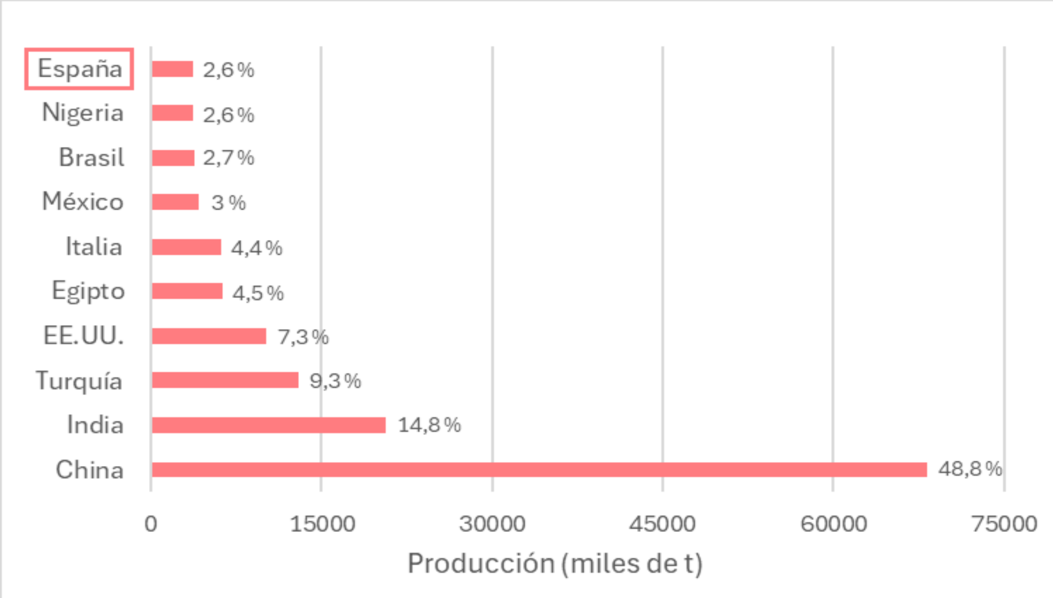


Figura 19. Top 10 países productores de tomate (miles de toneladas) a nivel mundial en el año 2022. El % representado está calculado en base a la producción total de los 10 principales países. Fuente: FAOSTAT, consultada en agosto de 2024.

La evolución de la producción de tomate en España ha disminuido levemente en los últimos años. Sin embargo, el valor del cultivo ha incrementado en 465 millones de euros en los últimos diez años a pesar del descenso ocurrido en la producción. Estas cifras nos indican la magnitud e importancia económica que posee el cultivo de tomate en España respecto a otros tipos de frutos (Figura 20).

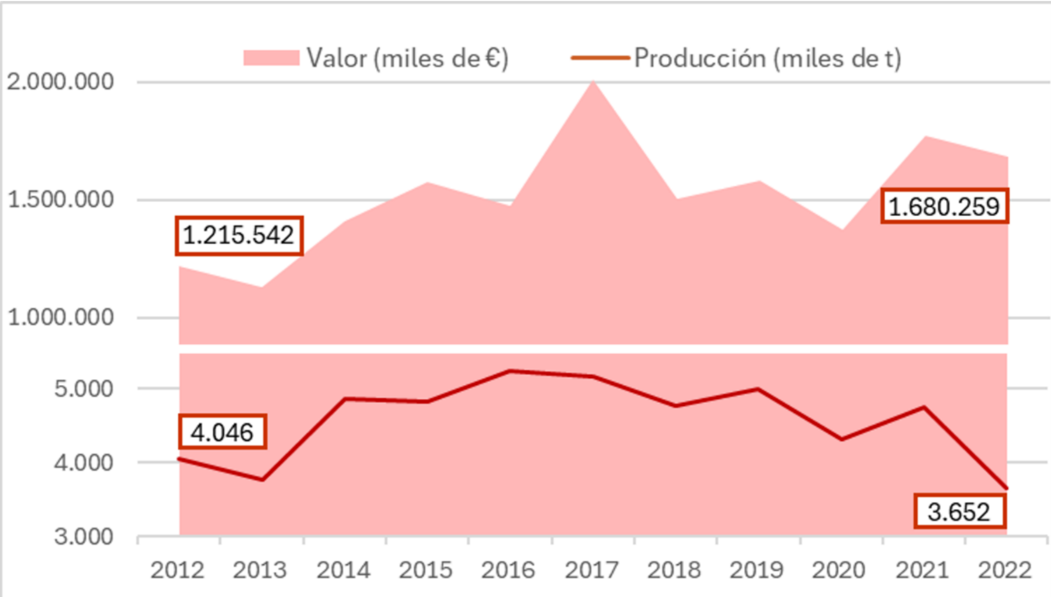


Figura 20. Evolución de la producción (miles de toneladas) y el valor (millones de euros) de tomate durante los últimos 10 años disponibles (2012-2022) en España. Fuente: MAPA, consultado en agosto de 2024.

El 78 % de la producción de tomate en España se centra en Extremadura (42 %) y Andalucía (36 %). En tercer lugar, se encuentra la Región de Murcia (6 %), seguido de Navarra (5 %), Castilla-La Mancha (3 %), Galicia (1,9 %) y la Comunidad Valenciana con un 1,7 % de la producción de tomate en 2022 (Figura 21).

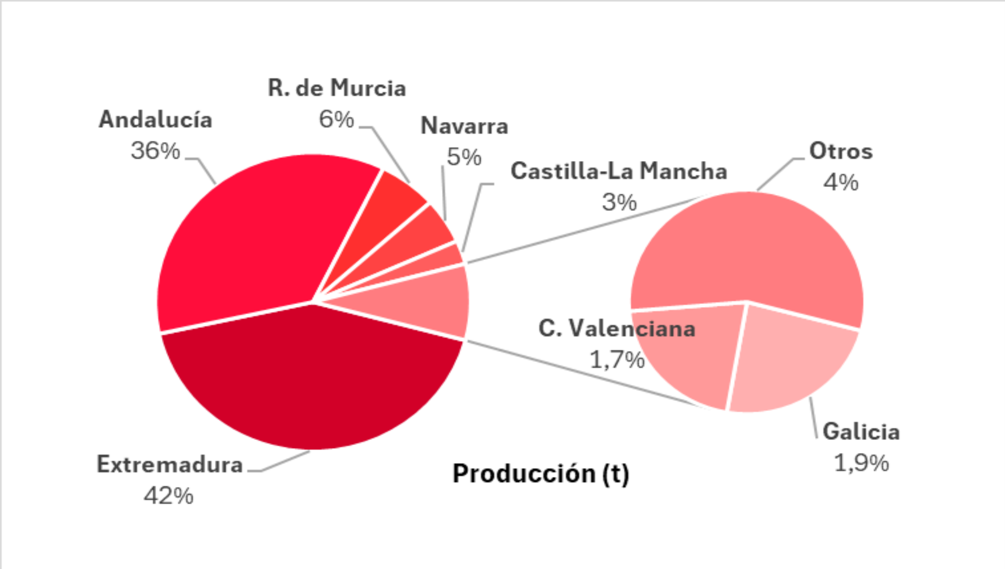


Figura 21. Distribución territorial de la producción total de tomate (toneladas) en España en el año 2022. Fuente: MAPA, consultado en agosto de 2024.

1.2. Desafíos en la producción y en la poscosecha de los cultivos

1.2.1. Cereza (*Prunus avium* L.)

La producción de cerezas es muy susceptible a las condiciones climáticas adversas y supone una preocupación para los productores debido a la escasa predictibilidad que se tiene sobre la climatología en los periodos de floración y desarrollo de las cerezas (Fernandez et al., 2023). De hecho, las caídas repentinas de temperatura primaverales son un desafío para los productores de cerezas, especialmente pueden provocar daños por heladas durante las etapas fenológicas de "botón floral" y "plena floración", causando una reducción del cuajado de frutos e impactando significativamente en el rendimiento de la producción (Kappel, 2010). Las heladas primaverales desintegran las estructuras celulares, lo que lleva a la muerte celular, causando daños y pérdidas significativas en el cultivo de la cereza (Kaya y Kose, 2022). Para prevenir el daño por heladas se han desarrollado estrategias con el fin de promover el crecimiento vegetativo y reproductivo de los tejidos. Se ha empleado el uso del riego deficitario (Blanco et al., 2020), estructuras protectoras para la lluvia (Pino et al., 2023) y polinizadores silvestres para mejorar el cuajado de los frutos (Mateos-Fierro et al., 2022). Los ésteres metílicos de ácidos grasos pueden regular el momento de la brotación y son una medida útil para evitar la coincidencia con las temperaturas adversas en los cerezos (Xu et al., 2023).

El agrietado es el desorden fisiológico más importante en la precosecha de las cerezas, causando pérdidas económicas importantes para los productores. El agrietado se puede dividir en macrogrietas (detectables mediante inspección visual) (Figura 22) y microgrietas (indetectables mediante inspección visual), siendo mayor el impacto económico de las macrogrietas (Schumann et al., 2020). Las frutas más grandes y firmes se ven más afectadas por el agrietado que las frutas pequeñas y de pulpa blanda (Schumann et al., 2019).

Figura 22. Macrogrietas en cereza.

El agrietado es el resultado de la absorción neta de agua en la fruta, lo que provoca un aumento en el volumen y el área superficial de la fruta, resultando en una piel tensa hasta provocar su ruptura (Knoche y Winkler, 2017; Pereira et al., 2020). El agrietado de la fruta puede representarse como una reacción en cadena (Figura 23) que comenzaría con la absorción localizada del agua, la ruptura de las células individuales, la liberación de ácido málico en el apoplasto, el hinchamiento de la epidermis y el debilitamiento de las células epidérmicas e hipodérmicas (Knoche y Peschel, 2006; Koumanov, 2015; Correia et al., 2018).

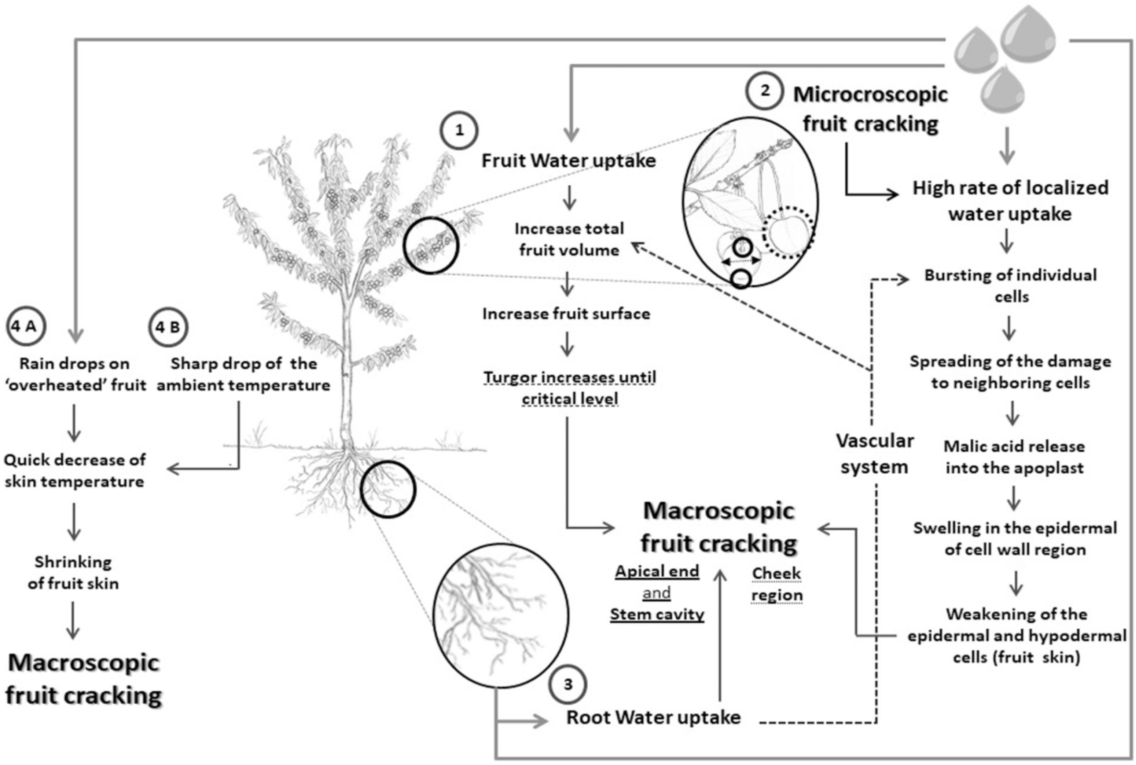


Figura 23. Representación esquemática de los diferentes mecanismos del agrietado de la cereza. Fuente: Correia et al. (2018).

Para reducir el agrietado se han estudiado las aplicaciones en precosecha con calcio (Matteo et al., 2022), silicato de sodio (Rombolà et al., 2023) y con reguladores del crecimiento como el ácido giberélico (AG) (Usenik et al., 2015) y el ABA (Balbontín et al., 2018). Sin embargo, las técnicas utilizadas son poco efectivas cuando la incidencia es alta, por ejemplo, por lluvias frecuentes. En consecuencia, es necesario desarrollar estrategias agronómicas que eviten o reduzcan la aparición final de macrofisuras, sin generar impactos adversos sobre los frutos y el árbol.

En cuanto al mantenimiento de la calidad de la cereza una vez recolectada, la temperatura de almacenamiento (0, 5 °C) y la gestión de una humedad relativa adecauda (90-95 %) son factores críticos para limitar la pérdida de agua y la deshidratación de la fruta y del pedicelo (Golding et al., 2017). Es posible extender la vida útil de las cerezas cuando se utilizan bajas temperaturas, especialmente combinadas con otras tecnologías como la atmósfera controlada, atmósfera modificada, aceites esenciales o recubrimientos comestibles (Wani et al., 2014; Hosseini et al., 2024; Zheng et al., 2024). También se ha evaluado el efecto de la aplicación poscosecha de compuestos de origen natural presentes en las plantas como el AG, el GABA, el JaMe y el ácido salicílico (AS) (Gu et al., 2022; Ozturk et al., 2022; Pan et al., 2022; Carrión-Antolí et al., 2024). Estos compuestos son capaces de inhibir la actividad enzimática que degrada los lípidos de la membrana, inducir los sistemas antioxidantes y reducir los daños producidos por el estrés oxidativo en cerezas.

1.2.2. Clavel (*Dianthus caryophyllus* L.)

En las etapas de la cosecha y la poscosecha, desde la recolección hasta el consumidor, se generan pérdidas en los productos vegetales con una magnitud considerable en todo el mundo. Mientras que en los países desarrollados las pérdidas ocurren principalmente en la venta al por menor y el consumidor, en los países en desarrollo se concentran en las etapas de cosecha, poscosecha y procesamiento, debido a bajos niveles tecnológicos y a una inversión limitada en los sistemas de producción (Prusky, 2011).

El objetivo de la conservación poscosecha es mantener la calidad y minimizar las pérdidas que se producen durante la conservación de los productos. Por ello, la mayoría de los productores y entre ellos los floricultores están involucrados en la poscosecha, ya que los consumidores demandan plantas ornamentales o comestibles con una apariencia atractiva y una vida extensa. Las propiedades estéticas y la vida útil en florero del clavel son indicadores de calidad y se determinan observando el enrollamiento y el marchitamiento de los pétalos (Satoh et al. 2005).

La disponibilidad de sustratos para mantener el requerimiento energético es un factor importante para la longevidad de las flores cortadas. La aplicación exógena de sacarosa proporciona a la flor los sustratos necesarios para la respiración y no solo prolonga la vida en florero, sino que también promueve la apertura de las flores (Ichimura et al., 2022). Cada etapa en el ciclo de vida de las flores está asociada con una serie de cambios bioquímicos, fisiológicos, hormonales y estructurales que están fuertemente modulados por los factores y estresores de origen biótico y abiótico (Figura 24).

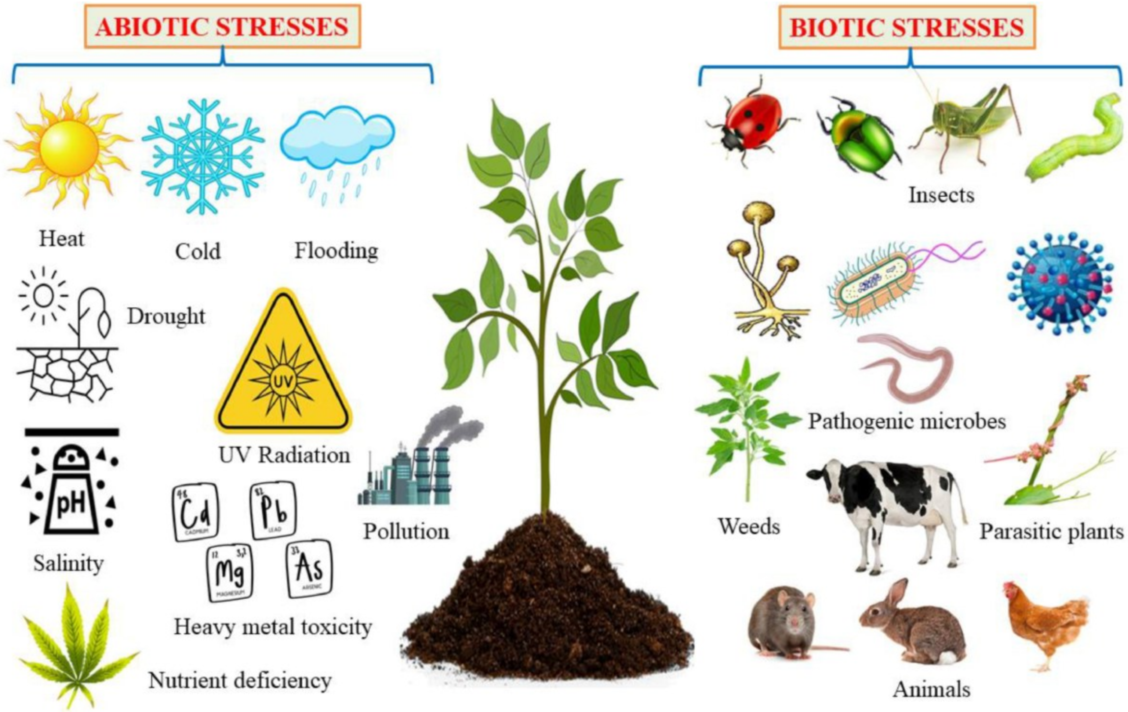


Figura 24. Factores abióticos y bióticos que causan respuesta de estrés en las plantas. Fuente: Kumar et al. (2023).

El estrés oxidativo y la polimerización de compuestos fenólicos producidos en respuesta a una herida conducen a la deposición de metabolitos secundarios en los extremos de los tallos de las flores cortadas para sanar los tejidos abiertos y prevenir la invasión microbiana. Sin embargo, esta deposición causa el bloqueo de los vasos, reduce la absorción de agua y acorta la vida en florero (Manzoor et al., 2024). Para intentar eliminar este problema se ha recomendado la adición de agentes antibióticos en la solución como el sulfato de 8-hidroxiquinoleína, ácido bórico, ácido aminooxiacético y nanopartículas de plata (Naing et al., 2020; Sarhan et al., 2023).

Además, el almacenamiento a baja temperatura es el procedimiento de conservación más importante para retrasar la senescencia de flores cortadas. Sin embargo, los floricultores a menudo dañan las flores en su intento de almacenarlas a bajas temperaturas. La respuesta bioquímica al daño por frío induce estrés oxidativo en las células como resultado de la disfunción de la membrana plasmática. Durante este proceso la membrana sufre una degradación de lípidos que conduce a un aumento de la fuga de electrolitos, de producción de etileno y de daños en el fotosistema (Wang, 2007; Costa et al., 2011).

Se ha investigado el uso de diferentes espectros de luz (Aliniaeifard et al., 2020) o el uso de compuestos como el 1-MCP (Phetsirikoon et al., 2012) con efectos positivos en la prevención de los daños por frío. También se han utilizado aceites esenciales, extractos vegetales, quitosano y ácido ascórbico como compuestos alternativos a los productos químicos (Solgi, 2018; Hassan y Fetouh, 2019; Akhtar et al., 2021; Mohammadi et al., 2023). Los tratamientos con elicitores y hormonas como el ABA (Pompodakis et al., 2010), el AS (Aghdam et al., 2016a; Pereira et al., 2018) y el GABA (Aghdam et al., 2016b) han sido evaluados positivamente en la prevención de los síntomas causados por las bajas temperaturas y en la vida útil de flores cortadas.

1.2.3. Kiwi (*Actinidia deliciosa* (A. Chev.))

Las principales causas del deterioro del kiwi durante su conservación son la deshidratación, pérdida de peso, cambios de color, ablandamiento, picado de la superficie, pardeamiento, pérdida de acidez y deterioro microbiano (Wei et al., 2019; Ebrahimi et al., 2024). El uso de mohos y levaduras como bioagentes reduce las infecciones fúngicas y aumenta las actividades de enzimas relacionadas con la defensa en el kiwi (Nian et al., 2023; Zhao et al., 2023). Se han utilizado también tratamientos poscosecha como choques térmicos, la irradiación de electrones, el ozono y los aceites esenciales para estimular las respuestas defensivas frente a los hongos en kiwi (Luo et al., 2019; Li et al., 2021a; Yang et al., 2023; Wang et al., 2024).

El kiwi puede almacenarse entre 0 y 0,5 °C con una humedad relativa del 90–95 % hasta 6 meses, sin embargo, si se mantienen temperaturas cercanas a la congelación por mucho tiempo aparecen síntomas de daño por frío (Choi et al., 2022). Los síntomas aparecen como un aspecto de tejido granular y translúcido en el pericarpio externo e interno, así como la lignificación de la pulpa (Jin et al., 2021). Los kiwis son frutas climatéricas y el aumento en la producción de etileno contribuye a acortar la vida útil como resultado de la sobremaduración (Chai et al., 2022). El 1-MCP actúa como un inhibidor del etileno reduciendo la tasa de respiración, la maduración, el ablandamiento y la descomposición por *Botrytis cinerea* durante el almacenamiento del kiwi (Xia et al., 2021; Xiong et al., 2023). Evita los daños por frío y el desarrollo del sabor desagradable al inhibir las enzimas involucradas en la fermentación del etanol (Quillehauquy et al., 2020; Ali et al., 2021; Liu et al., 2021a) (Figura 25).

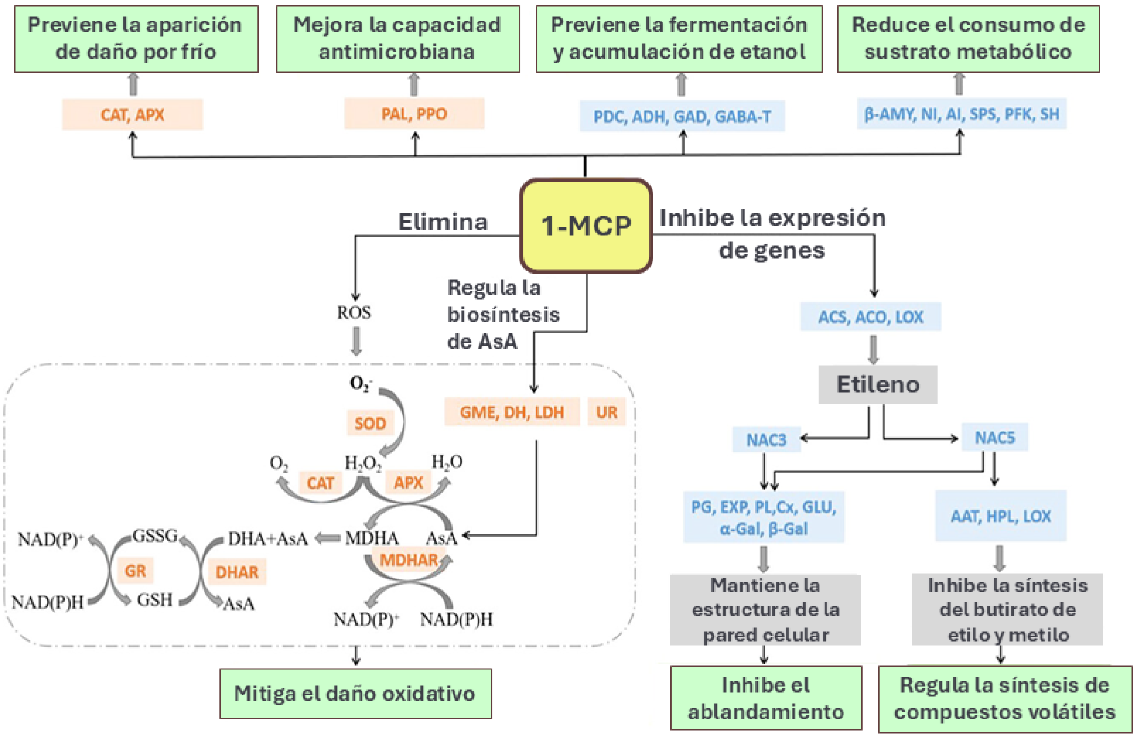


Figura 25. Efecto del 1-MCP sobre el metabolismo del kiwi. Fuente: adaptado de Xia et al. (2024).

La aplicación de hormonas vegetales como el ABA disminuye la lignificación en la pulpa del kiwi y mitiga los síntomas de la mancha negra (Jin et al., 2021). El tratamiento con JaMe, PUT o GABA induce tolerancia al frío debido al mayor contenido de ácido ascórbico, lo que resulta en una mejor protección de la membrana celular y el alivio del daño relacionado con el estrés oxidativo (Öztürk y Yücedağ, 2021; Taş et al., 2022; Liu et al., 2023).

1.2.4. Aguacate (*Persea americana* Mill.)

El deterioro de los aguacates durante la poscosecha es causado por factores intrínsecos de la fruta como el metabolismo, especialmente la respiración y la producción de etileno, y por factores externos como la temperatura, la composición del aire, el contenido de humedad y el daño mecánico (Álvarez-Herrera et al., 2021; Morales-Solis et al., 2024). Las pérdidas en la producción de aguacate se deben principalmente a microorganismos fitoaptogenos que aprovechan los nutrientes para proliferar y acelerar la descomposición a través de las lesiones externas causadas en el fruto (Bill et al., 2022; Geremu et al., 2022).

Almacenar los aguacates a baja temperatura ayuda a retrasar su ablandamiento y extiende su vida útil (Figura 26). Sin embargo, el aguacate expuesto a 3-5 °C más de dos semanas puede presentar desórdenes fisiológicos de daños por frío como el pardeamiento de la pulpa, problemas de maduración irregular y mayor susceptibilidad a ataques de patógenos (Chirinos et al., 2022). Estos signos son más evidentes cuando el aguacate se traslada a temperatura ambiente. El control adecuado de la temperatura en la preservación del aguacate es necesario, pero no se considera totalmente efectivo. Por ello, se han aplicado otras estrategias de conservación como el etileno exógeno, 1-MCP, aceites esenciales, antagonistas microbianos, recubrimientos comestibles y envasado en atmósfera modificada (Pesis et al., 2002; Careli-Gondim et al., 2020; Fuentealba et al., 2022; Olivares et al., 2022; Mpeluza et al., 2023; Gago et al., 2024).

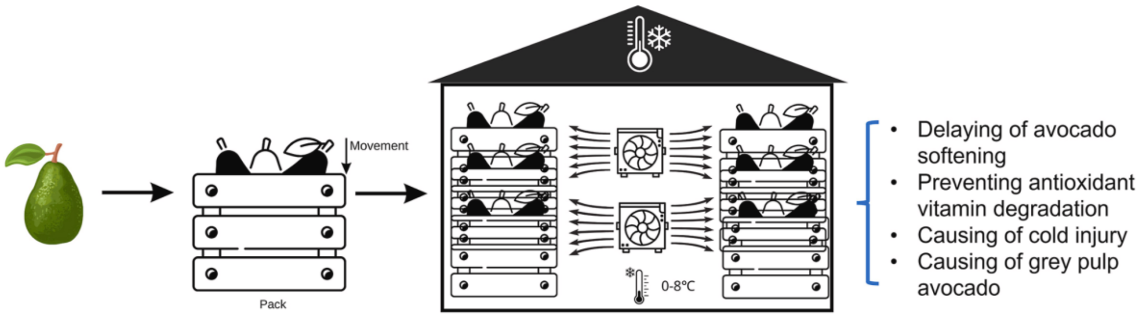


Figura 26. Efectos del almacenamiento del aguacate a baja temperatura. Fuente: Lieu et al. (2024).

Sin embargo, el uso de la tecnología 1-MCP bloquea el proceso de maduración evitando el desarrollo de los parámetros de calidad organoléptica deseados. Por tanto, el 1-MCP debe optimizarse o combinarse con estrategias de aplicación poscosecha para revertir este comportamiento e inducir o reactivar la capacidad de maduración de los frutos (Dias et al., 2021). Entre estas estrategias, se puede considerar el enfoque ecológico con el uso de sustancias de origen natural, que podrían ser tan efectivos como los productos químicos sintéticos.

Los aceites esenciales, los ácidos fenólicos y los elicitores son compuestos seguros y biodegradables usados en aguacate para reemplazar los productos químicos (Munhuweyi et al., 2020; Herrera-González et al., 2021). El AS, el JaMe y la MT son sustancias endógenas del crecimiento de las plantas que pueden influir significativamente en el desarrollo y las respuestas al estrés biótico y abiótico de la planta (Guillén et al., 2022). Cuando estos compuestos se aplican a la fruta estimulan eficazmente las defensas naturales contra el estrés y reducen la incidencia de la enfermedad de antracnosis en aguacate (Shikwambana et al., 2021; Osondu et al., 2022; Sanches y Repolho, 2022). También se produce una mejora en la actividad antioxidante del fruto contribuyendo a la eliminación de las especies reactivas de oxígeno (ROS; reactive oxygen species) y aliviar la peroxidación lipídica de la membrana del aguacate (Magri et al., 2022; Moreno-Pérez et al., 2024).

1.2.5. Tomate (*Solanum lycopersicum* L.)

Los tomates son susceptibles a lesiones físicas durante la cosecha y las prácticas de manejo posteriores, incluido el transporte (Zhang et al., 2018; Zewdie et al., 2021). Para preservar la calidad y prolongar la vida útil durante el almacenamiento poscosecha es crucial el desarrollo de diferentes actividades como la recolección, manipulación, limpieza, desinfección, enfriamiento inicial, clasificación y embalaje (Li et al., 2021b).

Los frutos climatéricos, entre ellos los tomates, tienen procesos respiratorios y metabólicos que están estrechamente correlacionados con la temperatura (Thole et al., 2021; Delgado-Vargas et al., 2023). Por tanto, el control de la temperatura es una de las técnicas más importantes para minimizar las tasas de procesos de deterioro y, por ende, prolongar la vida útil de los tomates. Sin embargo, la exposición a bajas temperaturas afecta negativamente al tomate puesto que es un fruto vulnerable al daño por frío cuando se expone a temperaturas inferiores a los 12-13 °C. Este daño es un proceso irreversible con efectos acumulativos y directamente proporcionales al tiempo de exposición y a la temperatura (Rai et al., 2022). El aumento de la pudrición poscosecha, el desarrollo de sabores indeseables, el oscurecimiento de las semillas, las lesiones de aspecto acuoso, el picado de la superficie, el ablandamiento prematuro y el desarrollo irregular del color son las consecuencias negativas del daño por frío en los tomates (David et al., 2022; Shan et al., 2022).

La generación de etileno y los efectos asociados durante la maduración y la senescencia de los frutos se ha controlado con éxito mediante la aplicación de 1-MCP. Los tomates tratados con 1-MCP muestran una tasa de respiración reducida, menor generación de etileno y pérdida de peso, junto con una acumulación más lenta de licopeno y de los cambios en el color exterior (Mata et al., 2021; Wu et al., 2023a; Horváth-Mezőfi et al., 2024).

Para prolongar la vida útil, reducir las pérdidas poscosecha y preservar el contenido nutricional de los tomates, se han investigado varios métodos y técnicas innovadoras de origen natural como los elicitores o fitohormonas. La aplicación de AS disminuye la fuga de electrolitos, la concentración de MDA y las actividades enzimáticas en los tomates (Aghdam et al., 2012). El tratamiento con JaMe y MT en tomates induce el aumento de los niveles de arginina, prolina y el sistema enzimático antioxidante, reduciendo así los síntomas de daño por frío en la fruta (Zhang et al., 2012a; Yan et al., 2022). La reducción del daño por frío a través de la MT puede estar asociada con la mejora del sistema antioxidante que favorece la integridad de la membrana junto con el suministro de suficiente ATP intracelular (Jannatizadeh et al., 2019; Li et al., 2019a).

1.3. Elicitores como herramienta precosecha y poscosecha para mejorar la producción y conservación de los productos vegetales.

La necesidad de desarrollar estrategias respetuosas con el medio ambiente para mejorar la defensa de las plantas y mejorar el valor nutricional de los productos hortofrutícolas ha estimulado el uso de elicitores como herramienta para modular las características de las plantas (Chowdhury et al., 2023; Zhang et al., 2023). El uso de estos compuestos se considera seguro y respetuoso con el medio ambiente, ya que son sustancias biodegradables con baja toxicidad (Artés-Hernández et al., 2024).

Los elicitores son compuestos que inducen respuestas de las plantas frente al estrés y estimulan diferentes clases de metabolitos secundarios. La resistencia inducida retrasa el declive de la inmunidad innata después de la cosecha y aumenta la producción de respuestas defensivas, confiriendo una mayor protección contra el deterioro poscosecha (Prusky y Romanazzi, 2023).

La concentración de estos metabolitos es más dependiente de la genética de la planta (especie y variedad) que de la naturaleza del elicitor (Malik et al., 2020; Guru et al., 2022). Los elicitores son reconocidos por las células vegetales al interactuar con receptores específicos en las membranas plasmáticas de las plantas (Figura 27). El nexo entre el elicitor y sus receptores es altamente específico y complejo, de hecho, el mecanismo exacto de interacción aún no se conoce completamente (Thakur et al., 2019; Meena et al., 2022).

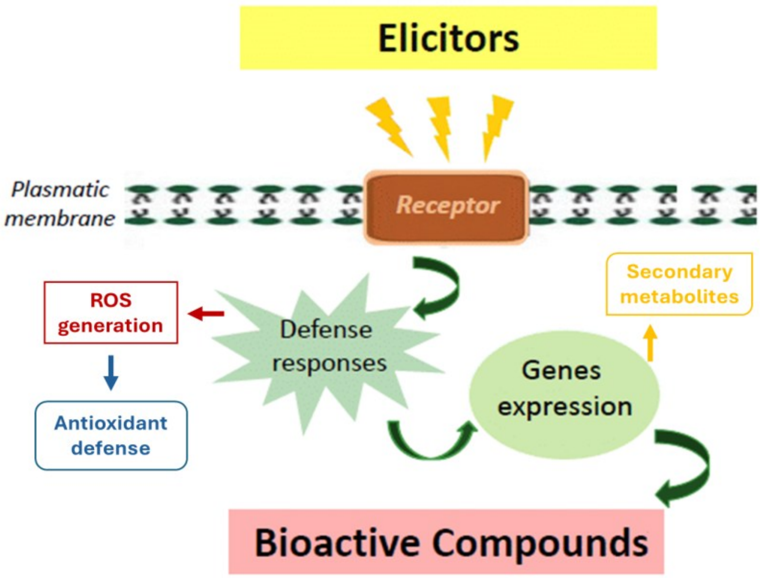


Figura 27. Respuesta de la planta frente a los elicitores. Fuente: Baenas et al. (2014).

1.3.1. Putrescina

Las poliaminas (PAs) son un grupo de compuestos naturales de aminas alifáticas similares a las fitohormonas que están presentes en los organismos vivos, incluidas las plantas. Entre las PAs, la putrescina (PUT) tiene más importancia, ya que es el componente principal y el precursor de las PAs terciarias y cuaternarias (González-Hernández et al., 2022). Las PAs son reguladores del crecimiento de las plantas y se sugiere que están involucradas en un amplio espectro de procesos fisiológicos, como la tolerancia al estrés, la embriogénesis, la división celular, la morfogénesis y el desarrollo. Los principales mecanismos descritos están asociados con la eliminación de ROS, la regulación de los niveles de ABA, la prevención de la peroxidación lipídica, el mantenimiento del pH celular, el equilibrio iónico y la regulación de los canales catiónicos (Thomas et al., 2020; Shao et al., 2022).

El efecto de las PAs en el retraso del ablandamiento del fruto (Tabla 1A, B) podría atribuirse a su unión en la pared celular y su papel en la estabilización de las paredes y las membranas celulares. Las PAs mantienen la firmeza de la fruta durante el almacenamiento al desarrollar una unión con las sustancias pécticas cargadas negativamente en las paredes celulares de las frutas. Además, se ha demostrado que el suministro exógeno de PAs estimula, en lugar de inhibir, la producción de etileno (Kuznetsov y Shevyakova, 2007; Farooq et al., 2009). Esto podría ser debido a que las PAs aplicadas exógenamente aumentan las PAs endógenas, que actúan como un inhibidor de retroalimentación en la vía de biosíntesis de PAs. En la vía biosintética, el etileno y las PAs tienen un precursor común S-adenosil metionina (SAM), por ello, si se reduce o inhibe el uso de SAM en la vía de las PAs, esto lleva a una mayor contribución de SAM para la producción de etileno (Sharma et al., 2017).

El uso de PAs en precosecha para estimular la respuesta de defensa de los frutos y vegetales frente a diferentes estreses abióticos ha sido ampliamente estudiado (Tabla 1A). En plántulas de tomate, el tratamiento con PUT 1 mM aumenta significativamente la tolerancia al calor al elevar las concentraciones y suprimir la actividad de las clorofilasas, modular el contenido de PAs libre endógenas, aumentar la eficiencia de la defensa antioxidante e incrementar la expresión de genes relacionados con el choque térmico (Jahan et al., 2022). El tratamiento con PUT (0,25, 0,5 y 1 mM) en cilantro mitigó el estrés químico provocado por metales como el cadmio al modular los antioxidantes y la actividad fotosintética de las plantas (Sardar et al., 2022). El estrés hídrico también ha sido estudiado en plántulas de uva por Zhao et al. (2021) y en trigo por Hussein et al. (2023), siendo capaz de aliviar los efectos negativos de la sequía al promover la eliminación de las ROS, mejorar el sistema antioxidante y aumentar el metabolismo de las PAs endógenas al regular positivamente la síntesis de sus genes. En plántulas de guayaba se ha observado que la PUT es capaz de ofrecer mayor tolerancia frente al estrés salino al aumentar el contenido de catalasa (CAT), polifenol oxidasa (PPO), carotenoides y prolina (Ghalati et al., 2020).

Respecto a las aplicaciones de PUT en poscosecha, en albaricoque se ha observado que fue capaz de mantener la firmeza de la fruta y reducir las magulladuras causadas por el daño mecánico inducido (Martínez-Romero et al., 2002). Además, PUT 1 mM redujo eficazmente la incidencia y gravedad de la antracnosis en mango a través de un efecto fungicida directo y un mecanismo de resistencia inducida indirectamente (Song et al., 2023). En papayas también se ha observado que la PUT a concentraciones de 2 mM suprimió la incidencia de podredumbres durante todo el período de almacenamiento (Hanif et al., 2020). Esto pudo deberse a las propiedades antipatógenas de las PAs, que tienen la capacidad de formar enlaces fuertes con fenoles y HCAA (amida del ácido hidroxicinámico), los cuales indujeron resistencias contra los patógenos reduciendo así la incidencia de frutos podridos (Tabla 1B).

Las PAs, como moléculas policatiónicas a pH fisiológico, pueden unirse fuertemente a los componentes aniónicos de las membranas celulares, como los fosfolípidos, lo que conduce a la estabilización de la superficie de la bicapa. Las PAs mantienen la estabilidad de la membrana, que a bajas temperaturas supone un factor importante para la resistencia de la planta al estrés por frío (Tabla 1A, B). Por ello, las PAs han demostrado eficacia en la reducción de los daños por frío de los frutos durante el almacenamiento. Además, las PAs proporcionan actividad antioxidante al eliminar las ROS, lo que conduce a una mayor estabilidad e integridad de la membrana bajo estrés por frío (Hussain et al., 2011).

En precosecha se ha estudiado el efecto de las aplicaciones de PUT 2 mM y 3 mM en peras, observándose una reducción de la conductividad eléctrica y del contenido de MDA, que son indicadores del daño por frío (Singh et al., 2023). En la poscosecha de calabacín, los efectos del tratamiento con PUT incrementaron la tolerancia al frío de los frutos a través del aumento de las concentraciones de betaína y prolina (Palma et al., 2015). El daño por frío se redujo en los albaricoque tratados con PUT al aumentar la actividad de las enzimas antioxidantes CAT, peroxidasa (POD) y superóxido dismutasa (SOD) las cuales participan en la protección de las plantas contra el daño causado por las ROS a baja temperatura (Saba et al., 2012). En arilos de granada, la aplicación de PUT 1 mM y 3 mM redujo la severidad de los daños por frío al incrementar los niveles de PUT endógenos, induciendo la aclimatación de la granada a las bajas temperaturas como mecanismo de protección (Mirdehghan et al., 2007; Fawole et al., 2020).

Tabla 1A. Revisión bibliográfica sobre los efectos de la putrescina en la precosecha y poscosecha de diferentes cultivos.

Putrescina		
Efectos	**Aplicación**	**Cultivo, concentración del elicitor* y referencia**
Fortalecimiento de la respuesta de defensa contra el estrés biótico y/o abiótico	Precosecha	Guayaba (0,5 mM); Ghalati et al., 2020 Coliflor (2,5 mM); Collado-González et al., 2021 Uva (0,1 mM); Zhao et al., 2021 Cereza (100 ppm); Sabir et al., 2021 Tomate (1 mM); Jahan et al., 2022 Cilantro (1 mM); Sardar et al., 2022 Trigo (1 mM); Hussein et al., 2023
	Poscosecha	Albaricoque (1 mM); Martinez-Romero et al., 2002
Aumento de la vida útil, retraso en la maduración y/o mejora de la calidad en el momento de la cosecha y/o durante el almacenamiento	Precosecha	Mango (1 mM); Malik y Singh, 2005 Ciruela (2 mM); Khan et al., 2008 Albaricoque (10^{-5} mM); Ali et al., 2010 Dátil (0,45 mM); Mohamed y Saleh, 2013
	Poscosecha	Fresas (2 mM); Khosroshahi et al., 2007
Reducción de los síntomas de daño por frío, fuga de electrolitos, permeabilidad de la membrana y/o contenido de MDA	Precosecha	Gerbera (2 mM); Rakbar et al., 2022 Pera (3 mM); Singh et al., 2023

*En algunos de los estudios se han probado diferentes concentraciones, pero se muestra sólo la que los autores establecen como la más efectiva para el efecto esperado.

Tabla 1B. Revisión bibliográfica sobre los efectos de la putrescina en la precosecha y poscosecha de diferentes cultivos.

Putrescina		
Efectos	**Aplicación**	**Cultivo, concentración del elicitor* y referencia**
Reducción de los síntomas de daño por frío, fuga de electrolitos, permeabilidad de la membrana y/o contenido de MDA	Precosecha	Gerbera (2 mM); Rakbar et al., 2022 Pera (3 mM); Singh et al., 2023
	Poscosecha	Granada (1 mM); Mirdehghan et al., 2007 Albaricoque (1 mM); Saba et al., 2012 Calabacín (1 mM); Palma et al., 2015 Granada (3 mM); Fawole et al., 2020
Incremento de los compuestos bioactivos y/o de la actividad antioxidante relacionada con compuestos antioxidantes enzimáticos y/o no enzimáticos	Precosecha	Uva (2 mM); Mirdehghan y Rahimi, 2016 Ciruela india (2 mM); Shanbehpour et al., 2020
	Poscosecha	Uva (2 mM); Shiri et al., 2013 Melocotón (1,6 mM); Kibar et al., 2021 Higo (4 mM); Kucuker et al., 2023
Inducción de la resistencia a patógenos, control de los desórdenes fisiológicos y/o de la podredumbre durante el almacenamiento	Poscosecha	Papaya (2 mM); Hanif et al., 2020 Mango (1 mM); Song et al., 2023

*En algunos de los estudios se han probado diferentes concentraciones, pero se muestra sólo la que los autores establecen como la más efectiva para el efecto esperado.

1.3.2. Melatonina y ácido γ-aminobutírico

La melatonina (MT) y el ácido γ-aminobutírico (GABA) son dos moléculas endógenas con múltiples funciones celulares y fisiológicas que participan en la regulación de las respuestas de las plantas a las variables ambientales (Arnao y Hernández-Ruiz, 2015; Debnath et al. 2019). El contenido de MT varía entre diferentes especies, tejidos, etapas de desarrollo, condiciones de crecimiento y grado de maduración de las plantas (Zhang y Zhang, 2021). Varios metabolitos como aminoácidos, azúcares y GABA endógeno son regulados positivamente con el tratamiento de GABA exógeno (Li et al., 2021c). Los reguladores del crecimiento similares a las fitohormonas como la MT, el GABA, los jasmonatos y las PAs utilizadas de forma exógena en las plantas han demostrado tener un importante potencial para mejorar la tolerancia al estrés abiótico (Figura 28).

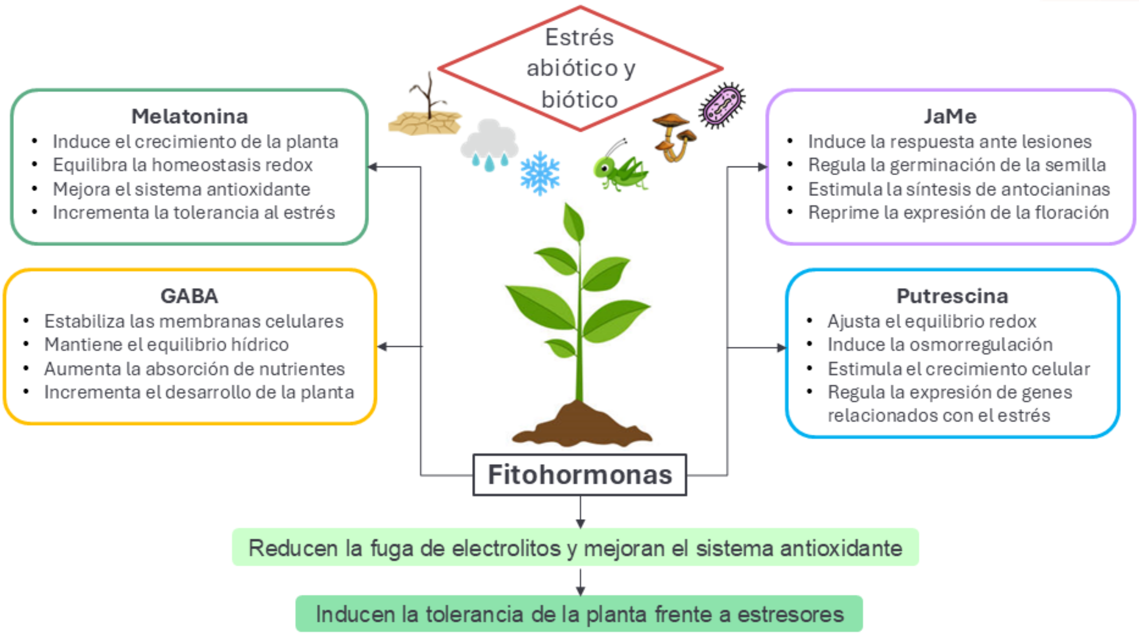


Figura 28. Fitohormonas como tratamiento exógeno en plantas para la mitigación de factores de estrés abióticos en cultivos. Fuente: adaptado de Huang y Jin (2024).

La interacción de la MT con otras moléculas de señalización es efectiva para el crecimiento y la productividad de las plantas (Figura 29). Se han descrito las interacciones que se producen entre la MT y otras fitohormonas y su influencia en la biosíntesis de moléculas como el GABA (Iqbal et al., 2021). La aplicación de GABA también aumenta los niveles de la mayoría de las fitohormonas a través de la expresión de sus genes biosintéticos (Sharma et al., 2024).

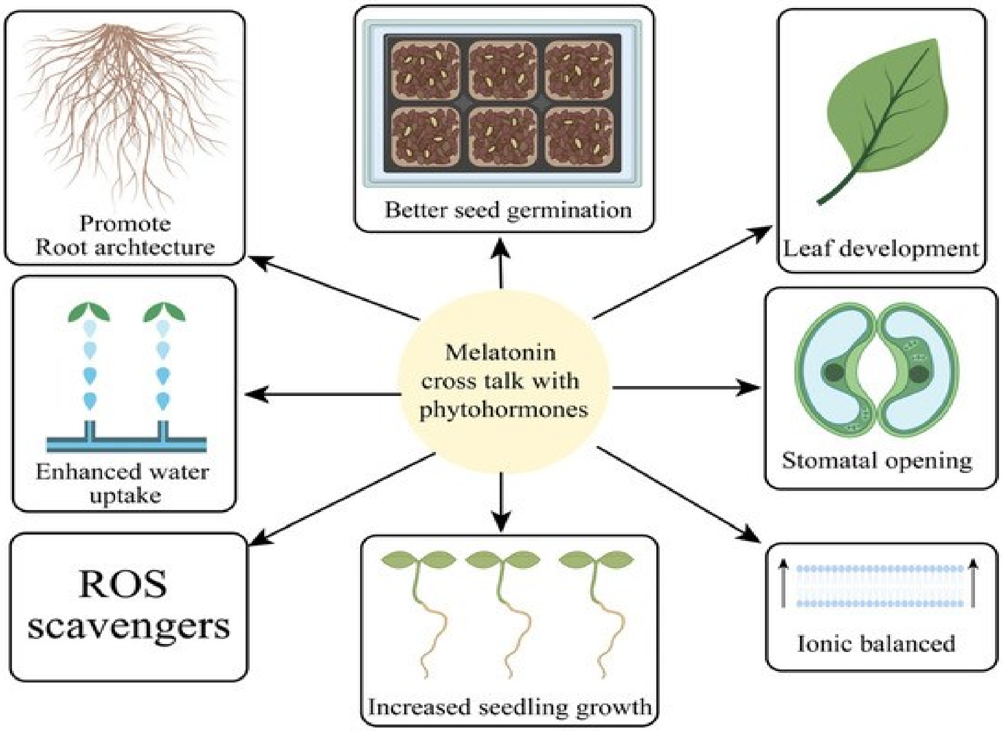


Figura 29. Interacción de MT con otras fitohormonas para la regulación de la fotosíntesis y otras funciones fisiológicas de la planta. Fuente: Huang y Jin (2024).

Los efectos interactivos del GABA y la MT se han estudiado ampliamente en diversos frutos como la fresa, tomate, pepino, mango, calabacín, melocotón y pera (Aghdam y Fard, 2017; Sharafi et al., 2019; Madebo et al., 2021; Bhardwaj et al., 2022; Ali et al., 2023; Wu et al., 2023b; Liu et al., 2024). El tratamiento con MT desencadena la respuesta de defensa del fruto al estrés por frío a través de una mayor expresión génica de la ruta de biosíntesis del GABA mediante un incremento en la actividad de las enzimas ácido γ-aminobutírico transaminasa (GABA-T) y glutamato descarboxilasa (GAD). Además, aumenta la expresión de genes de biosíntesis del GABA (*PpGAD1 y PpGAD4*) y suprime la expresión del gen de degradación del GABA (*PpGABA-T*), lo que resulta en la acumulación de GABA endógeno (Iqbal et al., 2021; Sharma et al., 2024).

La aplicación exógena de MT y GABA mejora la actividad enzimática y la expresión de genes antioxidantes en respuesta a condiciones de estrés (Tabla 2A), protegiendo así a las células del estrés oxidativo (Zheng et al., 2023a; Huang et al., 2024). En el cultivo de maíz, la MT mejora la tolerancia al estrés por sequía al retrasar la degradación de la clorofila, mejorar la fotosíntesis y aumentar la actividad de las enzimas antioxidantes (Huang et al., 2019b). En tomates, la MT exógena (0,1 mM) induce la biosíntesis de fitoquelatinas y quelatos adicionales de cadmio, aumentando así la distribución de cadmio en las paredes celulares y vacuolas y mejorando la tolerancia de la planta a este metal (Hasan et al. 2015). La aplicación de GABA (2 mM) mejoró el metabolismo del nitrógeno, la asimilación del azufre, la homeostasis iónica, el crecimiento y la fotosíntesis en condiciones de estrés salino en trigo (Khanna et al., 2021). La función reproductiva de las plantas de judías sometidas a estrés térmico tratadas con GABA mejoró significativamente en términos de germinación y viabilidad del óvulo (Priya et al., 2019).

La MT y el GABA regulan la inmunidad innata de la planta a través de respuestas de defensa que previenen y controlan enfermedades poscosecha en frutas y verduras (Tabla 2B). Por ello, se ha observado que el tratamiento con MT 0,25 mM aumentó la resistencia del algodón al hongo *Verticillium dahlia* al aumentar el flujo metabólico en las vías de fenilpropanoide, mevalonato y gosipol (Li et al., 2019). En lichi, el tratamiento con MT induce la resistencia a patógenos al reducir el daño en las mitocondrias y modular las vías de los fenilpropanoides y las pentosas fosfato, así como el metabolismo de la fruta (Zhang et al., 2021b). Asimismo, GABA también actúa en la estimulación del sistema de defensa, reduciendo eficazmente la incidencia de *B. cinerea* y el diámetro de las lesiones en tomates a través de las vías de señalización del etileno y del ácido jasmónico, que están involucradas en la respuesta a la defensa (Li et al., 2024b). Yu et al. (2023) demostraron que el tratamiento con GABA 10 mM inhibió el crecimiento y el impacto de *A. alternata*, destruyendo la estructura celular del hongo y reduciendo la actividad de las enzimas de degradación de la pared celular.

Con respecto a la reducción de los daños por frío en la poscosecha de frutos (Tabla 2B), el tratamiento con MT fue eficaz en melocotón (Wu et al., 2023b) y calabacín (Ali et al., 2023) al reducir el contenido de MDA y la fuga de electrolitos. Además, los frutos tratados con MT mejoraron el sistema antioxidante no enzimático y aumentaron la actividad de enzimas antioxidantes. Los compuestos fenólicos y la capacidad antioxidante aumentaron en las uvas tratadas con 40 mM de GABA debido a una menor actividad de la enzima PPO (Asgarian et al., 2022). Respecto al efecto del GABA en los daños por frío de los frutos, se observó un aumento en la tolerancia a las bajas temperaturas en pepino y pera a través de la regulación de las PAs, prolina y el GABA endógeno (Madebo et al., 2021; Liu et al., 2024). La aplicación de GABA 1 mM redujo el daño por frío en calabacín a través de la inducción de la biosíntesis del GABA al aumentar las actividades de las enzimas GABA-T y GAD. El GABA está involucrado en el suministro de metabolitos para producir energía y ayudar a la fruta a hacer frente al estrés por frío a largo plazo (Palma et al., 2019).

Tabla 2A. Revisión bibliográfica sobre los efectos de la melatonina y el GABA en la precosecha y poscosecha de diferentes cultivos.

Melatonina y GABA			
Efectos	**Aplicación**	**Elicitor**	**Cultivo, concentración del elicitor* y referencia**
Fortalecimiento de la respuesta de defensa contra el estrés biótico y/o abiótico	Precosecha	MT	Tomate (0,1 mM); Hasan et al., 2015 Manzana (0,2 mM); Li et al. 2018b Maiz (0,1 mM); Huang et al., 2019b
		GABA	Arroz (0,5 mM); Kumar et al., 2019 Judías (1 mM); Priya et al., 2019 Trigo (2 mM); Khanna et al., 2021
Aumento de la vida útil, retraso en la maduración y/o mejora de la calidad en el momento de la cosecha y/o durante el almacenamiento	Precosecha	MT	Tomate (0,1 mM); Liu et al., 2016 Granada (0,1 mM); Medina-Santamarina et al., 2021 Cereza (0,3 mM); Carrión-Antolí et al., 2022a
		GABA	Limón (50 mM); Badiche-El Hilali et al., 2023 Granada (100 mM); Lorente-Mento et al., 2023
	Poscosecha	MT	Fresa (0,1 mM); Aghdam y Fard, 2017 Nectarina (1 mM); Bal, 2021
		GABA	Naranja (0,5 mM); Sheng et al., 2017 Cereza (5 mM); Aghdam et al., 2019a
Reducción de los síntomas de daño por frío, fuga de electrolitos, permeabilidad de la membrana y/o contenido de MDA	Precosecha	MT	Albahaca (0,4 mM); Albornoz et al., 2023 Narciso (0,7 mM); Zulfiqar et al., 2023
		GABA	Tomate (10 mM); Zarei et al., 2020 Gerbera (1mM); Mohammadi et al., 2021

*En algunos de los estudios se han probado diferentes concentraciones, pero se muestra sólo la que los autores establecen como la más efectiva para el efecto esperado.

Tabla 2B. Revisión bibliográfica sobre los efectos de la melatonina y el GABA en la precosecha y poscosecha de diferentes cultivos.

Melatonina y GABA			
Efectos	**Aplicación**	**Elicitor**	**Cultivo, concentración del elicitor* y referencia**
Reducción de los síntomas de daño por frío, fuga de electrolitos, permeabilidad de la membrana y/o contenido de MDA	Poscosecha	MT	Pepino (0,1 mM); Madebo et al., 2021 Calabacín (0,2 mM); Ali et al., 2023 Pera (5 mM); Liu et al., 2024
		GABA	Plátano (20 mM); Wang et al., 2014 Calabacín (1 mM); Palma et al., 2019
Incremento de los compuestos bioactivos y/o de la actividad antioxidante relacionada con compuestos antioxidantes enzimáticos y/o no enzimáticos	Precosecha	MT	Cereza (0,5 mM); Michailidis et al., 2021 Mora (0,1 mM); Shah et al., 2023
		GABA	Cereza (50 mM); Carrión-Antolí et al., 2023 Pistacho (20 mM); Jalali et al., 2023
	Poscosecha	MT	Tomate (0,1 mM); Sharafi et al., 2021 Melocotón (5 mM); Wu et al., 2023b
		GABA	Uva (40 mM); Asgarian et al., 2022 Pera (5 mM); Li et al., 2019c
Inducción de la resistencia a patógenos, control de los desórdenes fisiológicos y/o de la podredumbre durante el almacenamiento	Precosecha	MT	Algodón (250 mM); Li et al., 2019b Tomate (0,1 mM); Li et al., 2022a
		GABA	Manzana (0,5 mM); Liu et al., 2022
	Poscosecha	MT	Mandarina (0,05 mM); Lin et al., 2019 Lichi (0,25 mM); Zhang et al., 2021b
		GABA	Pera (0,1 mM); Yu et al., 2014 Tomate (10 mM); Yu et al., 2023; Li et al., 2024b

*En algunos de los estudios se han probado diferentes concentraciones, pero se muestra sólo la que los autores establecen como la más efectiva para el efecto esperado.

1.3.3. Jasmonato de metilo

Debido a su naturaleza volátil, así como a la capacidad de permear a través de las membranas biológicas, el jasmonato de metilo (JaMe) activa diferentes vías de señalización y alteraciones metabólicas (Ho et al., 2020; Jeyasri et al., 2023).

Se ha evaluado el efecto de las aplicaciones de JaMe en precosecha sobre las respuestas relacionadas con la defensa y la mejora de la calidad del fruto (Tabla 3A, B). La aplicación antes de la cosecha con JaMe (5 y 7,5 mM) mejora el color de la piel de las naranjas, presentando mayores niveles de carotenoides (Rehman et al., 2021). Los resultados en ciruela muestran que el JaMe es eficaz para aumentar el tamaño, el color, la firmeza y el peso de la fruta en la cosecha (Martínez-Esplá et al., 2014). La aplicación de JaMe en plantas de frambuesa aumentó significativamente los compuestos beneficiosos para la salud como el ácido elágico, la quercetina y la miricetina, debido a un efecto promotor sobre la actividad de la enzima fenilalanina amonio liasa (PAL) (Flores y Castillo, 2014). Serna-Escolano et al. (2021) observaron que JaMe (0,1 mM) aplicado en limón aumentó los compuestos antioxidantes fenólicos, además de las actividades de las enzimas CAT, POD y ascorbato peroxidasa (APX). Por otro lado, se ha descrito que las aplicaciones de JaMe en la precosecha mejoran la resistencia a enfermedades poscosecha en la fruta (Tabla 3B), reduciendo la incidencia de podredumbres (Yao y Tian, 2005; Saavedra et al., 2017; Minh, 2022).

La mayoría de los tratamientos con JaMe en poscosecha (Tabla 3A, B) se dirigen a mejorar la resistencia de la fruta frente a los efectos perjudiciales producidos durante el almacenamiento. La tolerancia a los daños por frío en melocotón, piña y pimiento es estimulada a través de una disminución en la insaturación lipídica presente en las membranas celulares, reduciendo la fuga de electrolitos y aumentando la actividad de la enzima PAL (Chen et al., 2019; Sangprayoon et al., 2019; Ma et al., 2020). La incidencia y la gravedad de los daños por frío se redujeron significativamente en el aguacate expuesto a JaMe 0,1 mM, a través de una mayor integridad de la membrana producida por la alteración del contenido y la composición de los ácidos grasos y la reducción de la actividad de la lipoxigenasa (LOX) (Glowacz et al., 2017). Se ha utilizado el JaMe en la precosecha de granada (1 y 2 mM) y limón (0,3 mM) para tratar los daños por frío que se producen en la poscosecha a través de la inhibición de las actividades de fosfolipasa D (PLD) y LOX, reduciendo la fuga de electrolitos y el contenido de MDA (Saba y Zarei, 2019; Liao et al., 2022). Otras investigaciones han demostrado que los tratamientos poscosecha con JaMe mejoran la actividad antioxidante al aumentar los compuestos bioactivos en las granadas (Sayyari et al., 2011), arándanos (Wang et al., 2019) y albaricoques (Ezzat et al., 2020), promoviendo compuestos beneficiosos para la salud (Tabla 3B).

Tabla 3A. Revisión bibliográfica sobre los efectos del jasmonato de metilo en la precosecha y poscosecha de diferentes cultivos.

Jasmonato de metilo		
Efectos	**Aplicación**	**Cultivo, concentración del elicitor* y referencia**
Fortalecimiento de la respuesta de defensa contra el estrés biótico y/o abiótico	Precosecha	Guisante (0,2 mM); Shahzad et al., 2015 Cereza (0,4 mM); Balbontín et al., 2018 Tomate (0,025 mM); Kamakshi et al., 2023
	Poscosecha	Pitaya (0,1 mM); Li et al., 2018
Aumento de la vida útil, retraso en la maduración y/o mejora de la calidad en el momento de la cosecha y/o durante el almacenamiento	Precosecha	Manzana (0,5 mM); Rudell et al., 2005 Ciruela (0,5 mM); Martínez-Esplá et al., 2014 Champiñón (0,1 mM); Yang et al., 2019 Naranja (5 mM); Rehman et al., 2021
	Poscosecha	Berenjena (0,005 mM); Fan et al., 2016 Mandarina (1 mM); Baswal et al., 2020 Caqui (0,024 mM); Bagheri y Esna-Ashari, 2022 Naranja (0,05 mM); Habibi et al., 2020
Reducción de los síntomas de daño por frío, fuga de electrolitos, permeabilidad de la membrana y/o contenido de MDA	Precosecha	Granada (2 mM); Saba y Zarei, 2019 Limón (0,3 mM); Liao et al., 2022

*En algunos de los estudios se han probado diferentes concentraciones, pero se muestra sólo la que los autores establecen como la más efectiva para el efecto esperado.

Tabla 3B. Revisión bibliográfica sobre los efectos del jasmonato de metilo en la precosecha y poscosecha de diferentes cultivos.

Jasmonato de metilo		
Efectos	**Aplicación**	**Cultivo, concentración del elicitor* y referencia**
Reducción de los síntomas de daño por frío, fuga de electrolitos, permeabilidad de la membrana y/o contenido de MDA	Poscosecha	Aguacate (0,1 mM); Glowacz et al., 2017 Melocotón (0,01 mM); Chen et al., 2019 Piña (0,01 mM); Sangprayoon et al., 2019 Pimiento verde (0,001 mM); Ma et al., 2020
Incremento de los compuestos bioactivos y/o de la actividad antioxidante relacionada con compuestos antioxidantes enzimáticos y/o no enzimáticos	Precosecha	Frambuesa (0,1 mM); Flores y Castillo, 2014 Pak Choi (0,5 mM); Baek et al., 2021 Limón (0,1 mM); Serna-Escolano et al., 2021 Cebollino (0,5 mM); Wang et al., 2022
	Poscosecha	Granada (0,01 mM); Sayyari et al., 2011 Arándano (0,1 mM); Wang et al., 2019 Albaricoque (0,2 mM); Ezzat et al., 2020
Inducción de la resistencia a patógenos, control de los desórdenes fisiológicos y/o de la podredumbre durante el almacenamiento	Precosecha	Cereza (0,2 mM); Yao y Tian, 2005 Fresa (0,25 mM); Saavedra et al., 2017 Melón (3 mM); Minh, 2022
	Poscosecha	Uva (0,01 mM); Jiang et al., 2015 Kiwi (0,1 mM); Pan et al., 2020 Rosa (0,2 mM); Naeemi et al., 2022 Cereza (0,05 mM); Pan et al., 2022

*En algunos de los estudios se han probado diferentes concentraciones, pero se muestra sólo la que los autores establecen como la más efectiva para el efecto esperado.

1.4. Aplicaciones con 1-metilciclopropeno y sus efectos sobre la calidad y vida útil de los productos vegetales

El etileno es la fitohormona gaseosa clave asociada con los fenómenos de maduración en frutas y hortalizas. Sin embargo, su producción acelera la senescencia y disminuye la vida útil de los productos vegetales (Pattyn et al., 2021). Por ello, se utiliza ampliamente el 1-metilciclopropeno (1-MCP), que es un derivado del ciclopropeno y un inhibidor competitivo de la acción del etileno, interactuando de forma irreversible con sus receptores (Figura 30). La eficacia de la inhibición de la maduración y senescencia de frutas y hortalizas depende de la concentración de 1-MCP aplicada, hasta la saturación de los receptores de unión (Watkins, 2006; Baswal y Ramezanian, 2021).

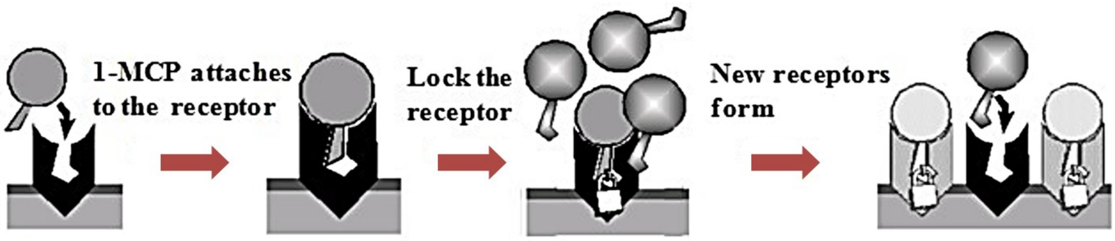


Figura 30. Acción del 1-MCP en los receptores de etileno. Fuente: Hu et al. (2017).

Cuando se produce una interacción irreversible del 1-MCP con los receptores del etileno, se bloquea el proceso de maduración en frutas climatéricas, impidiendo el desarrollo de las características sensoriales esperadas por los consumidores (Satekge y Magwaza, 2022). Las limitaciones con el 1-MCP han creado la necesidad de buscar tratamientos que se apliquen conjuntamente y que sean capaces de reactivar la capacidad de maduración. Los estudios sobre la fisiología de la fruta climatérica han descubierto que algunas hormonas como las auxinas, el ABA y el JaMe pueden reestablecer la maduración y podrían utilizarse de forma combinada con el 1-MCP (Dias et al., 2021).

La mayor parte de la investigación con el 1-MCP se ha realizado como tratamiento poscosecha (Tabla 4A, B). Aunque se ha desarrollado una formulación que se disuelve en agua y se aplica como pulverización, la investigación en este campo sigue siendo escasa comparada con su uso en poscosecha (Valero et al., 2016). La mayoría de los estudios en precosecha se han centrado en la capacidad del 1-MCP para retrasar el proceso de maduración del fruto en el árbol (Tabla 4A, B). La aplicación de 1-MCP a 400 nL/L retrasó significativamente el cambio de color de la cáscara de plátano y prolongó la vida útil del plátano hasta 19 días (Manigo y Matuginas, 2020). Lee et al (2020) observaron un retraso en la firmeza, la producción del etileno y la respiración durante el almacenamiento de melocotones después de la aplicación foliar de 1-MCP. En manzana, el desarrollo de color rojo y la desverdización se inhibieron con el 1-MCP, y se retrasó entre 6 y 15 días el tiempo para que la fruta alcanzara la firmeza para cosechar (Amarante et al., 2022). El 1-MCP en precosecha también retrasa el ablandamiento de la fruta inducido por el Etefón y extiende el período de cosecha en caqui (Vilhena et al., 2022). La actividad de la enzima PPO, el contenido de polifenoles y el MDA fueron retrasados significativamente con el 1-MCP en el momento de la cosecha de la pera (Li et al., 2022b).

El beneficio más importante de la aplicación del 1-MCP en poscosecha es el retraso de la maduración para extender los períodos de almacenamiento y comercialización sin la aparición de desórdenes fisiológicos (Tabla 4A, B). En pera, el tratamiento con 1-MCP consiguió controlar el pardeamiento interno que reduce la resistencia a las enfermedades poscosecha (Xu et al., 2020) y mejoró la textura y el color a través de una reducción en la tasa de respiración y el etileno (Arias et al., 2009). El 1-MCP redujo el desarrollo de la lesión y la incidencia de la descomposición por la podredumbre azul en los jínjoles. La resistencia inducida por 1-MCP está relacionada con el aumento de las enzimas involucradas en la eliminación de ROS y las asociadas con el metabolismo de compuestos fenólicos que producen productos altamente tóxicos contra la invasión de patógenos (Zhang et al., 2012b). En este sentido, el 1-MCP redujo eficazmente las podredumbres en kiwi causadas por *Phomopsis spp*., mejoró la capacidad antioxidante al aumentar la actividad enzimática de la SOD y CAT relacionada con la defensa y elevó los contenidos de compuestos fenólicos (Xia et al., 2021).

Las plantas del algodón sometidas a estrés hídrico y tratadas con 1-MCP tuvieron una mayor actividad de las enzimas antioxidantes con una mejora en el mantenimiento de la integridad de la membrana (Kawakami et al., 2010). En plantas de soja, la aplicación de 1-MCP redujo la producción de etileno y el contenido de ROS, retrasó la senescencia de las hojas, disminuyó la abscisión de las flores y aumentó el porcentaje de formación de las vainas (Djanaguiraman et al., 2011). Además el 1-MCP actúa también en la tolerancia del fruto a las bajas temperaturas, observándose que dosis múltiples de 1-MCP en nectarinas redujeron de forma más efectiva los daños por frío que el tratamiento convencional con una única dosis (Zhang et al., 2020). El tratamiento con 1-MCP redujo el índice de daño por frío, la tasa de fuga de electrolitos y el contenido de MDA de las judías verdes. También mostraron una mayor capacidad antioxidante total con la mejora de la acumulación de compuestos fenólicos al regular las actividades de las enzimas (Lv et al., 2023). En melocotón, el tratamiento con 1-MCP mejora la tolerancia al frío al regular la biosíntesis de la fitohormona ácido indol-3-acético, la transducción de la señalización de auxina y la degradación de la pared celular (Zheng et al., 2023b) (Tabla 4A, B). Los efectos positivos descritos tanto en precosecha como en poscosecha en diferentes especies vegetales hacen del 1-MCP una importante herramienta comercial.

Tabla 4A. Revisión bibliográfica sobre los efectos del 1-MCP en la precosecha y poscosecha de diferentes cultivos.

1-MCP		
Efectos	**Aplicación**	**Cultivo, concentración de 1-MCP* y referencia**
Fortalecimiento de la respuesta de defensa contra el estrés biótico y/o abiótico	Precosecha	Algodón (0,37 % v/v); Kawakami et al., 2010 Soja (1 µg /L); Djanaguiraman et al., 2011
	Poscosecha	Albaricoque (0,5 µL/L); De Martino et al., 2006
Aumento de la vida útil, retraso en la maduración y/o mejora de la calidad en el momento de la cosecha y/o durante el almacenamiento	Precosecha	Pitahaya (400 µg/L); Serna et al., 2012 Plátano (0,4 µL/L); Manigo y Matuginas, 2020 Melocotón (240 mg/L); Lee et al., 2020 Manzana (300 mg/L); Amarante et al., 2022 Caqui (12 g/L); Vilhena et al., 2022
	Poscosecha	Pera (300 nL/L); Arias et al., 2009 Kiwi (1 µL/L); Ali et al., 2021 Aguacate (0,03 µL/L); Pachón et al., 2022 Tomate (1 µL/L); Wu et al., 2023a
Reducción de los síntomas de daño por frío, fuga de electrolitos, permeabilidad de la membrana y/o contenido de MDA	Precosecha	Pera (300 mg/L); Li et al., 2022b
	Poscosecha	Orquídea (0,1 µL /L); Phetsirikoon et al., 2012 Nectarina (0,25 µL/L); Zhang et al., 2020 Kiwi (0,25 µL/L); Liu et al., 2021a Aguacate (300 g/mol); Shikwambana et al., 2021 Melocotón (10 µL/L); Zheng et al., 2023b

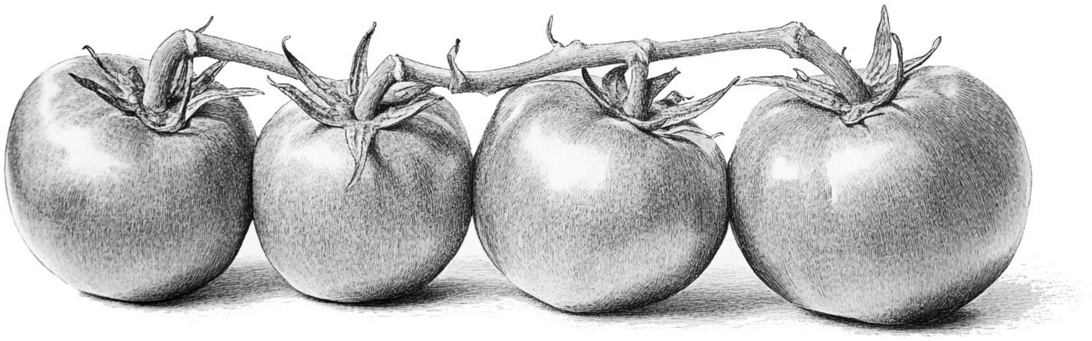
*En algunos de los estudios se han probado diferentes concentraciones, pero se muestra sólo la que los autores establecen como la más efectiva para el efecto esperado.

Tabla 4B. Revisión bibliográfica sobre los efectos del 1-MCP en la precosecha y poscosecha de diferentes cultivos.

1-MCP		
Efectos	**Aplicación**	**Cultivo, concentración de 1-MCP* y referencia**
Incremento de los compuestos bioactivos y/o de la actividad antioxidante relacionada con compuestos antioxidantes enzimáticos y/o no enzimáticos	Poscosecha	Kale (10 µL/L); Sun et al., 2012 Col (1 µL/L); Hu et al., 2021 Calabaza (0,25 µL/L); Kurubaş et al., 2021 Melocotón (1,2 µL/L); Gao et al., 2022 Judías verdes (1 µL/L); Lv et al., 2023
Inducción de la resistencia a patógenos, control de los desórdenes fisiológicos y/o de la podredumbre durante el almacenamiento	Poscosecha	Jínjol (1 µg/L); Zhang et al., 2012b Pera (1 µg/L); Xu et al., 2020 Plátano (10 µL/L); Kesari et al., 2010 Kiwi (0,8 µL/L); Xia et al., 2021 Caqui (1,35 µL/L); Zeng et al., 2021

*En algunos de los estudios se han probado diferentes concentraciones, pero se muestra sólo la que los autores establecen como la más efectiva para el efecto esperado.

2. OBJETIVOS



2. OBJETIVOS

El sector hortofrutícola en España es el sector más importante en el conjunto del sector agrario representando un 43 % de la producción total vegetal y el 25 % de la producción agraria. El valor del sector de frutas y hortalizas superó en 2022 los 15.860 millones de euros, un 3,6 % más que en el año anterior. España es el primer productor de la UE de frutas y hortalizas con más del 25 % de la producción europea y el séptimo a nivel mundial.

Por otro lado, la producción de flor cortada a nivel nacional muestra una tendencia creciente en los últimos años con un valor de 780 millones de euros en 2022, el 12 % frente al año anterior. La producción de flor cortada se concentra fundamentalmente en el clavel y la rosa, representando ambas el 51 % del total nacional. España es el quinto productor de la UE de flor cortada y planta ornamental con el 11 % del valor total comunitario de la producción europea.

Estos productos vegetales se deterioran rápidamente después de la cosecha y durante el transporte y la comercialización, en algunos casos, no llegan a los consumidores con la calidad óptima. La pérdida de calidad del producto influye en la aceptación de los consumidores y en los beneficios atribuidos para la salud de sus compuestos bioactivos. El manejo de enfermedades poscosecha se ha llevado a cabo durante muchos años mediante el uso de productos de origen artificial, pero una mayor conciencia en la población sobre los riesgos asociados a estos compuestos está cambiando esta tendencia. Se están explorando tecnologías más respetuosas con el medio ambiente y sostenibles para sus aplicaciones en la conservación de estos productos vegetales. Entre estas tecnologías se encuentras los elicitores, que están presentes de forma natural en las plantas y son moléculas señalizadoras que actúan generando una respuesta de defensa en la planta. Esta respuesta da como resultado la síntesis de metabolitos que reducen el daño y aumentan la resistencia a diferentes estreses abióticos y bióticos.

La creciente importancia económica a nivel nacional de los cultivos y la pérdida de calidad y la susceptibilidad a sufrir desórdenes fisiológicos hacen necesaria la ampliación del conocimiento para facilitar la conservación y mantener el estado óptimo de los productos. Por ello, ampliar la investigación sobre los tratamientos precosecha y poscosecha en los productos vegetales expuestos en esta Tesis y comunicar a los consumidores los beneficios que conllevan para la salud el consumo de estos productos es un factor esencial para sostener la demanda del producto.

Por todo ello, el objetivo general de esta Tesis Doctoral es estudiar el impacto de los elicitores sobre la calidad y la vida útil de diferentes productos vegetales aplicados durante el desarrollo del fruto en el árbol y una vez recolectados. El uso de compuestos de origen natural de forma individual o combinado con tecnologías de origen artificial ampliamente usadas permitirá extender el conocimiento sobre sus posibles efectos sinérgicos con el fin de reducir la cantidad de compuestos utilizados artificiales en la conservación de los productos vegetales estudiados en esta Tesis.

Para la consecución de este fin se establecieron los siguientes objetivos específicos para la reducción de las fisiopatías en precosecha:

I. Determinar los efectos del jasmonato de metilo, putrescina y melatonina sobre el agrietado y el cuajado de las cerezas durante el desarrollo en el árbol.

II. Incrementar los parámetros de calidad en el momento de la cosecha de las cerezas tratadas con los elicitores.

Para evaluar el efecto de los elicitores en la mejora de la calidad y vida útil en poscosecha, se establecieron los siguientes objetivos específicos:

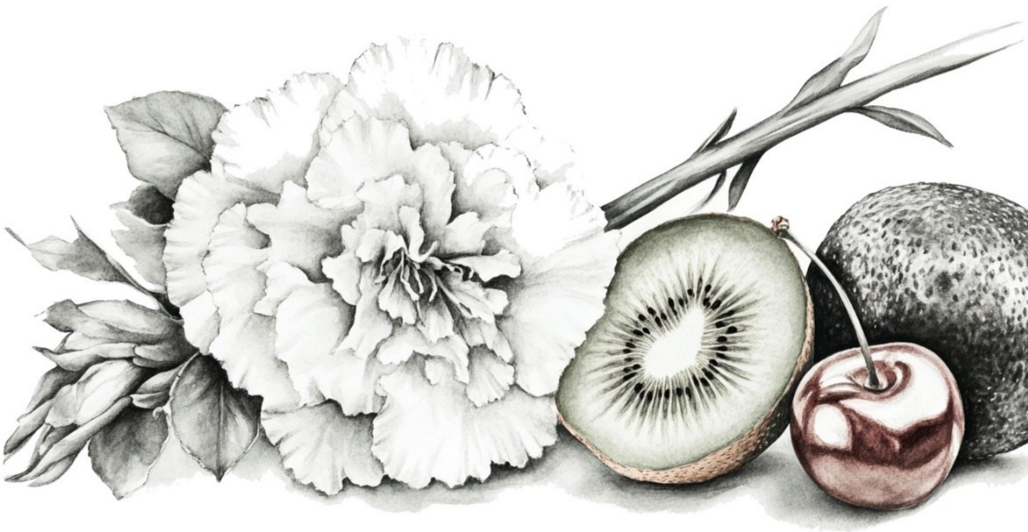
III. Evaluar la capacidad antisenescente de la melatonina sobre flores comestibles u ornamentales.

IV. Estudiar los efectos sobre los daños por frío durante el almacenamiento en frío de los frutos cuando los elicitores son aplicados de forma individual o en combinación con 1-MCP.

V. Aumentar la vida útil y reducir las podredumbres en la poscosecha de los frutos tratados con elicitores y/o 1-MCP.

VI. Determinar los efectos sobre los compuestos bioactivos y la capacidad antioxidante de los productos vegetales tratados con elicitores y/o 1-MCP tanto en precosecha como en poscosecha.

Con la información recogida en esta Tesis, se conseguirá incrementar el conocimiento sobre las posibilidades de los elicitores en el aumento de la vida útil de productos vegetales y en la reducción de fisiopatías tanto en precosecha como en poscosecha.



3. MATERIALES Y MÉTODOS

3. MATERIALES Y MÉTODOS

3.1. Material vegetal y diseño experimental

3.1.1. Aplicaciones precosecha en cereza

Los experimentos se realizaron en diferentes parcelas de campo ubicadas en Alcoy (Mas de Roc Cooperativa Agrícola, Alcoy, España) con cerezos (*Prunus avium* L.) que fueron injertados en portainjertos SL-64. Todos los cerezos se cultivaron bajo las prácticas agronómicas habituales y uniformes para los diferentes ciclos productivos estudiados. Los tratamientos se realizaron aplicando 3 L por árbol mediante pulverización foliar con las soluciones recién preparadas y con agua destilada para los árboles control. Se añadió 1 mL L^{-1} de Tween 20 a las disoluciones como surfactante. Para cada tratamiento y variedad, se utilizaron tres grupos de tres árboles como réplicas con un total de nueve árboles (Figura 31).

Figura 31. Infografía del diseño experimental realizado en precosecha de cereza.

- **Aplicaciones con JaMe**

El tratamiento con JaMe 0,5 mM (Sigma-Aldrich, España, ≥ 98 % W341002) se aplicó en el estadio de endurecimiento del hueso (E3, BBCH 77) y al inicio de los cambios de color (E4, BBCH 81) según la escala BBCH (Biologische Bundesantalt Bundessortenamt und Chemische Industrie) durante cuatro ciclos productivos (2019-2022) (Figura 32). La selección del número de aplicaciones se basó en los resultados observados en 3 grupos de árboles estudiados en la temporada 2019 para las variedades 'Prime Giant' y 'Early Lory'. Estos grupos de árboles fueron tratados con 1, 2 y 3 aplicaciones, sin embargo, la tercera aplicación no aumentó significativamente el potencial de los tratamientos con JaMe para los diferentes parámetros evaluados.

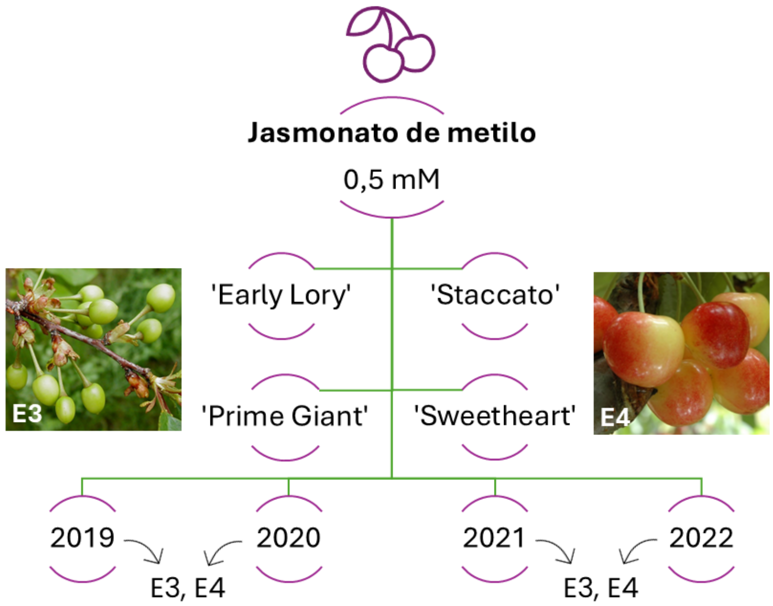


Figura 32. Diagrama de la aplicación del tratamiento con JaMe en cereza. Etapas fenológicas E3: endurecimiento del hueso (BBCH 77) y E4: inicio del cambio de color (BBCH 81).

- **Aplicaciones con PUT**

Para el estudio con PUT se utilizaron las variedades 'Prime Giant' y 'Sweetheart' durante cuatro ciclos de producción (2020-2023). Los tratamientos con PUT (Sigma-Aldrich, Alemania, ≥ 99 % D13208) se aplicaron a concentraciones de 1 y 10 mM, escogidas por las ensayadas por otros autores anteriormente en diferentes especies vegetales (Alburquerque et al., 2006; Mirdehghan y Rahimi, 2016; Singh et al., 2022). Se realizaron cuatro aplicaciones durante 2020 y 2021 en 4 estadios fenológicos. En 2022 y 2023 se omitió el estadio de plena floración (E2) para optimizar el número de aplicaciones y se utilizó la concentración más baja de 1 mM en base a los resultados obtenidos en 2020 y 2021. Los primeros años sirvieron como fase de selección para la concentración y número de aplicaciones óptimas en los parámetros analizados de la cereza (Figura 33).

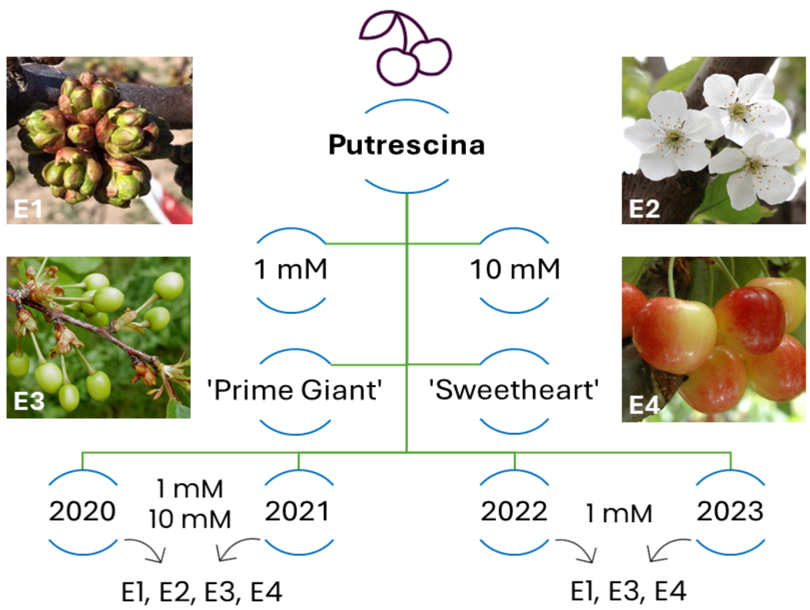


Figura 33. Diagrama de la aplicación del tratamiento con PUT en cereza. Etapas fenológicas E1: botón floral (BBCH 54), E2: plena floración (BBCH 65), E3 y E4 descritas en la Figura 32.

- **Aplicaciones con MT**

De 2020 a 2023, se llevó a cabo el estudio de cuatro años con el tratamiento de MT en las variedades 'Prime Giant' y 'Sweetheart'. Durante el período 2020-2022 se seleccionaron diferentes árboles y parcelas, y en 2022 y 2023 se utilizó la misma parcela. Se utilizaron concentraciones de 0,01, 0,05 y 0,1 mM de MT (Sigma-Aldrich, Alemania, ≥ 98 % M5250). Estas concentraciones se determinaron en base a estudios previos del grupo de investigación (Carrión-Antolí et al., 2022a).

Se pulverizaron cuatro aplicaciones por cada concentración de MT durante las temporadas de 2020 y 2021 en los cuatro estadios fenológicos de crecimiento del fruto. En 2022 y 2023, sólo se realizaron tres aplicaciones, excluyendo la etapa de plena floración (E2) para optimizar el número de aplicaciones. Solo se aplicó la concentración más baja 0,01 mM durante los ciclos productivos de 2022 y 2023 (Figura 34).

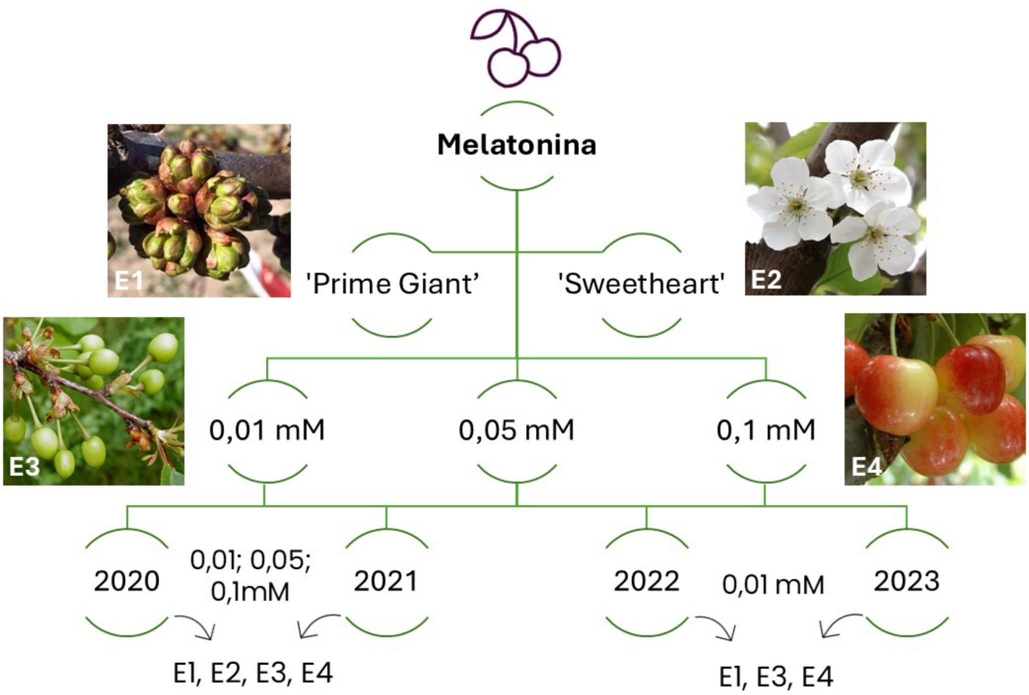


Figura 34. Diagrama de la aplicación del tratamiento con MT en cereza. Etapas fenológicas (E1, E2, E3 y E4) descritas en la Figura 32 y 33.

3.1.2. Aplicaciones poscosecha en clavel, kiwi, aguacate y tomate

Los claveles (*Dianthus caryophyllus* cv. Báltico) se recolectaron de un huerto comercial en Murcia (España) en la etapa de cosecha comercial. El mismo día de la cosecha, las flores se transportaron al laboratorio con los tallos sumergidos en agua. Después en el laboratorio, se descartó cualquier flor con defectos visuales y se seleccionaron 66 flores en la etapa de desarrollo S3 para cada tratamiento (0, 0,01, 0,1 y 1 mM) (Figura 35).

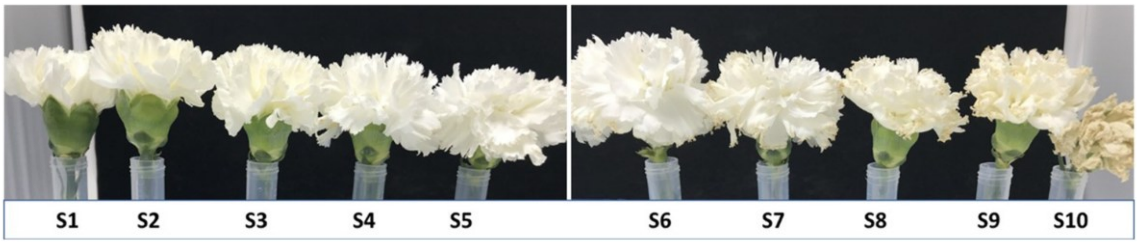


Figura 35. Etapas de desarrollo y senescencia del clavel cv. Báltico.

Para seleccionar las concentraciones óptimas de MT en esta flor, se realizó un estudio previo y se probaron diferentes concentraciones de MT en pétalos de clavel 'Báltico'. Se evaluaron 20 pétalos de forma individual por cada concentración de MT (0, 0,005, 0,01, 0,1 y 1 mM) a temperatura ambiente y se compararon con 20 pétalos en agua destilada como control (Figura 36B). Se observó que el peso fresco (PF) de los pétalos no se redujo para la concentración de MT de 0,1 mM (Figura 36A). Se seleccionaron las concentraciones más altas y la concentración más baja fue descartada por mostrar la mayor pérdida de peso.

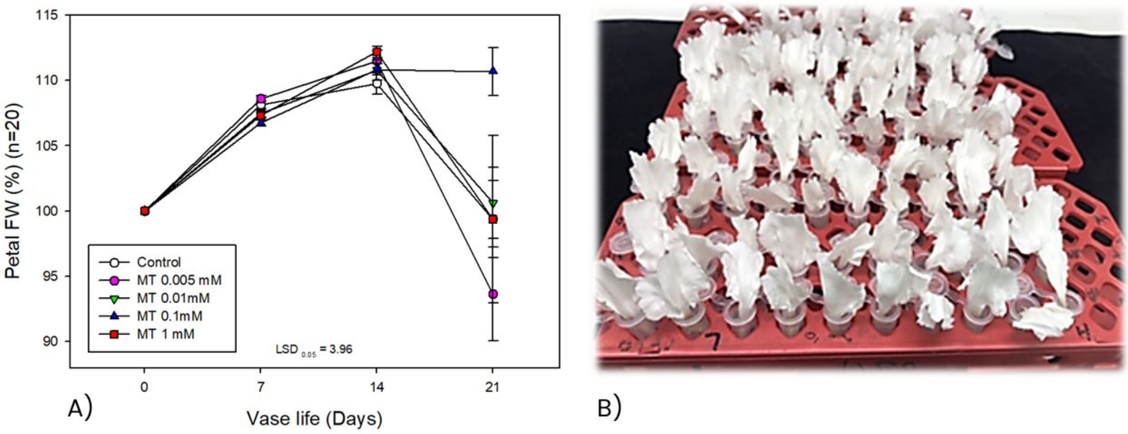


Figura 36. A) Evolución del PF de los pétalos (n=20) almacenados a 20 ºC y B) pétalos individuales con las diferentes concentraciones de MT.

La MT (Sigma-Aldrich, EE. UU., ≥ 98 % M5250) se disolvió previamente para cada concentración con 0,5 mL de etanol. El mismo volumen de etanol se agregó a la solución control. Los tallos se cortaron con una longitud de 10 cm y se colocaron individualmente en tubos falcon con 10 mL de las diferentes disoluciones. Los claveles se mantuvieron a temperatura ambiente de aproximadamente 20 °C, humedad relativa de 65–70 % y un fotoperiodo de 12 h utilizando luz fluorescente blanca con una intensidad de 80,5 μmol m^{-2} s^{-1}. Estas condiciones se mantuvieron durante algo más de cuatro semanas (30 días) y se añadió agua destilada o soluciones de MT a los tubos falcon cuando fue necesario (Figura 37).

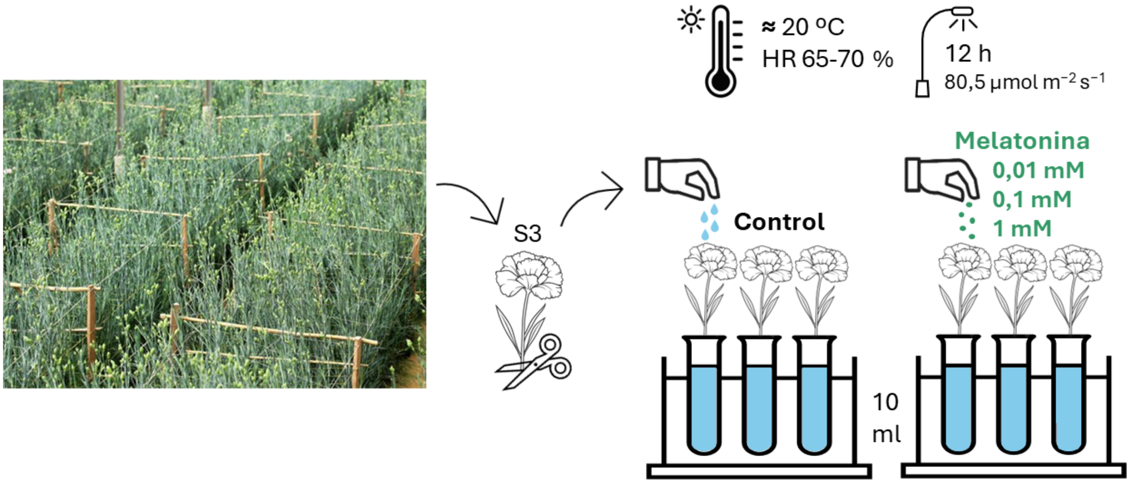


Figura 37. Infografía del diseño experimental del clavel de la variedad 'Báltico'. Proceso de recolección en huerto y su posterior acondicionamiento en el laboratorio para su almacenamiento y correspondientes análisis.

Se realizaron mediciones no destructivas como la vida en florero, el PF y la absorción de solución en florero (ASF) casi a diario en un lote de 30 flores por tratamiento. Para la tasa de respiración y la producción de etileno, se utilizó otro lote de 6 flores por tratamiento exclusivamente para evaluar estos parámetros. Adicionalmente, 30 flores por tratamiento fueron divididas en 5 lotes de 6 flores cada uno, para evaluar parámetros destructivos como el índice de estabilidad de membrana (IEM), el contenido total de polifenoles (CTP), la actividad antioxidante total en pétalos (AAT) o el contenido total de clorofila (CTC) en sépalos después de 5, 10, 15, 20 y 25 días de almacenamiento a temperatura ambiente.

Además, los diferentes parámetros fueron evaluados en un lote extra de 10 claveles el día de la cosecha. Todos los parámetros fueron evaluados en muestras frescas excepto el contenido de AAT y CTC que fueron medidos en pétalos y cáliz respectivamente, separados de 6 flores para cada tratamiento, mezclados, pulverizados en nitrógeno líquido y almacenados a -80 °C para su posterior análisis.

Los kiwis (*Actinidia deliciosa*) cv. Hayward se cosecharon manualmente en una parcela comercial en Carlet (Valencia, España), cuando la fruta alcanzó la madurez de cosecha (6-7 °Brix) (Wang et al., 2021). Se clasificó y seleccionó un total de 315 kiwis sin defectos o daños mecánicos para obtener una muestra uniforme (Figura 38). Después de la selección, los kiwis se agruparon en 3 réplicas de 5 frutos por tratamiento y día de muestreo.

Figura 38. Kiwis 'Hayward'.

Los aguacates (*Persea americana* Mill.) cv. Hass se cosecharon en una finca comercial en Granada (España) y transportaron al laboratorio el mismo día de la cosecha.

Figura 39. Aguacates cv. Hass dispuestos para su selección.

En el laboratorio, los frutos fueron seleccionados visualmente considerando parámetros como tamaño y color homogéneos y ausencia de defectos (Figura 39). Un total de 360 aguacates fueron agrupados en 3 réplicas de 5 frutos para cada tratamiento y día de muestreo.

De forma análoga, se cosecharon tomates cv. Conquista tipo RAF (*Solanum lycopersicum* L.) en una parcela comercial en Almería, España. Fueron transportados al laboratorio el mismo día de la cosecha, donde se seleccionaron 255 frutos uniformes sin defectos, con características de tamaño y color similares (Figura 40). Inicialmente, un grupo de 15 frutos fueron analizados el día 0, mientras que los restantes se organizaron en 3 réplicas de 5 frutos cada una para los diferentes tratamientos y días de muestreo.

Figura 40. Tomates a su llegada al laboratorio.

En los experimentos poscosecha de kiwi, aguacate y tomate se aplicaron inmersiones en 10 litros de agua destilada durante 10 min tanto para el lote control como para el lote tratado únicamente con 1-MCP. Estas inmersiones se realizaron para todos los frutos con el objetivo de igualar las condiciones del experimento respecto a los frutos en los que se realizaron los tratamientos inmersivos con MT o GABA (Figura 41). Todas las soluciones de inmersión incluían Tween 20 a una concentración del 0,05 % (Sigma-Aldrich, España, P1379).

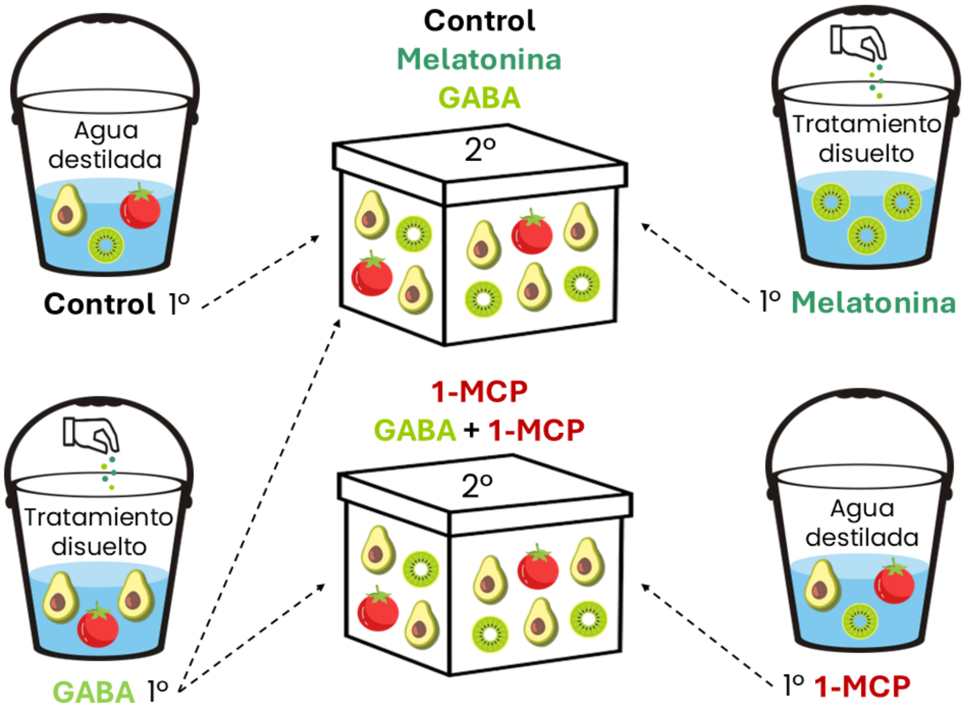


Figura 41. Diseño experimental poscosecha en kiwi, aguacate y tomate. Primero se realizaron las inmersiones y una vez secados los frutos se transfieren a los contenedores para la aplicación con 1-MCP o en condiciones de atmósfera normal para los lotes control y tratados únicamente con MT o GABA.

Para el tratamiento de MT en kiwi, se realizaron inmersiones en soluciones de 0,1 mM (Sigma-Aldrich, Alemania, ≥ 98 % M5250) durante 10 min siguiendo las condiciones óptimas en estudios previos (Jiao et al., 2022). En aguacate, para el tratamiento con GABA (Sigma-Aldrich, España, ≥ 99 % A2129) se utilizaron soluciones recién preparadas de 1 mM con inmersión durante 10 min. Este tratamiento se seleccionó entre diferentes concentraciones de GABA (1–10 mM) y tiempos de inmersión ensayados (10 min y 1 h) en experimentos previos (Figuras 42 y 43). El tratamiento con GABA en tomate se realizó con una concentración de 10 mM escogida en un ensayo visual preliminar en el que se probaron las mismas concentraciones (1–10 mM) y tiempos (10 min y 1 h) que en aguacate.

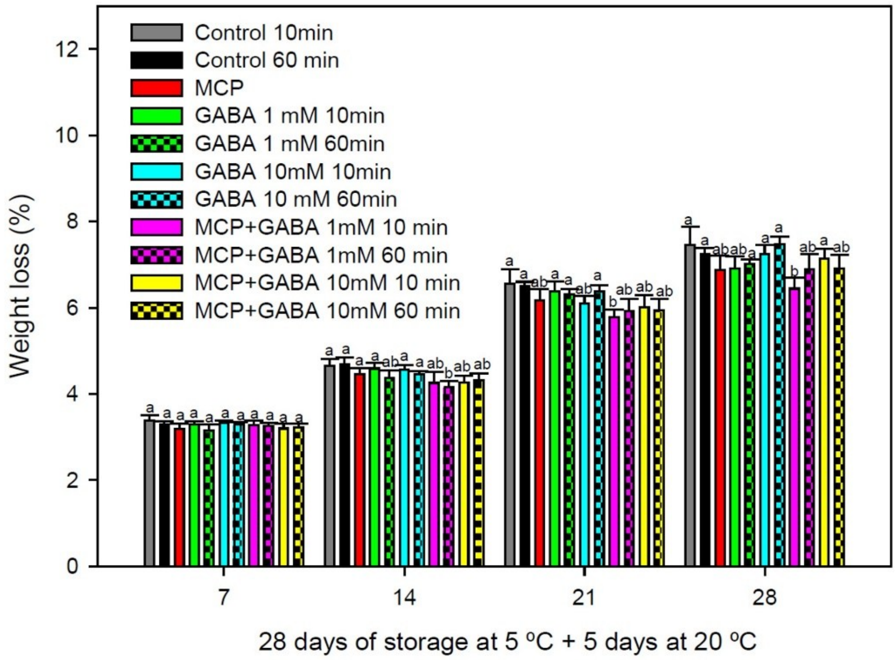


Figura 42. Experimento preliminar. Evolución de la pérdida de peso (%) del aguacate 'Hass' tratado tras las inmersiones de 10 o 60 minutos con agua destilada en los lotes control, tratados con GABA (1 y 10 mM) y con 1-MCP (0,3 µL L^{-1}) solo o combinado con GABA durante 28 días de almacenamiento a 5 °C más 5 días a 20 °C. Los datos son la media ± EE (n = 3). Letras minúsculas diferentes indican diferencias significativas (*p* < 0,05) entre los tratamientos.

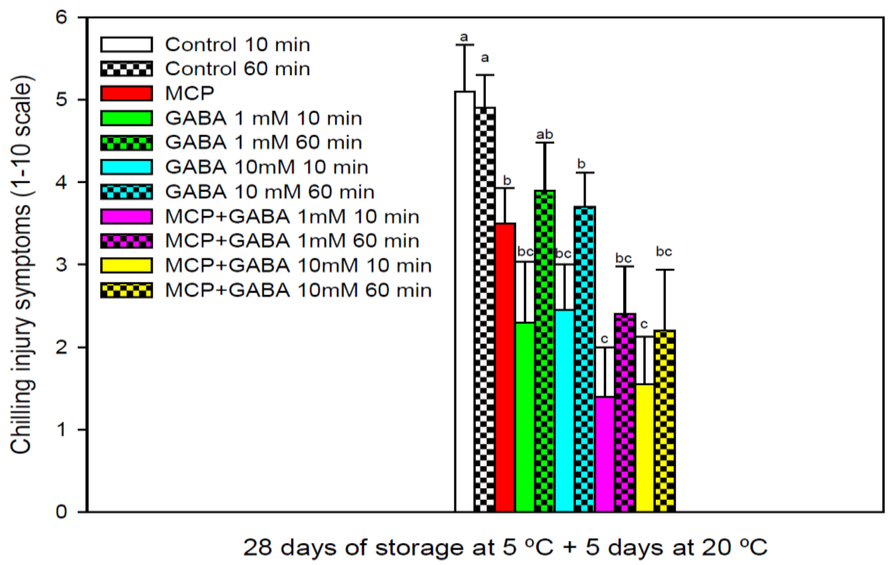


Figura 43. Experimento preliminar. Daño por frío (escala 0-10) del aguacate 'Hass' tratado durante inmersiones de 10 o 60 minutos con agua destilada en los lotes control, tratados con GABA (1 y 10 mM) y con 1-MCP (0,3 µL L^{-1}) solo o combinado con GABA después de 28 días de almacenamiento a 5 °C más 5 días a 20 °C. Los datos son la media ± EE (n = 3). Letras minúsculas diferentes indican diferencias significativas (*p* < 0,05) entre los tratamientos.

Los tratamientos con 1-MCP en aguacate se aplicaron mezclando tabletas comerciales que liberaban 0,3 μL L^{-1} (Defilippi et al., 2018) con una solución activadora comercial proporcionada por SmartFresh (Agro-Fresh Inc., Philadelphia, PA, USA). En kiwi, se escogió la concentración óptima de 0,5 μL L^{-1} en base a estudios previos (Koukounaras y Sfakiotakis, 2007). De la misma forma se seleccionó la concentración de 1-MCP para tomate (Guillén et al., 2006).

Después de los tratamientos por inmersión, todas las frutas se dejaron secar al aire a 20 °C durante una hora antes de ser transferidas a contenedores plásticos individuales de 130 litros, asegurando condiciones uniformes para los diferentes lotes (Figura 44). Los contenedores se cerraron y sellaron herméticamente, permitiendo que el 1-MCP actuase durante 24 horas a 20 °C en los casos de los tratamientos individuales y combinados. Para los tratamientos que no contienen 1-MCP, como el control o los tratados únicamente con MT y GABA, los contenedores se cerraron y sellaron en las mismas condiciones (Figura 44).

Figura 44. Contenedores plásticos con los diferentes tratamientos.

Posteriormente, los kiwis se almacenaron durante 84 días a 2 °C y 90 % de humedad relativa (HR) más un período adicional de 5 días a 20 °C para realizar las determinaciones de vida útil. Los aguacates se almacenaron durante 35 días a 5 °C y 90 % de HR, seguidos de un período adicional de 5 días a 20 °C. Los tomates tuvieron un periodo de almacenamiento de 21 días a 4 °C con una HR del 90 %, seguidos de un periodo adicional de 7 días a 20 °C. Todos los reactivos utilizados en este estudio fueron de pureza analítica.

3.2. Evaluación de los desórdenes fisiológicos de la cereza en precosecha

3.2.1. Resiliencia a las heladas primaverales

La resistencia a las heladas primaverales fue evaluada para los tratamientos precosecha de PUT y MT. En marzo y abril de 2020, durante el inicio de la pandemia de COVID-19, las circunstancias no permitieron evaluar la tolerancia a las heladas en cerezos. En el mes de abril de los ciclos productivos de 2021, 2022 y 2023, durante la etapa de floración, se registraron temperaturas de -0, 4, -0,7 y -3,4 °C, respectivamente. Cabe destacar que, en 2023, la temperatura más baja registrada (-3,4 °C) coincidió con la variedad 'Prime Giant' en la etapa de plena floración, sin mostrar resiliencia frente a temperaturas subóptimas. Como consecuencia, el cuajado de los frutos para esta variedad fue significativamente menor en comparación con el resto de los ciclos productivos evaluados.

En 2021 y 2022 para el tratamiento con PUT y MT, el contenido de MDA se evaluó en el tejido de los botones florales una semana después de que los árboles fueron tratados. Para ello, se recolectaron un conjunto de botones en el E1 botón floral (BBCH 55) de cuatro ramas diferentes y equidistantes ubicadas a ambos lados de cada árbol (Figura 45A, B).

Figura 45. Fotografías tomadas de las ramas de cerezo etiquetadas con el tratamiento de A) PUT 1 mM y B) MT 0,01 mM.

El porcentaje de pistilos no dañados se evaluó en 2022 y 2023 debido al impacto significativo de las bajas temperaturas en los pistilos durante el estadio de plena floración (BBCH 65) en las variedades 'Prime Giant' y 'Sweetheart'. Se examinaron individualmente cien flores de cada árbol (50 flores seleccionadas en cada lado del árbol) para determinar la presencia de pistilos verdes o si este tejido estaba afectado por un pardeamiento interno como resultado del daño por las heladas. Los pistilos dañados por las heladas exhibieron signos de necrosis o daño interno. La proporción de flores no dañadas se expresó como un porcentaje. Este porcentaje se obtuvo de tres réplicas de tres árboles (n = 3) y representa la proporción de botones florales con pistilos verdes que podrían tolerar condiciones de heladas y ser potencialmente funcionales para desarrollarse en frutos.

El cuajado de los frutos se determinó contando los botones florales en cuatro segmentos de ramas homogéneamente divididas en ambos lados del árbol (n = 3). Los botones florales que aún no se habían abierto representaban las flores que potencialmente podrían convertirse en frutos y se contaron cuidadosamente en cada rama etiquetada durante los últimos tres años de esta investigación (2021, 2022 y 2023). Después del cuajado de los frutos (BBCH 75) y antes del cuarto tratamiento previo a la cosecha (BBCH 77), se registró la cantidad de cerezas desarrolladas para cada segmento de la rama señalada anteriormente. El porcentaje de cuajado de los frutos se determinó calculando la proporción de cerezas completamente desarrolladas en relación con el número de botones florales registrado previamente dentro del segmento de rama correspondiente.

3.2.2. Evaluación del agrietado del fruto

Para las cerezas tratadas con JaMe se realizó una evaluación del agrietado de estos frutos en la cosecha para las variedades 'Early Lory' en 2019, 'Staccato' en 2020, 'Prime Giant' (2019, 2021 y 2022) y 'Sweetheart' (2020, 2021 y 2022). Las circunstancias de la pandemia del COVID-19 impidieron las evaluaciones durante varios meses críticos del desarrollo del fruto en el 2020 en variedades de recolección temprana. También se investigó durante diferentes ciclos productivos el efecto del tratamiento con JaMe (2019-2021), PUT (2020-2023) y MT (2020-2023) en el agrietado de las cerezas a medida que la fruta maduraba en el árbol. En 2023, la variedad 'Prime Giant' no fue evaluada para el tratamiento con PUT y MT porque la producción de frutos no fue lo suficientemente alta debido al daño por heladas experimentado a principios de la primavera de ese año.

Las diferentes etapas de maduración seleccionadas para investigar el impacto del tratamiento sobre el agrietado de 'Prime Giant' fueron las coincidentes con el inicio de los cambios de color (S1), la aparición del color rosa (S2), el color rojo brillante (S3) y el color rojo oscuro (S4). Para 'Sweetheart', las etapas de maduración coincidieron con el color verde-amarillo inmaduro (S1), el inicio de los cambios de color (S2), el color rosado (S3), el color rojo brillante (S4) y el color rojo oscuro (S5).

La incidencia del agrietado en el árbol se evaluó en 4 ramas etiquetadas opuestamente en cada árbol en un total de 100 frutos por árbol, y los resultados se expresaron como porcentaje de agrietado. Por otra parte, se evaluó el agrietado inducido en condiciones controladas utilizando inmersiones en agua destilada en frutos sanos simulando una fuerte lluvia artificial adicional de 6 horas según el método descrito por Christensen (1996). Se realizó en el momento de la cosecha en tres réplicas de 50 frutos por cada tratamiento y los valores obtenidos se registraron como un índice de agrietado.

3.3. Parámetros de calidad determinados tras la recolección y durante la conservación poscosecha

3.3.1. Evolución del peso del producto durante el almacenamiento

La pérdida de peso de los diferentes frutos individuales se expresó como la diferencia con relación al peso inicial obtenido en el mismo día de la cosecha expresado en porcentaje. Los resultados obtenidos se representaron como la media ± EE de 5 frutos por cada réplica (n = 3). Para los claveles, el peso fresco (PF) de cada flor se expresó como el porcentaje en relación con su peso inicial, el cual se asumió como el 100 %. Los resultados fueron la media ± EE de 30 flores. Para evaluar el % de absorción de la solución en florero se pesó por separado el falcón vacío, el falcón con la solución sin la flor y la flor por separado obteniendo el peso de la solución. Los resultados se expresaron como la media ± EE de 30 flores por tratamiento y se utilizó la siguiente fórmula para calcular esta absorción (mL d^{-1} g^{-1} PF) = ($W_{(t-1)}$ -$W_{(t)}$) / $PF_{t=0}$), donde W_t = peso de la solución (g) en t días (3, 4, 5, etc.), W_{t-1} = peso de la solución (g) del día anterior y $PF_{t=0}$ = PF de la flor (g) del día 0.

3.3.2. Tasa de respiración y producción de etileno

Los niveles de respiración y etileno se midieron por triplicado colocando 5 frutos de cada réplica en recipientes de 3,4 L para kiwi o 4,6 L para aguacate y tomate sellados herméticamente con un tapón de goma durante 30 minutos (tomate) o 60 minutos (kiwi y aguacate) a temperatura ambiente utilizando el método estático (Figura 46A). En clavel, la tasa de respiración se midió en 6 flores por tratamiento colocando cada flor en un frasco de vidrio de 1 L herméticamente cerrado con un tapón de goma durante 2 horas (Figura 46B).

Figura 46. A) Tomates y B) claveles colocados en recipientes.

Posteriormente, una muestra de gas de 1 mL por duplicado (n = 3) extraída del espacio de cabeza de los recipientes se inyectó en un cromatógrafo de gases Shimadzu GC-14B (Shimadzu Europa GmbH, Duisburg, Alemania) para determinar la concentración de CO_2, y la producción de etileno se evaluó con un cromatógrafo de gases Shimadzu GC-2010 (Shimadzu Europa GmbH, Duisburg, Alemania) equipado con un detector FID. Los parámetros cromatográficos fueron detallados por Medina-Santamarina et al. (2021). La tasa de respiración y la producción de etileno se expresaron como mg de CO_2 kg^{-1} h^{-1} y nL g^{-1} h^{-1}, respectivamente. En flor, la tasa de respiración se expresó como nmol de CO_2 liberado por kg^{-1} s^{-1}.

3.3.3. Color externo e interno

La medición de color se realizó en el momento de la cosecha para cada fruto utilizando un colorímetro de reflectancia Minolta (CRC400, Minolta Camera Co., Kantō, Tokio, Japón) equipado con un iluminante D65 y un observador estándar CIE 2° que mide a través de una apertura de 8 mm. Se tomaron tres medidas de color para cada fruto, obteniendo 15 medidas para cada réplica (n = 3) en tres puntos equidistantes a lo largo del área ecuatorial según las coordenadas CIELab (CIE L^*, CIE a^* y CIE b^*). Se calcularon los valores de CIE *hue** (180 + $\tan^{-1}$ (b^*/a^*), si $a^* < 0$), luminosidad (CIE L^*), *Croma** ($(a^{*2} + b^{*2})^{1/2}$) y la diferencia de color total ΔE ($(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$ descrita por Lombardelli et al. (2021).

3.3.4. Firmeza

La firmeza de la cereza, kiwi, aguacate y tomate se determinó individualmente utilizando un analizador de textura TX-XT2i (Stable Microsystems, Godalming, Reino Unido) equipado con una sonda de plato plano con un diámetro de 100 mm (Figura 47). La velocidad de descenso del disco fue de 20 mm min^{-1} hasta alcanzar una deformación del 5 %. Los frutos se evaluaron individualmente en la región ecuatorial. La firmeza del fruto se expresó como la relación entre la fuerza aplicada y la distancia recorrida (N mm^{-1}).

Figura 47. Determinación de la firmeza del tomate en el analizador de textura.

3.3.5. Sólidos solubles totales y acidez titulable

El contenido de sólido solubles totales (SST) se determinó por duplicado utilizando aproximadamente 50 g de fruto y obteniendo el zumo filtrado a través de dos capas de tela de algodón y extraído de la mezcla de 5 mitades de kiwi, aguacate o tomate, y en cereza se utilizó la pulpa de 20 frutos para cada réplica. En aguacate, dada su naturaleza grasa, la extracción se efectuó después de mezclar 10 g de pulpa de aguacate con 10 mL de agua desionizada y homogeneizar finamente (Ultraturrax, T18 basic, IKA, Berlín, Alemania). Los SST se midieron en zumo filtrado y centrifugado (10.000 × g) a 4 °C durante 20 minutos y utilizando un refractómetro digital Atago PR-101 (Atago Co., Ltd. Tokio, Japón) a 20 °C. Los resultados de SST se expresaron en g 100 g^{-1}.

La acidez titulable (AT) se determinó por duplicado mediante titulación automática (785 DMP Titrino, Metrohm, Herisau, Suiza) con NaOH 0,1 N hasta alcanzar pH 8,1, con 1 mL de zumo en 25 mL de agua destilada. Los resultados se expresaron como g 100 g^{-1} del ácido orgánico predominante, equivalentes de ácido málico para todos los frutos excepto para kiwi que corresponde a ácido cítrico. Se utilizó la siguiente fórmula: (M × 0,067 o 0,064 × N × D) /m) × 100, donde M es la cantidad de NaOH utilizada en mL, 0,067 es el factor de conversión de miliequivalentes de ácido málico y 0,064 de ácido cítrico, N es la normalidad de NaOH, D es el factor de dilución utilizado para la extracción y m es la cantidad de muestra evaluada.

3.3.6. Determinación de compuestos bioactivos

El contenido de clorofila se evaluó extrayendo una mezcla homogénea de epicarpio de 5 mitades en aguacate y de 5 mitades de mesocarpio en kiwi y tomate para cada réplica por lote siguiendo el método descrito por Vu et al. (2019). La extracción del pigmento se logró mediante homogeneización en metanol durante 2 minutos, después las muestras se centrifugaron (Figura 48). El sobrenadante se evaluó por duplicado utilizando un espectrofotómetro (1900 UV/Vis, Shimadzu, Kyoto, Japón). Las longitudes de onda fueron de 652 y 665 nm y el resultado se expresó como miligramos por 100 g^{-1} de muestra para carotenoides y clorofilas.

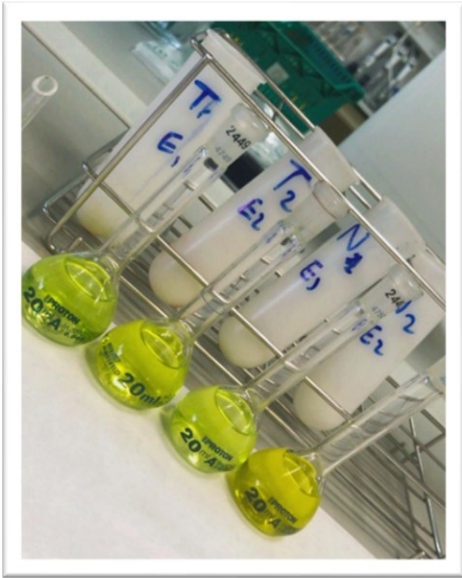


Figura 48. Extracción de clorofilas de la piel de aguacate.

Para la medición de la clorofila en los sépalos del clavel, se perforaron seis discos de cáliz de cada flor, cada uno de 6,25 mm de diámetro. Los discos se colocaron inmediatamente en 8 mL de metanol al 100 % y se dejó que los pigmentos se extrajeran en la oscuridad a 30 °C durante 24 horas. La absorbancia del extracto se midió a 652 y 665 nm (Porra et al., 1989).

Los flavonoides en tomate se midieron siguiendo el método de Woisky y Salatino (1998), donde 500 µL del extracto de la muestra se mezclaron por duplicado con 1,5 mL de metanol al 95 %, 0,1 mL de $AlCl_3$ al 10 % (m/v), 0,1 mL de acetato de sodio 1 M, y 2,8 mL de agua destilada. Después de la incubación en oscuridad a temperatura ambiente durante 30 minutos, se centrifugaron y se midió la absorbancia a 415 nm utilizando un espectrofotómetro (1900 UV/Vis, Shimadzu, Kioto, Japón). Los resultados se expresaron en miligramos equivalentes de quercetina-3-rutinosido por 100 gramos de materia seca, en referencia a la curva de calibración de quercetina-3-rutinosido.

Los polifenoles totales se extrajeron homogeneizando en Ultraturrax (T18 basic, IKA, Berlín, Alemania) 5 g de tejido congelado en cereza y 0,5 g de tejido de pétalos en clavel con 10 mL y 20 mL respectivamente de agua en metanol (2:8) que contenía 2 mM de NaF (para inactivar la actividad de la PPO y prevenir la degradación fenólica). A continuación, los extractos se centrifugaron a 10.000 rpm durante 10 min a 4 °C, y los compuestos fenólicos totales se midieron por duplicado en el sobrenadante utilizando el reactivo de Folin-Ciocalteu (FC) con el método descrito por Lezoul et al. (2020). Los resultados se expresaron como miligramos de ácido gálico equivalente por 100 g^{-1} en cereza y por kg^{-1} en clavel.

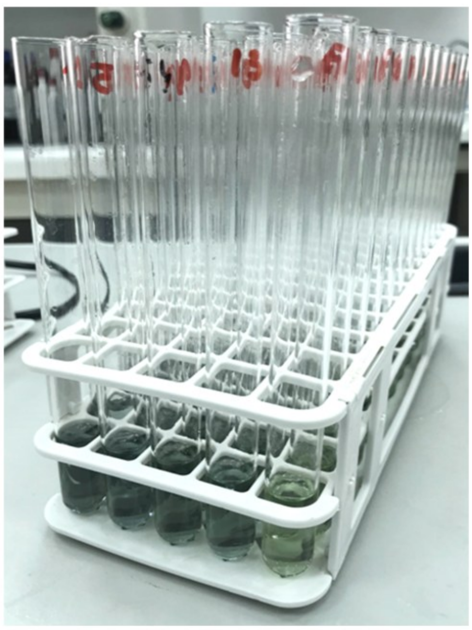


Figura 49. Análisis de polifenoles mediante FC.

El análisis de polifenoles en tomate se evaluó utilizando el método de FC descrito por Lezoul et al. (2020). Se tomaron 200 µL de cada extracto por duplicado (5 g de muestra y 10 mL extractante) y se combinaron con 300 µL de solución tampón de fosfato 50 mM, 2,5 mL de reactivo FC y 2 mL de Na_2CO_3 al 1 N. Después de agitar y realizar la incubación en un baño de agua a 50 °C durante 5 minutos, los extractos se centrifugaron y la absorbancia se midió a 760 nm utilizando un espectrofotómetro (1900 UV/Vis, Shimadzu, Kioto, Japón) (Figura 49). Los resultados se expresaron en mg equivalentes de ácido gálico por 100 g.

3.3.7. Actividad antioxidante

En clavel se añadió 10 mL de tampón fosfato 50 mM y 10 mL de acetato de etilo a 1 g de tejido de pétalos en 3 réplicas y la mezcla se homogeneizó durante 1 minuto. En clavel y tomate, la actividad antioxidante total (AAT) se evaluó utilizando el ensayo ABTS descrito por Lezoul et al. (2020). Se mezclaron 2 g de la muestra de tomate con 10 mL de tampón fosfato 50 mM y 6 mL de acetato de etilo. Después de la homogeneización y centrifugación (10.000 rpm durante 20 minutos a 4 °C), se separó la fase hidrofílica (AAT-H) y lipofílica (AAT-L), y se midieron por duplicado para cada extracto. La AAT-H se determinó con 890 µL de solución tampón de glicina 50 mM, 30 µL de ABTS 10 mM, 30 µL de H_2O_2 1 mM y 25 µL de POD 10 µM, mientras que la AAT-L se evaluó con 30 µL de ABTS 10 mM, 30 µL de H_2O_2 1 mM, 25 µL de POD 10 µM y 850 µL de etanol. Los resultados se expresaron en mg o g de Trolox por 100 g en tomate o por kg de PF en flores.

3.3.8. Contenido en malondialdehído

El contenido de MDA se determinó utilizando el protocolo descrito por Zhang et al. (2019) con algunas modificaciones. Se homogeneizó una muestra de tejido fresco de 1 g en el caso de los botones florales de la cereza y 5 g para los tomates en 10 mL de solución de ácido tricloroacético (TCA) al 10 %. Posteriormente se centrifugó a 10.000 rpm durante 20 minutos a 4 °C. Después de la centrifugación, se mezclaron 2 mL del sobrenadante con 6 mL de solución de ácido tiobarbitúrico (TBA) al 0,67 % por duplicado (n=3). Los tubos de ensayo se sometieron a 100 °C durante 20 minutos, se enfriaron rápidamente y se centrifugaron a 10.000 rpm durante 10 minutos. Finalmente, se midió la absorbancia de las muestras con un espectrofotómetro (1900 UV/Vis, Shimadzu, Kyoto, Japón) con mediciones de absorbancia a 450, 532 y 600 nm, expresadas en base al PF como µmol kg^{-1} (n = 3) siguiendo la formula descrita por Zhang et al. (2019).

3.3.9. Integridad de membrana

La fuga de electrolitos se evaluó siguiendo el método descrito por Lorente-Mento et al. (2024) con ligeras modificaciones. Se extrajeron 15 discos de cada réplica de 5 frutos de 0,5 cm (kiwi) o 1 cm (aguacate y tomate) de diámetro (Figura 50). Después de agitar 3 veces por 3 minutos con agua desionizada, los discos se sometieron a agitación continua durante 30 (kiwi) y 60 minutos (aguacate y tomate) con 50 mL de agua desionizada a temperatura ambiente.

Figura 50. Fuga de electrolitos en tomate.

Después se midió la conductividad eléctrica inicial (C1) a 20 °C utilizando un conductímetro Crison. Posteriormente, los discos se calentaron a 100 y 121 °C durante 10 y 15 minutos para tomate y kiwi y aguacate respectivamente. Una vez atemperadas las muestras a temperatura ambiente (20 °C), se midió la conductividad total (C2) y los resultados se expresaron como (C1/C2) × 100.

En claveles se midió esta fuga de electrolitos como el índice de estabilidad de la membrana. De cada una de las 6 flores de cada tratamiento y por cada fecha de muestreo se tomaron cuatro pétalos de la segunda línea de pétalos de la corola y de cada pétalo se cortó un disco de 1 cm de diámetro y se colocó en tubos de ensayo. A estos tubos se les añadió 15 mL de agua desionizada y 6 tubos se colocaron en un baño de 40 °C durante 30 minutos, se dejaron enfriar a 25 °C y luego se midió la conductividad eléctrica (CE) (C1). Una segunda serie de 6 tubos de ensayo se colocó en un baño a 100 °C durante 20 min y luego se leyó la conductividad eléctrica después de enfriarse rápidamente en un baño de hielo hasta 25 °C (C2). La estabilidad de la membrana se calculó utilizando la siguiente fórmula: [1 - (C1/C2)] * 100.

3.3.10. Daños sufridos en la cosecha y durante el almacenamiento

En cereza se midió el pardeamiento que sufre el pedicelo en el momento de la cosecha. Las cerezas con síntomas visibles de oscurecimiento del pedicelo se contabilizaron de acuerdo con una escala de 0 a 4: pedicelo sin síntomas visibles (0), pedicelos afectados por oscurecimiento < 25 % (1), 26–50 % (2), 51–75 % (3), y > 75 % del área del pedicelo afectada por oscurecimiento (4).

El clavel sufre senescencia una vez recolectado y la vida útil en florero es un indicador del marchitamiento de la flor. La vida en florero se evaluó diariamente en cada flor individualmente y se determinó por el número de días en que las flores mantuvieron sus propiedades decorativas, hasta que los claveles perdieron su valor ornamental debido al aspecto de los pétalos enrollados o pardeados de las puntas de los pétalos. Para ello, se evaluó los claveles siguiendo la escala de la Figura 35.

La determinación de la susceptibilidad de los diferentes frutos a los daños por frío se realizó con una evaluación visual. En kiwi se observó la pulpa después de quitarle la piel (Mao et al., 2007), en aguacate se midió la decoloración del mesocarpio de acuerdo con el método descrito por Hershkovitz et al. (2005) y en tomate se inspeccionó el picado superficial como síntoma de daños por frío (Ding et al., 2002).

En kiwi la evaluación visual se realizó individualmente en cada fruta utilizando una escala de 5 puntos (escala 0-4) de acuerdo con el área de superficie de picaduras y manchas acuosas oscuras: 0 (sin síntomas), 1 (< 25 %), 2 (25-50 %), 3 (51-75 %) y 4 (> 75 %). El daño por frío se expresó como Σ [(escala × N) / número total de frutas]. N es el número de frutas en la escala correspondiente. Los aguacates se cortaron longitudinalmente en dos mitades para inspeccionar la apariencia de la pulpa, y el daño interno se evaluó utilizando una escala de 10 puntos de la siguiente manera: 0 para ningún daño, 1 para daño menor, 5 para daño moderado y 10 para daño severo (Figura 51).

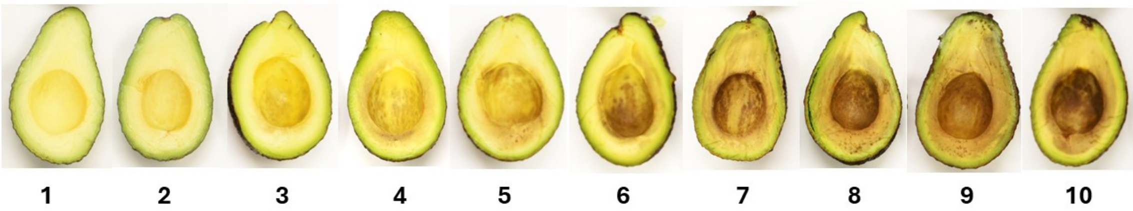


Figura 51. Escala de 1 al 10 para evaluar visualmente los daños por frío en aguacate.

La severidad de los síntomas en tomate se evaluó en una escala de 0 a 4 puntos: 0 para ausencia de picado, 1 representa unas pocas depresiones dispersas, 2 indica picado que cubre hasta el 5 % de la superficie del fruto, 3 significa picado que cubre entre > 5 % y < 25 % de la superficie del fruto, y 4 denota picado extenso que cubre más del 25 % de la superficie del fruto. La incidencia de frutos podridos en el tomate se cuantificó en función del porcentaje de frutos afectados en relación con el número total de frutos.

3.3.11. Análisis estadístico

Los experimentos se realizaron utilizando un diseño completamente aleatorio. Respecto a los experimentos precosecha de cereza se empleó el análisis de varianza de una vía, donde (*) indica diferencias significativas entre las muestras tratadas y el control (prueba t de Student no pareada; * $p < 0,05$, ** $p < 0,01$). Los resultados se expresaron como media ± EE. En clavel los valores se compararon mediante una prueba de diferencia mínima significativa (LSD) con un valor de $p < 0,05$. Para el resto de los experimentos poscosecha en kiwi, aguacate y tomate, los datos se sometieron a un análisis de varianza (ANOVA). Las comparaciones de medias se realizaron utilizando una prueba de rango múltiple (prueba HSD de Tukey) para encontrar diferencias significativas ($p < 0,05$) entre tratamientos para cada día de muestreo. Diferentes letras minúsculas indican una diferencia significativa entre tratamientos en la misma fecha de muestreo. Todos los análisis se realizaron con el paquete de software SPSS, versión 22 (IBM Corp., Armonk, NY, EE. UU.).

4. PUBLICACIONES

4. Publicaciones

4.1. Publicación I (Transcripción literal)

I

Putrescine increases frost tolerance and effectively mitigates sweet cherry (*Prunus avium* L.) cracking: a study of four different growing cycles

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Article

Putrescine Increases Frost Tolerance and Effectively Mitigates Sweet Cherry (*Prunus avium* L.) Cracking: A Study of Four Different Growing Cycles

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Abstract: Sweet cherry producers must deal with different climactic challenges annually, specifically the impact of spring frost and the inherent risk of fruit cracking. This susceptibility arises from the simultaneous occurrence of spring frost during the bloom stage or the sweet cherry cracking at vulnerable maturity stages in sweet cherry trees during persistent rainfall. Given the change in climatic patterns, the implementation of new strategies and innovative approaches becomes imperative to alleviate potential damage from these climatic adversities. This study aims to explore—for the first time—the effectiveness of preharvest putrescine applications during the flowering stage and ripening on-tree to increase tolerance in sweet cherry against adverse climatic events throughout its on-tree development and at the time of harvest. In this context, foliar applications of putrescine at concentrations of 1 and 10 mM were administered to distinct sweet cherry cultivars, namely, 'Prime Giant' and 'Sweetheart'. Over the course of four growing seasons, our investigation focused on evaluating the influence of this natural elicitor on the frost resilience of flower buds during the preharvest period and its impact on reducing fruit cracking in these selected cultivars. In this sense, the overall malondialdehyde content exhibited a reduction in flower buds treated with putrescine, and the fruit set experienced an increase across the majority of evaluated growing seasons. On the other hand, the incidence of sweet cherry cracking in putrescine-treated sweet cherries showed a consistent reduction in all the studied growing seasons. Our results indicate that preharvest treatments with putrescine effectively alleviate the susceptibility of flower buds to spring frost and significantly diminish fruit cracking, thereby enhancing the overall tolerance to abiotic stress. Furthermore, we evaluated different quality parameters at the time of harvest, including fruit firmness, external color, total soluble solids, and total acidity. Generally, the observed changes in these parameters were delayed in putrescine-treated fruit as compared to the control batch or remained unaffected. For this reason, the implementation of preharvest treatments based on putrescine emerges as a valuable strategy for adapting to climate change and mitigating the impact of abiotic stress, potentially increasing sweet cherry production.

Keywords: climate change; cracking; preharvest; putrescine; *Prunus avium* L.; ripening stage; spring frost

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1. Introduction

Sweet cherry production is a system highly susceptible to adverse climatic conditions. Over the past years sweet cherry growers have witnessed a substantial drop in production, largely attributed to the detrimental impact of spring frost and the occurrence of sweet cherry cracking. This decline in production serves as clear evidence of the anticipated consequences of climate change on the Mediterranean area, and adverse climate conditions become less predictable [1]. Spring frost is a recurrent challenge for sweet cherry growers, especially during the 'open cluster stage' and 'full bloom' phenological stages. Sudden temperature drops during these stages can lead to frost damage, causing reduced fruit set and significantly impacting the overall yield [2]. Climate change can also have an additional impact on sweet cherry production due to different factors, such as heavy rainfall and high humidity, which contribute to fruit cracking [3]. These environmental influences, in addition to sudden temperature changes, affect the final production. On the other hand, a multifactorial process is involved in sweet cherry cracking, but factors such as sudden rainfalls, high humidity [4,5], or the stage of ripeness at which sweet cherries are exposed to these conditions [6] play a crucial role and can lead to significant losses for producers. As a result, climate change becomes an additional challenge for sweet cherry growers, necessitating the development of innovative strategies to mitigate the impact of these abiotic events.

To prevent frost damage in sweet cherry trees, only a limited number of management approaches have been assayed. Bioregulators have been shown to delay the developmental process. In this regard, the use of aminoethoxyvinylglycine (AVG, ReTain), an ethylene inhibitor, extends ovule functionality [7]. The timing of bud break can also be modulated by methyl esters of fatty acids [8]. Additionally, seaweed extracts have increased production when applied before fruit set [9]. Other methods aim to regulate plant temperature, such as the preharvest application of coatings made from cellulose nanocrystals [10] or traditional frost protection candles [11]. Furthermore, irrigation and protective structures, such as rain covers, impact orchard conditions and may enhance resilience against frost damage [12,13]. To mitigate sweet cherry cracking, the number of strategies developed is as limited as those to prevent frost damage. Researchers have applied different preharvest technologies in sweet cherry orchards. Among these approaches are the use of protective cover structures [14,15], the application of calcium-based sprays [4,16], sodium silicate [17], and the implementation of treatments involving seaweed extracts [18]. Growth regulators, including gibberellic acid [19], glycine betaine [20], and methyl jasmonate (MeJA) [6,21], also have been shown to control sweet cherry cracking.

The preharvest application of plant-derived hormones, such as polyamines, can impact a multitude of metabolic and physiological processes in plant tissues. These processes are as diverse as the flowering, fruit set, and embryogenesis or protection against certain types of stress [22]. The application of putrescine before fruit set has been studied, and these treatments were able to maintain the number of flowers in apricot trees with satisfactory results. This was attributed to the fact that polyamines stimulate fruit sets, playing a role in floral stimulation and the formation of several organs of the flower, including the ovary development [22,23]. Putrescine, like many other polyamines, has exhibited remarkable effectiveness in delaying fruit ripening and senescence as a preharvest or postharvest strategy [24]. It helps to maintain antioxidant balance and delays tissue disintegration in climacteric [25,26] and non-climacteric fruit [27]. Additionally, as with many other elicitors, putrescine has been demonstrated to be involved in the stimulation of energy supply pathways, which is critical in energy-demanding periods, such as abiotic stress and senescence [28]. Positive effects on fruit quality have been demonstrated through the application of preharvest treatments based on putrescine in mango [29], plum [30,31], jujube [32], and pears [33] with additional cold storage tolerance benefits in this fruit [34]. This effect has been associated with an increase in bioactive compounds and antioxidant levels. Similarly, in other non-climacteric fruits, such as table grapes, the preharvest anti-senescence effects of putrescine have been observed [27] and associated with an increased antioxidant balance. However, there is no previous report in which putrescine has been evaluated to control frost tolerance or sweet cherry cracking. In this study, the main goal has been to evaluate the effectiveness of preharvest putrescine treatments for enhancing frost tolerance up to the fruit set stage and reducing fruit cracking during on-tree fruit development across four distinct growing seasons (2020–2023).

2. Materials and Methods

2.1. Plant Material and Experimental Design

Over the period from 2020 to 2023, this four-year investigation was carried out in diverse field plots situated in Alcoy (Mas de Roc Coop. Agrícola, Alcoi, Spain). Sweet cherry trees (*Prunus avium* L.) of the 'Prime Giant' and 'Sweet-heart' cultivars, grafted onto SL-64 rootstock, were utilized for the study. Different trees and orchards were selected during the 2020–2022 period, and in 2023, the same plot as in 2022 was chosen. The sweet cherry trees were subjected to consistent agronomic practices throughout the different growing seasons.

Putrescine (Sigma-Aldrich, Germany, >99% D13208) treatments (3 L per tree) were applied using a manual sprayer machine (2020, 2021, and 2023 growing cycles). In 2022, the different solutions were applied using a spraying tractor following regular agronomic practices. Freshly prepared putrescine solutions were applied at concentrations of 1 and 10 mM, with the addition of 1 mL L^{-1} of Tween 20 as a surfactant. These concentrations were proposed and increased after studying those assayed by previous authors in different species [27,33]. Control trees were treated with 3 L of distilled water containing 1 mL L^{-1} Tween 20. For every treatment and cultivar, three sets of 3 trees were utilized as replicates. Each treatment involved four spray applications during the 2020 and 2021 growing cycles at key moments or phenological stages using the BBCH scale (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie): before open cluster stage (BBCH 54), full bloom (BBCH 65), pit hardening (BBCH 77), and the beginning of color changes (BBCH 81). In 2022 and 2023, only three applications were applied, omitting the full bloom stage in order to optimize the number of applications. Only the lower concentration was applied in the 2022 and 2023 periods. Notably, 2020 and 2021 served as a screening phase for concentrations involving two sweet cherry cultivars.

For the MDA and fruit set evaluations, a total of 36 branch segments (equally divided around the tree and at opposite sides of the canopy) were labeled into three groups, with three trees of each cultivar. The evaluation of cracking incidence on the tree was carried out on four specified branches of each tree located at opposite sides of the tree, with 100 fruits evaluated for this disorder in each tree. This evaluation was assayed only for two consecutive growing seasons (2021 and 2022).

2.2. Sweet Cherry Frost Tolerance Evaluation

In March and April of 2020, during the onset of the COVID-19 pandemic, circumstances did not allow the evaluation of frost tolerance in sweet cherry trees. In 2021, 2022, and 2023, the recorded freezing temperatures in April during the flowering stage were −0.4, −0.7, and −3.4 °C, respectively. Remarkably, in 2023, the lowest temperature recorded (−3.4 °C) coincided with the 'Prime Giant' cultivar in the full bloom stage, showing no resilience against suboptimal temperature. For this reason, the fruit set was very low in this cultivar as compared with the rest of the growing seasons evaluated.

MDA content was evaluated in flower bud tissues one week after trees were treated in 2021 and 2022. Initially, a set of flower buds (BBCH 55) was taken from four different and equidistant branches located at both sides of each tree. MDA was assayed following the method of Zhang et al. [35], with some modifications. The fresh sample was homogenized in three different replicates (n = 3) of three trees per replica.

A tissue sample (1.0 g) was homogenized in a 10 mL solution of 10% trichloroacetic acid and subsequently centrifuged at 10,000× g for 10 min. Following centrifugation, 2 mL of the supernatant was combined with 6 mL of a 0.6% thiobarbituric acid solution for each duplicate and thoroughly mixed. The resulting test tubes were then subjected to a temperature of 95 °C for a duration of 20 min. Then, after cooling, they were left to temper at room temperature and measured using a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan) with absorbance measurements taken at 450, 532, and 600 nm. The calculation of MDA content was carried out according to previously established equations [35] and expressed as μmol kg^{-1} (n = 3).

The percentage of undamaged pistils was evaluated in 2022 and 2023 following the important impact of suboptimal temperatures on pistils of both cultivars at the full blossom stage (BBCH 65). One hundred flowers from each tree (taken from both tree sides) were individually checked to determine the presence of green pistils or if this tissue was affected by internal browning as a result of frost damage. Pistils affected by frost damage exhibited signs of necrosis or internal damage. The proportion of non-damaged flowers was expressed as a percentage. This percentage was obtained from three replicates of three trees ($n = 3$) and represents the proportion of flowers that could tolerate frosting conditions and potentially be functional.

Fruit set percentage was determined based on flower bud counts in four branch segments equally divided at both sides of the tree branches ($n = 3$). Flower buds that had not yet opened represented the flowers that could potentially develop into fruits and were carefully counted in each labeled branch during the last three years of this research (2021–2023). After the fruit set (BBCH 75) and before the fourth preharvest treatment (BBCH 77), the number of developed sweet cherries was counted. The percentage of fruit set was quantified as the ratio of mature sweet cherries to the previously reported number of flower buds within the corresponding branch segment.

2.3. Sweet Cherry Cracking Evaluation

In 2020, we conducted the assessment of sweet cherry cracking only at harvest. The COVID-19 pandemic circumstances did not allow the evaluation of fruit cracking during development. Subsequently, in the consecutive growing seasons of 2021, 2022, and 2023, we investigated the effect of putrescine treatments on sweet cherry cracking as the fruit developed through several ripening stages while still on the tree. The selection of these ripening stages was determined as follows: the 'Prime Giant' cultivar was coincident with the onset of color change (Stage 1), the appearance of pink color (Stage 2), bright red color (Stage 3), and dark red color (Stage 4). In the case of the 'Sweetheart' cultivar, the ripening stages at which sweet cherry cracking was studied corresponded to the stages of immature green-yellow color (Stage 1), the beginning of color change (Stage 2), pink color surface (Stage 3), bright red color (Stage 4), and dark red color (Stage 5). The evaluation of sweet cherry cracking on a tree was carried out on four specified branches of each tree, with 100 fruits evaluated for this disorder in each tree, and the results were reported as a percentage. Coincident with these measurements, we also assessed sweet cherry cracking in undamaged fruits with the traditional methodology described by different authors [21,36]. In this sense, each treatment batch comprised three replicates, each consisting of 50 fruits subjected to distilled water immersions simulating a heavy rainfall and expressed as a cracking index. In 2023, the 'Prime Giant' cultivar was not evaluated since it did not show any production after frost damage during early spring in this production cycle.

2.4. Fruit Quality Parameters at Harvest

The experiment was assayed across a four-year duration, covering the years 2020 to 2023. Sweet cherries were harvested at their respective commercial ripening stages, following commercial standards, which are primarily determined by the skin color of each different cultivar studied. Subsequently, for each replicate of 3 trees per treatment, sweet cherry lots were blended, and the resulting mixture was promptly transported to the laboratory. Then, at room temperature, three sets of 20 uniformly sized and colored fruits from each replicate and treatment in the field without apparent defects were chosen randomly. These sweet cherries were selected for the analytical assessments. Results were organized, displaying the effect of 1 mM concentration as the common and effective preharvest treatment throughout all seasons and cultivars under investigation, being also the lowest concentration assayed in this study.

For the evaluation of fruit firmness, each individual sweet cherry was measured using a TX-XT2i texture analyzer (Stable Microsystems, Godalming, UK) equipped with a flat plate probe. The descent rate of the disc was set at 20 mm min^{-1} until a 5% deformation was achieved. Fruit firmness was calculated as the ratio of the applied force to the distance traveled (N mm^{-1}).

Color measurements were conducted individually for each fruit using a Minolta colorimeter (CR-C400, Konica Minolta Camera Co.; Kanto¯, Tokyo, Japan). Two measurements were taken for each fruit at two opposing and equidistant points in the equatorial zone. These measurements were expressed in terms of CIE *hue** (arctg b^*/a^*) based on CIELab coordinates.

To evaluate the total soluble solids (TSS) and titratable acidity (TA), the flesh from 20 cherries in each replicate was homogenized to obtain a consistent sample, and approximately 50 g of this sample was passed through two layers of cotton cloth. The resulting juice was used for measurements of TSS and TA. Both parameters were evaluated in duplicate for each filtered juice extracted, as previously described [37], in each replicate per batch. TSS in sweet cherry juice was measured at 20 °C using an Atago PR-101 digital refractometer (Atago Co., Ltd., Tokyo, Japan), with results expressed as g per 100 g^{-1}. TA was also determined in each sample per duplicate through automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) and reported as g of malic acid equivalents per 100 g^{-1}.

2.5. Statistical Analysis

The experiments were conducted using a completely randomized design. Statistical analyses were performed using the SPSS package program, version 22 (IBM Corp., Armonk, NY, USA). During the 2020–2021 period, results were expressed as mean ± SE, and the data were subjected to analysis of variance (ANOVA). Mean comparisons were conducted using a multiple-range test (Tukey's HSD test) to identify significant differences ($p < 0.05$) among treatments for the same sampling date. These differences were represented for treatments on the same sampling date using lowercase letters. For the 2022–2023 results, a one-way analysis of variance was employed, and the data are presented as mean ± standard error ($n = 3$). (*) indicates significant differences between putrescine-treated and control samples, determined through Student's unpaired *t*-test; * $p < 0.05$, ** $p < 0.01$.

3. Results and Discussion

3.1. Effect of Exogenous Putrescine on Sweet Cherry Frost Tolerance: MDA, Ovary Integrity, and Fruit Set

MDA is a recognized biomarker for oxidative stress, reflecting the peroxidation of plasma membranes and its direct association with the structural integrity of plant tissues [38]. The observed levels of MDA in flower buds treated with putrescine exhibited a significant decrease ($p < 0.05$) in MDA content compared to control flower buds for both studied cultivars (Figure 1).

Although there was no dose-dependent effect in the 'Prime Giant' cultivar (Figure 1A), this effect was indeed observed in the 'Sweetheart' cultivar (Figure 1B). In this cultivar, the 10 mM putrescine concentration exhibited a significantly ($p < 0.05$) greater impact on reducing MDA levels compared to the concentration tested (1 mM). In a previous study, the application of diverse preharvest concentrations of putrescine was observed to decrease the generation of hydrogen peroxide and enhance the antioxidant activity of citrus leaves exposed to frost stress [39]. Additionally, in both growing cycles studied (2021 and 2022), putrescine applications reduced MDA content, with the putrescine treatment having a similar effect in the 2021 growing season as compared to the 2022 results. The occurrence of spring frosts is linked to considerable stress, triggering the synthesis of oxidative radicals (ROS), as reported by Sachdev et al. [40]. Cold stress dramatically increases the generation of ROS, leading to the formation of highly active radicals such as MDA. These radicals have the potential to exert detrimental effects on cellular tissues [41]; hence, the variations in MDA content among different growing seasons could be directly associated with the abiotic conditions in each growing season. The observed reduction in MDA content in putrescine-treated trees may be associated with better control of the antioxidant balance in the evaluated tissues, as reported previously [25–34].

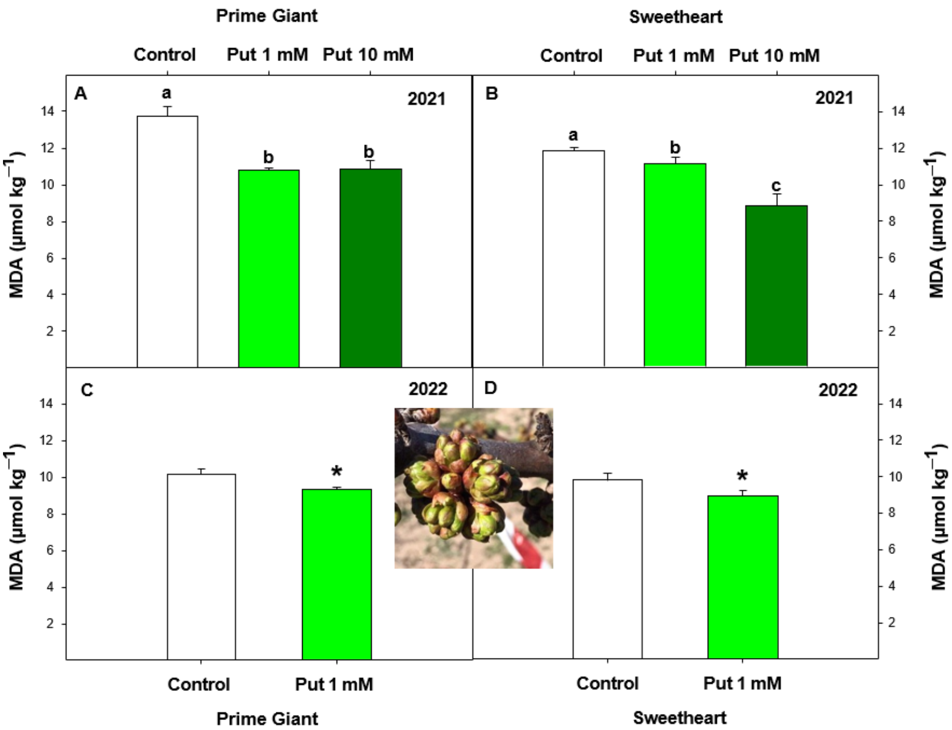

Figure 1. (**A**,**C**) MDA content (μmol kg^{-1}) in 'Prime Giant' and (**B**,**D**) 'Sweetheart' cultivars in flower buds treated with putrescine (1 and 10 mM) and control samples evaluated in 2021 and 2022. Data are the mean ± standard error ($n = 3$). In 2021, different lowercase letters indicate significant differences ($p < 0.05$) among treatments for each cultivar. In 2022 study, (*) indicates significant differences between putrescine-treated and control samples for each studied cultivar (Student's unpaired *t*-test; * $p < 0.05$).

The impact of spring frosts on the reproductive organs of sweet cherries is frequently related to internal and external irregularities that disrupt the usual fruit development process. Indeed, sweet cherry flower buds affected by spring frosts typically display a browning of the pistil [42]. In this study, only important frost events occurred in 2022 and 2023 in periods close to the full bloom stage. Spring frost dramatically affected this species, compromising the whole production in this growing season. Probably because of the important effect on pistil browning (90–98%), we did not find significant differences ($p > 0.05$) related to the exogenous preharvest putrescine treatment applied in flower buds harvested in these two different cycles (2022 and 2023) (Figure 2).

Although a higher percentage ($p > 0.05$) of green pistils was observed in putrescine-treated pistils in 2023 (Figure 2C,D), the previous growing cycle displayed a contrary pattern (Figure 2A,B). It seems that the putrescine content in flower bud tissues is related to the developmental stages, showing reduced levels of polyamines in advanced stages of development, as has been reported in other species, such as in apricot ovaries [23]. According to the authors, this decreased level contrasts with the higher levels observed during the early stages of ovary and ovule development.

The fruit set in sweet cherry depends on a combination of factors related to pollination, nutrition factors, management practices, and weather conditions [43]. Fruit set is a promising start to the development of sweet cherry fruit, but in both cultivars, fruit set was reduced across the growing cycles studied (Figure 3). This reduced fruit set could be associated with adverse effects on flower organs during consecutive growing seasons, as reported previously in this study.

In terms of fruit set percentages, significant differences were not observed ($p > 0.05$) between putrescine-treated and control 'Prime Giant' trees in the first period study (2021) (Figure 3A). However, 'Sweetheart' trees displayed significant differences ($p < 0.05$) in fruit set percentages after preharvest treatments, with a 10 mM putrescine concentration showing a higher fruit set as compared to the rest of the batches (Figure 3B). In contrast, in the subsequent 2022 growing cycle, significant differences ($p < 0.05$) were observed in fruit set percentages for both cultivars when treated with 1 mM of putrescine. In this sense, an important increase in fruit set for both cultivars under these conditions was observed (Figure 3C,D). Nevertheless, the adverse effects of spring frost in 2023 significantly diminished the fruit set percentage in both cultivars, with a particularly pronounced impact on the 'Prime Giant' cultivar (Figure 3E). These findings emphasize the substantial variability observed in fruit set percentages across different growing seasons and cultivars, as reported in previous studies [35], which is a problem in commercial production. In sweet cherry cultivation, an inadequate fruit set represents a significant physiological issue triggered by climatic factors, which has a considerable impact on yield [35,44,45]. For sweet cherries, achieving a favorable fruit-setting ratio ranging between 25% and 40% is essential for optimal production [46]. This optimal fruit setting range was only reached in 2021 (Figure 3A,B). The increase in fruit set observed by putrescine has also been observed in other studies for different species [47] and has been related to improved ovule viability [23] and fruit retention on trees [48] in other fruit species, such as apricot and pear.

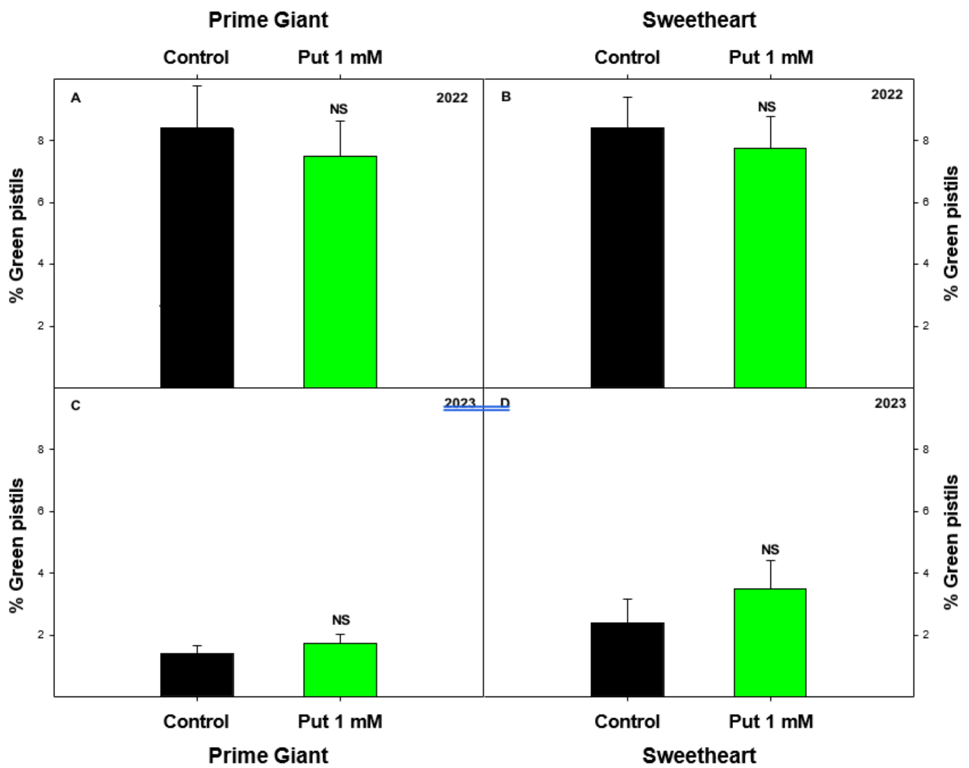

Figure 2. (**A**,**C**) Percentage of green pistils in 'Prime Giant' and (**B**,**D**) 'Sweetheart' cultivars in flower buds treated with putrescine (1 mM) and control samples evaluated in 2022 and 2023. (Student's unpaired *t*-test; NS: non-significant differences between putrescine-treated and control samples for each studied cultivar).

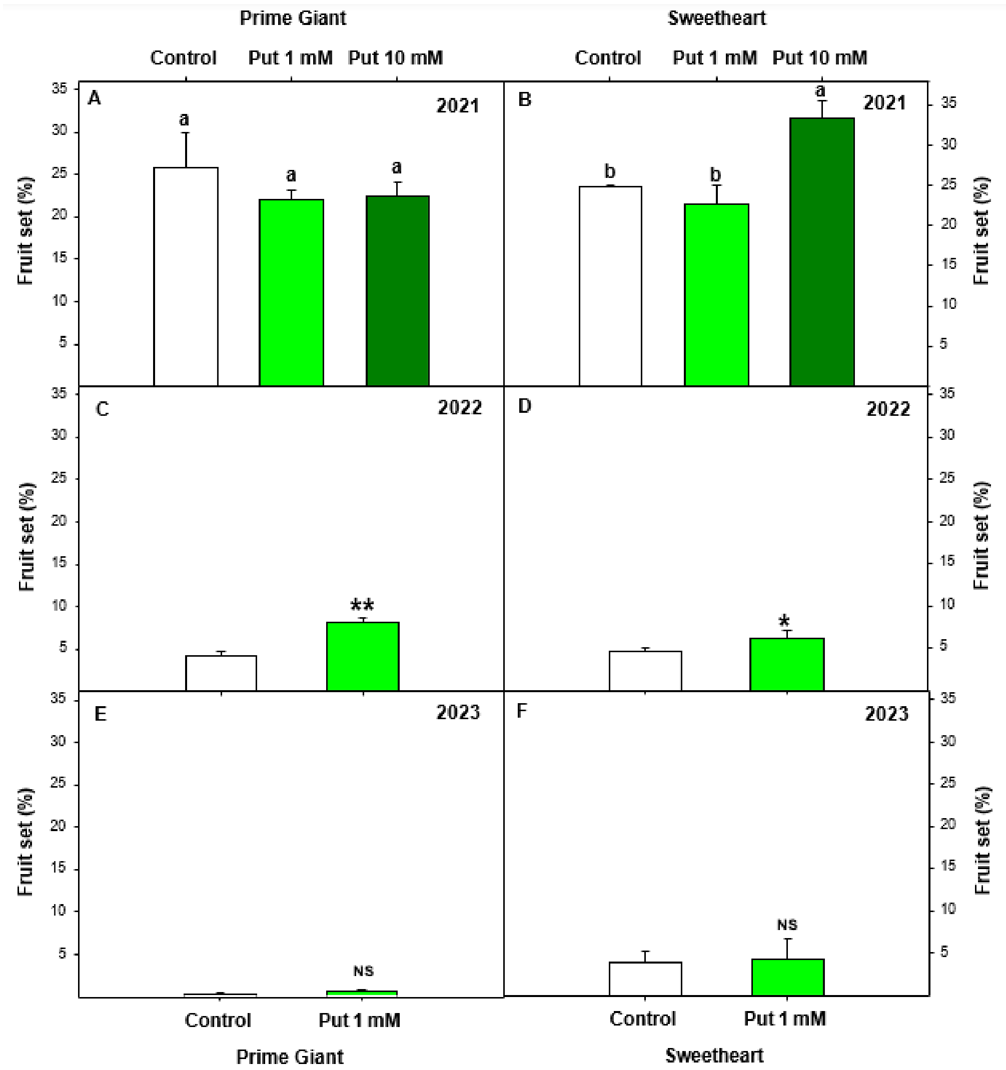

Figure 3. (**A**,**C**,**E**) Fruit set percentage in 'Prime Giant' and (**B**,**D**,**F**) 'Sweetheart' cultivars in fruit treated with putrescine (1 and 10 mM) and control samples evaluated in 2021, 2022, and 2023. In 2021, different lowercase letters indicate significant differences ($p < 0.05$) among treatments for each cultivar. In 2022 and 2023. (*) indicates significant differences between putrescine-treated and control samples for each studied cultivar (Student's unpaired *t*-test; * $p < 0.05$, ** $p < 0.01$, NS: non-significant).

3.2. Effect of Exogenous Putrescine on Sweet Cherry Cracking during Ripening on Tree

The occurrence of cracking is a significant problem for sweet cherry producers, as it has the potential to produce notable economic losses. Once the fruit undergoes cracking, its susceptibility to pests and diseases is higher, diminishing its quality and market value [49]. The factors contributing to the different cracking tolerance among cultivars are still not clear. However, it is worth noting that localized occurrences like rain cracking exert a notable influence on sweet cherry cracking [50,51]. Regarding this factor, the 'Sweetheart' cultivar was not coincident with substantial rainfall between 2020 and 2022, as it is atypical for significant precipitation to occur across the month of July, which is the usual harvest time for this cultivar. In fact, the absence of heavy rainfall in 2022 during the on-tree development, when sweet cherries are most susceptible, played a critical role in preventing cracking disorders in both cultivars (Figure 4E,F).

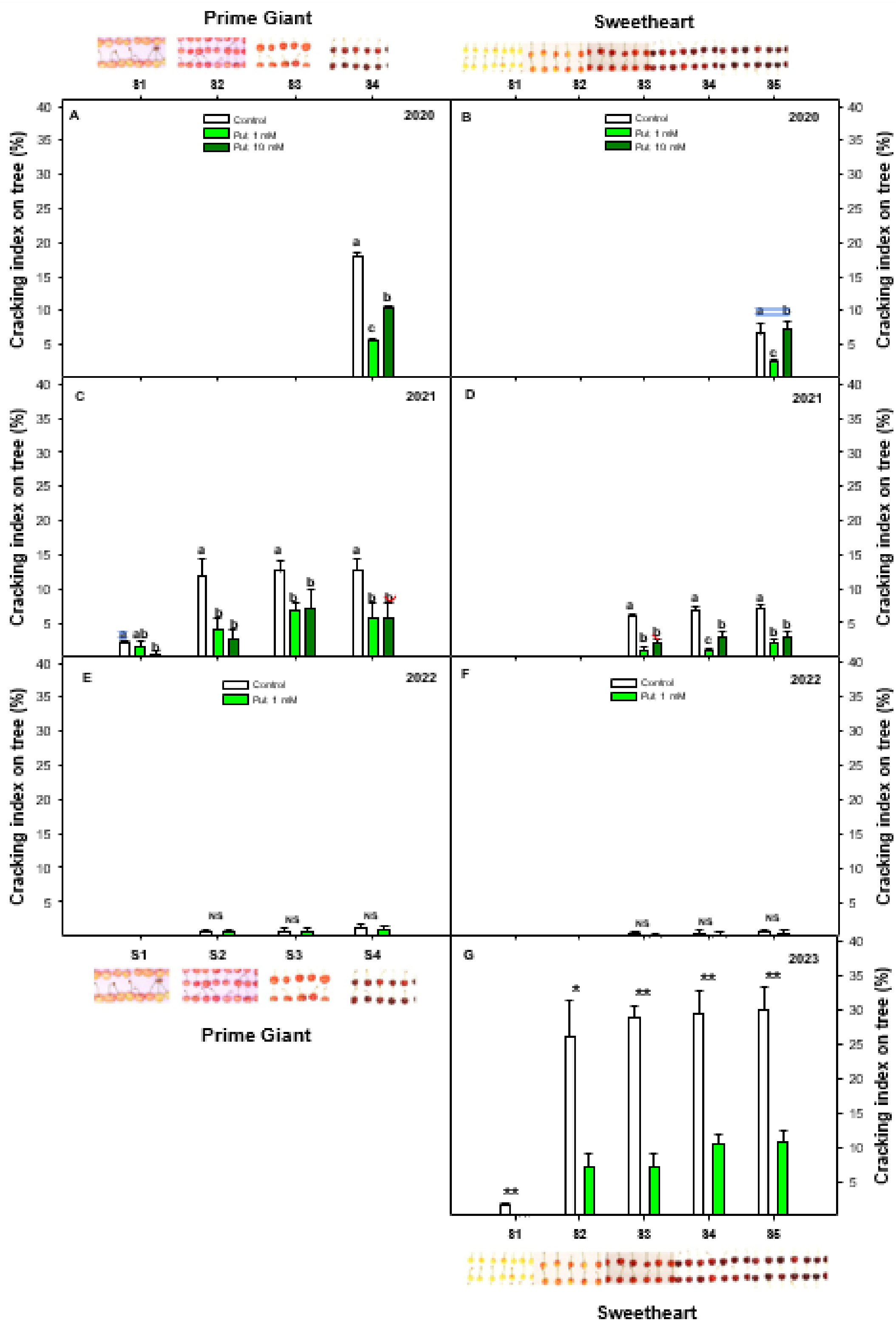

Figure 4. (**A**,**C**,**E**) Cracking incidence during development on tree (%) in 'Prime Giant' and (**B**,**D**,**F**,**G**) 'Sweetheart' cultivars in fruits treated with putrescine (1 and 10 mM) and control samples evaluated between 2020 and 2023. Data are the mean ± standard error (n = 3). In 2020 and 2021, different lowercase letters indicate significant differences (p < 0.05) among treatments for each cultivar. In 2022 and 2023, (*) indicates significant differences between putrescine-treated and control samples for each studied cultivar (Student's unpaired t-test; * p < 0.05, ** p < 0.01, NS: non-significant).

In 2023, the impact of the spring frost (Figure 3E) affected the entire 'Prime Giant' production, but we had the opportunity to evaluate 'Sweetheart' trees (Figure 4G). During this growing season, heavy rainfalls coincided with the development of this cultivar, leading to a notably high incidence of cracking, displaying the highest rate of fruit cracking among the different cultivars and growing seasons studied. In contrast, this cultivar showed the lowest incidence as compared to 'Prime Giant' during the previous growing seasons, both exposed to uncontrolled weather conditions (Figure 4). All the preharvest treatments containing putrescine successfully decreased the incidence of cracking, though they did not exhibit a dose-dependent effect in general when the putrescine concentration was increased up to 10 mM (Figure 4A–D). In the 'Prime Giant' cultivar, reductions in cracking at harvest reached 68.5% and 56.2% as compared to control trees in 2020 and 2021, respectively (Figure 4A,C). However, this effect was even more pronounced at harvest with the 'Sweetheart' cultivar, where reductions in fruit cracking of 62.1%, 71.8%, and 64.2% were observed in 2020, 2021, and 2023, respectively (Figure 4B,D,G).

Considering the distinct fruit ripening stages during on-tree development in both cultivars and their correlation with sweet cherry cracking from 2021 to 2023, the highest levels of fruit cracking were observed after the S2 developmental stage, coinciding with color changes. Significant increases in fruit cracking were not observed beyond this phenological stage during the consecutive periods studied.

Fruit cracking is a multifactorial event, and for this reason, it is very difficult to determine the specific reasons affecting the different resistance levels among distinct cultivars [50]. However, rainfall emerges as a predominant factor in the complex equation of fruit cracking, with a clear effect related to the degree of fruit ripeness coinciding with rain events [6,52,53]. Our present findings showed a reduction in sweet cherry cracking after preharvest putrescine treatments during on-tree ripening, although the impact on cracking incidence exhibits divergent patterns among the cultivars we examined. Applying putrescine, synchronized with the pit hardening stage (prior to S1), was effective in reducing cracking in the 'Prime Giant' and 'Sweetheart' cultivars, coinciding with changes in fruit color during on-tree development (S1 for 'Prime Giant' and S2 for 'Sweetheart'). On the other hand, our previous studies [6] demonstrated a strong correlation between sweet cherry fruit ripeness and susceptibility to abiotic stress conditions. In this context, the different efficacy of preharvest treatments against rain-induced cracking seems to be closely linked to the ripening stage [6,53]. Furthermore, the susceptibility to cracking appeared to be cultivar-dependent in the present study, which is consistent with earlier research findings [6,51] since cultivars with thinner skin are generally more susceptible to cracking compared to those with thicker skin.

Winkler et al. [52] have proposed that the occurrence of rain-induced cracking should not be mostly attributed to excessive water absorption through the tree roots. Instead, it appears to be triggered mainly by the time of contact with liquid water on the fruit skin. These findings elucidated the clear connection between rain cracking and the overall water balance of the fruit. For this reason, as fruit cracking on the tree appears to be directly influenced by various weather factors that can act synergistically under uncontrolled field conditions, we employed the immersion method [21,36] to evaluate the susceptibility of different cultivars at different ripening stages. In this sense, when healthy sweet cherries were exposed to water immersions, both cultivars showed a lower resilience to fruit cracking under controlled conditions (Figure 5) as compared to the cracking results obtained under weather conditions (Figure 4). However, on-tree cracking evaluations and cracking induced by immersions showed a similar pattern between them. The 'Prime Giant' cultivar exhibited the highest incidence of sweet cherry cracking in 2020 (Figure 5A) at the harvest stage. Notably, the application of putrescine (1 mM) resulted in a significant reduction ($p < 0.05$) in fruit cracking for both cultivars. Our results highlight the most pronounced effect in the 'Prime Giant' cultivar in 2022 and the 'Sweetheart' cultivar in 2020, 2021, and 2022, demonstrating the highest reduction in fruit cracking (around 75% in all these growing cycles) as compared to control fruit at the time of harvest (Figure 5B,D–F). There are no studies examining the effects of putrescine on the occurrence of sweet cherry cracking at harvest or during development. However, a similar effect was observed when it was evaluated on different fruit species as a preharvest treatment. In this sense, putrescine was able to reduce fruit cracking at harvest in litchi [54], and fruit drop in peach [55] increased fruit yield, as also observed in table grapes [56].

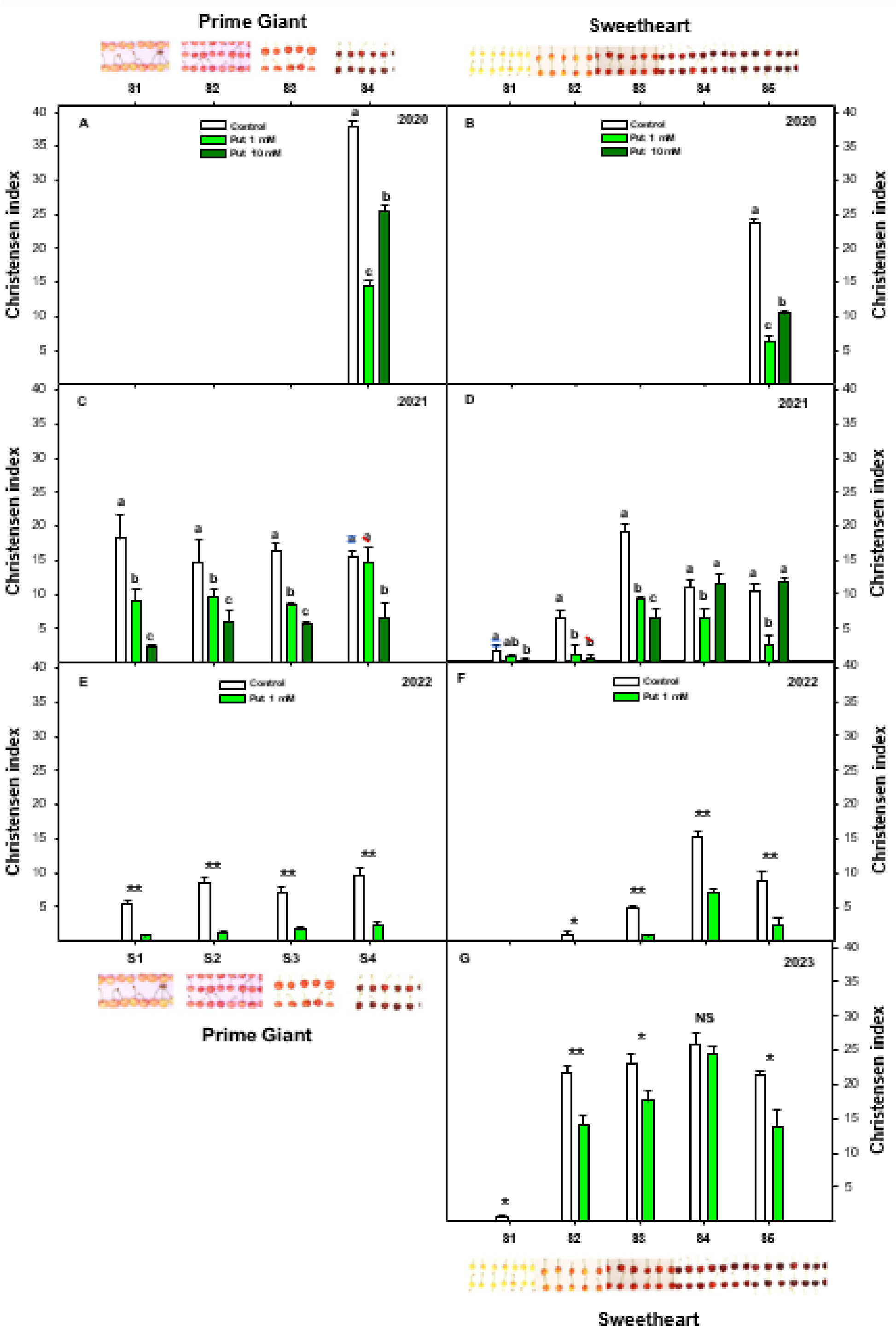

Figure 5. (**A**,**C**,**E**) Christensen index during development on tree in 'Prime Giant' and (**B**,**D**,**F**,**G**) 'Sweetheart' cultivars in fruit treated with putrescine (1 and 10 mM) and control samples evaluated between 2020 and 2023. Data are the mean ± standard error (n = 3). In 2020 and 2021, different lowercase letters indicate significant differences ($p < 0.05$) among treatments for each cultivar. In 2022 and 2023, (*) indicates significant differences between putrescine-treated and control samples for each studied cultivar (Student's unpaired t-test; * $p < 0.05$, ** $p < 0.01$, NS: non-significant).

It is worth noting that the 'Sweetheart' and 'Prime Giant' trees displayed a similar susceptibility to fruit cracking on trees in both cultivars. However, in practice, this equivalence is not frequently observed, primarily since 'Prime Giant' ripening starts earlier than 'Sweetheart,' thus more advanced ripening stages in 'Prime Giant' are coincident with the spring rainfall period. In this sense, immersion studies (Figure 5) have also allowed us to confirm that in both cultivars, sensitivity to cracking was similar, reaching its maximum at the beginning of the color changes in coincidence with the cracking counts on-tree (Figure 4). In the 'Sweetheart' cultivar, the earliest ripening stages (S1 and S2) or the most advanced ripening stages (S5) exhibit greater tolerance compared to ripening stages S3 or S4, in which the fruit adopts a uniform reddish color. However, in 'Prime Giant', resilience to fruit cracking remains similar after color changes during ripening on-tree (S1–S4). This observation matched our previous findings conducted on these same cultivars [6]. Consequently, controlled conditions through immersions in distilled water revealed that early and late stages of ripening exhibited reduced susceptibility to cracking in 'Sweetheart'. Coincidently, Faizy et al. [53] also did not observe a significantly higher cracking in control fruit after delaying harvest for a week in the '0900 Ziraat' cultivar.

In the 'Prime Giant' cultivar, the effects of putrescine preharvest treatments on fruit cracking were quite effective. In 2021, the 1 mM putrescine treatments resulted in a significant reduction of 50.5% and 33.5% in fruit cracking for the S1 and S2 ripening stages, respectively (Figure 5C). However, the treatments showed even greater effectiveness in 2022, with cracking reduced by 85.2% for the S1 stage and 86.04% for the S2 stage (Figure 5E). On the other hand, in the 'Sweetheart' cultivar, the putrescine treatments also demonstrated their effectiveness in 2021, leading to a 52.1% reduction in fruit cracking for the S3 stage and a 46.81% reduction for the S4 stage (Figure 5D). The following year, in 2022, the effectiveness of these treatments became even more pronounced, resulting in a remarkable 83.34% reduction in fruit cracking for the S3 stage and a 52.63% reduction for the S4 stage (Figure 5F). Hence, the impact of putrescine on reducing cracking was positive for both cultivars across consecutive growing seasons and ripening stages in this research.

It is important to highlight that the fruit's ripeness level, which may coincide with environmental stress factors such as heavy rainfalls during its maturation on the tree, will change from year to year. This fact is highly significant because, during cherry ripening, several intrinsic attributes undergo changes, such as fruit firmness, TSS, and the volume of the fruit [57]. These attributes are closely related to susceptibility to sweet cherry cracking [4,19,58]. For this reason, differences in ethylene production on-tree [5] and the level of firmness [21], along with reduced levels of soluble solids and acidity, may play a role in diminishing the fruit's vulnerability to cracking [59]. Sweet cherries tend to experience a lower occurrence of cracking when parenchyma cells in the epidermis and the hypodermis undergo an enlargement process during the later stages of ripening. This highlights the importance of the flexibility and integrity of the fruit skin to mitigate fruit cracking [4,60]. Consequently, the delayed fruit ripening will also impact the cracking incidence of sweet cherries, especially under the influence of persistent rainfalls, and may also be postponed. Furthermore, the vulnerability to cracking in sweet cherries is highly cultivar-dependent [51], and the impact of putrescine fruit quality parameters can be dependent on agronomical practices, weather conditions, species, and cultivars [22,52], and the number of applications. In this context, a comparable effect of putrescine was observed with four putrescine applications in both the 2020 and 2021 growing seasons and with three on-tree applications in 2022 and 2023.

3.3. Effect of the Preharvest Treatment with Putrescine on the Sweet Cherry Quality at Harvest

In the present study, different quality attributes were positively affected after putrescine preharvest treatments. In this regard, putrescine delayed on-tree fruit firmness evolution in both Prime Giant and Sweetheart cultivars across all growing seasons, displaying higher values at harvest across four consecutive growing cycles. However, statistical significance ($p < 0.05$) was observed only in specific periods evaluated (Table 1).

Table 1. The effect of the preharvest treatment with putrescine 1 mM on the physical and chemical quality of sweet cherries at harvest.

Fruit Firmness (N mm^{-1})			
Cultivar	Year	Control	Put 1 mM
'Prime Giant'	2020	1.82 ± 0.09	1.88 ± 0.09 ^{NS}
'Prime Giant'	2021	1.51 ± 0.07	1.66 ± 0.09 *
'Prime Giant'	2022	1.18 ± 0.05	1.20 ± 0.06 ^{NS}
'Sweetheart'	2020	2.08 ± 0.10	2.09 ± 0.10 ^{NS}
'Sweetheart'	2021	1.78 ± 0.09	2.03 ± 0.04 *
'Sweetheart'	2022	1.69 ± 0.07	1.80 ± 0.09 ^{NS}
'Sweetheart'	2023	1.89 ± 0.05	2.20 ± 0.09 *
Fruit color (CIE h°)			
Cultivar	Year	Control	Put 1 mM
'Prime Giant'	2020	21.15 ± 0.80	23.29 ± 0.97 *
'Prime Giant'	2021	13.36 ± 0.30	15.66 ± 0.34 **
'Prime Giant'	2022	23.37 ± 0.31	22.91 ± 0.43 ^{NS}
'Sweetheart'	2020	17.57 ± 0.58	18.63 ± 0.91 ^{NS}
'Sweetheart'	2021	20.65 ± 0.19	20.37 ± 0.16 ^{NS}
'Sweetheart'	2022	19.27 ± 0.25	18.77 ± 0.35 ^{NS}
'Sweetheart'	2023	19.22 ± 0.47	18.48 ± 0.56 ^{NS}
Total soluble solids (g 100 g^{-1})			
Cultivar	Year	Control	Put 1 mM
'Prime Giant'	2020	18.45 ± 0.12	18.18 ± 0.34 ^{NS}
'Prime Giant'	2021	17.76 ± 0.18	18.20 ± 0.54 ^{NS}
'Prime Giant'	2022	22.75 ± 0.41	23.01 ± 0.59 ^{NS}
'Sweetheart'	2020	22.42 ± 0.07	21.80 ± 0.23 *
'Sweetheart'	2021	18.90 ± 0.16	19.26 ± 0.29 ^{NS}
'Sweetheart'	2022	22.65 ± 0.44	23.07 ± 0.11 ^{NS}
'Sweetheart'	2023	21.56 ± 0.20	21.08 ± 0.04 *
Titratable acidity (g 100 g^{-1})			
Cultivar	Year	Control	Put 1 mM
'Prime Giant'	2020	1.00 ± 0.04	1.07 ± 0.06 ^{NS}
'Prime Giant'	2021	1.09 ± 0.01	1.10 ± 0.01 ^{NS}
'Prime Giant'	2022	1.58 ± 0.01	1.53 ± 0.02 ^{NS}
'Sweetheart'	2020	1.22 ± 0.02	1.36 ± 0.02 *
'Sweetheart'	2021	1.33 ± 0.02	1.43 ± 0.02 *
'Sweetheart'	2022	1.44 ± 0.03	1.60 ± 0.08 *
'Sweetheart'	2023	1.98 ± 0.08	1.89 ± 0.09 ^{NS}

Data are the mean ± standard error (n = 3). (*) indicates significant differences between putrescine-treated and control samples for each studied cultivar (Student's unpaired t-test; * $p < 0.05$, ** $p < 0.01$, NS: non-significant).

In 2021, there was a significant delay ($p < 0.05$) in fruit firmness at harvest in different growing seasons for 'Prime Giant' and 'Sweetheart' cultivars and in 2023 also for 'Sweetheart'. These findings were also reported in plums with a suppressed-climacteric genotype, such as the 'Golden Japan' plum, and in non-climacteric fruit, such as table grapes [27,31]. In these studies, similar concentrations of putrescine (1 and 3 mM) demonstrated greater efficacy as compared to lower concentrations (0.1 mM). In different climacteric fruits, such as 'Angelino' plums, jujube, and pear fruit [30,32,33], higher fruit firmness was reported in putrescine-treated fruit after preharvest treatments with similar concentrations (1–3) mM than the concentration applied in the present research study. Conversely, postharvest treatments with putrescine have demonstrated efficacy in delaying fruit firmness in various fruit species, including lemons, apricots, and plums, during cold storage [24]. The enhancement of firmness due to polyamines was attributed to their effect in cross-linking pectic molecules within the cell wall. This cross-linking process leads to an immediate solidification, as observed during postharvest storage [61], providing an inhibitory effect on wall-degrading enzymes. Additionally, polyamines maintain cell bio-membranes, protecting phospholipids and proteins from peroxidation [62]. This inhibition serves to mitigate fruit softening during storage, as documented by different authors [24,61,63].

As with fruit firmness, color development was significantly delayed ($p < 0.05$) in the 'Prime Giant' cultivar at harvest time in the 2020 and 2021 seasons. In mango, 'Golden Japan' plum, and jujube fruit [29,31,32], the preharvest putrescine effect displayed a delayed color at harvest, which was related to a delayed chlorophyll senescence. In fact, putrescine has been related to the maintenance of thylakoid membranes through the storage period [64]. The observed delay in color development has been noted not only at harvest time but also at postharvest in different fruit species, also increasing cold tolerance against chill- ing injury [26,65]. However, this delayed pattern of color evolution was not observed ($p > 0.05$) in the 'Sweetheart' cultivar. In a previous study, Mirdheghan and Rahimi [27] observed the same discrepancy between two different table grape cultivars after preharvest putrescine treatments. It seems that color could play a dynamic effect related to the cultivar studied, and for this reason, this effect deserves further investigation across other sweet cherry cultivars.

The preharvest treatment with 1 mM putrescine did not lead to a significant impact ($p > 0.05$) on the 'Prime Giant' cultivar at harvest concerning sugar accumulation and TA content as compared to control batches. However, a significant effect ($p < 0.05$) was observed for the 'Sweetheart' cultivar, resulting in a delay in TSS accumulation in putrescine-treated batches during two different growing seasons (2020 and 2023). On the other hand, no effect on delaying sugar accumulation at harvest was noted in 2021 and 2022 for this cultivar. Furthermore, the efficacy of putrescine in maintaining higher and significant ($p < 0.05$) TA levels (≈10%) in contrast with control fruit at harvest in most of the growing seasons studied for the 'Sweetheart' cultivar. In table grapes, 1 mM putrescine concentrations delayed sugar accumulation at harvest for the 'Rishbaba' cultivar, although not significantly in the 'Olhoghi' cultivar [27]. Nevertheless, a higher concentration (2 mM) effectively delayed ripening at harvest in both table grape cultivars, delaying sugar accumulation and with higher TA as compared to control batches. On the contrary, higher TSS accumulation after preharvest putrescine treatments was observed in peaches [66]. With respect to TA levels, Shanbehpour et al., [32] did not find any putrescine-related effect in this parameter in different jujube fruit genotypes, although a higher ascorbic acid level was reported in putrescine-treated batches. Additionally, though the putrescine effect at 1 mM or 2 mM was weak at harvest in different table grape cultivars and in pear fruit [27,33], delayed TSS and TA evolution during postharvest storage was evident in most of the reported studies [27,29,30,32,33,66]. Fruits treated with polyamines have exhibited an elevated starch content attributed to a lower starch hydrolysis to sugar and a reduced respiration maintaining organic acid content [26,32,66]. The observed differences among authors, genotypes, and applied concentrations could result from variations in the number of applications during sweet cherry ripening on trees, the ripening stage at harvest, and a cultivar-dependent effect in the mentioned studies.

4. Conclusions

Preharvest treatments with putrescine could effectively enhance frost tolerance, mitigating stress and consequently increasing fruit set in both 'Prime Giant' and 'Sweetheart' cultivars. This naturally occurring compound increased frost tolerance, reducing MDA content and increasing fruit set, also reducing fruit cracking during preharvest and at harvest. Earlier ripening stages and medium ripening stages during preharvest were found to be more susceptible to the occurrence of fruit cracking in 'Prime Giant' and 'Sweetheart', respectively. Furthermore, putrescine preharvest treatments delayed fruit ripening on the tree, resulting in higher fruit firmness and delayed color development at harvest. The content of soluble solids and acidity at harvest time was only delayed in the 'Sweetheart' cultivar following putrescine treatments. The overall results suggest that putrescine preharvest treatments could serve as a valuable tool to mitigate production losses, reducing the impact of weather-related stress on trees during fruit evolution. Additionally, it contributes to enhancing fruit quality at harvest.

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References

1. Fernandez, E.; Mojahid, H.; Fadón, E.; Egea, J.A.; Mimoun, M.B.; Kodad, O.; El Yaacoubi, A.; Ghrab, M.; Egea, J.; Benmoussa, H.; et al. Climate change impacts on winter chill in Mediterranean temperate fruit orchards. *Reg. Environ. Chang.* **2023**, *23*, 7. [CrossRef]
2. Kaya, O.; Kose, C. Sensitivity of some sweet cherry (*Prunus avium* L.) cultivars to late spring frosts during different phenological stages following bud burst. *Theor. Appl. Climatol.* **2022**, *148*, 1713–1725. [CrossRef]
3. Huang, W.; Gao, Z.; Wang, L.; Zhang, Y.; Fu, X. Advances in research on cherry fruit cracking: Causes, prevention and control. *Front. Plant Sci.* **2019**, *10*, 986.
4. Correia, S.; Schouten, R.; Silva, A.P.; Gonçalves, B. Sweet cherry fruit cracking mechanisms and prevention strategies: A review. *Sci. Hortic.* **2018**, *240*, 369–377. [CrossRef]
5. Knoche, M.; Peschel, S. Water on the surface aggravates microscopic cracking of the sweet cherry fruit cuticle. *J. Am. Soc. Hortic. Sci.* **2006**, *131*, 192–200. [CrossRef]
6. Ruiz-Aracil, M.C.; Valverde, J.M.; Lorente-Mento, J.M.; Carrión-Antolí, A.; Castillo, S.; Martínez-Romero, D.; Guillén, F. Cracking during development on the tree and at harvest sweet cherry (*Prunus avium* L.): The impact of methyl jasmonate on four different growing seasons. *Agriculture* **2023**, *13*, 1244. [CrossRef]
7. Bound, S.A.; Close, D.C.; Jones, J.E.; Whiting, M.D. Improving fruit set of 'Kordia' and 'Regina' sweet cherry with AVG. *Acta Hortic.* **2014**, *1042*, 285–292. [CrossRef]
8. Xu, H.; Ediger, D.; Sharifi, M. Horticultural practices in early spring to mitigate the adverse effect of low temperature on fruit set in 'Lapins' sweet cherry. *Plants* **2023**, *12*, 468. [CrossRef]
9. Ureta-Ovalle, A.; Atenas, C.; Larraín, P. Application of an *Ecklonia maxima* seaweed product at two different timings can improve the fruit set and yield in 'Bing' sweet cherry trees. *Acta Hortic.* **2019**, *1235*, 319–326. [CrossRef]
10. Alhamid, J.O.; Mo, C.; Zhang, X.; Wang, P.; Whiting, M.D.; Zhang, Q. Cellulose nanocrystals reduce cold damage to reproductive buds in fruit crops. *Biosyst. Eng.* **2018**, *172*, 124–133. [CrossRef]
11. Zago, M.; Ropelato, E.; Gobber, M.; Franchini, S. Defence against late frost with frost protection candles. *Laimburg J.* **2005**, *2*, 58–61.
12. Feldmane, D. Effects of irrigation and woodchip mulch on growth and habit of sour cherries. In Proceedings of the Annual 15th International Scientific Conference Research for Rural Development, Aguascalientes, Mexico, 18–21 June 2009; pp. 64–70.

13. Salvadores, Y.; Bastías, R.M. Environmental factors and physiological responses of sweet cherry production under protective cover systems: A review. *Chilean J. Agric. Res.* **2023**, *83*, 484–498. [CrossRef]
14. Pino, S.; Palma, M.; Sepúlveda, Á.; Sánchez-Contreras, J.; Moya, M.; Yuri, J.A. Effect of rain cover on tree physiology and fruit condition and quality of 'Rainier', 'Bing' and 'Sweetheart' sweet cherry trees. *Horticulturae* **2023**, *9*, 109. [CrossRef]
15. Blanco, V.; Zoffoli, J.P.; Ayala, M. High tunnel cultivation of sweet cherry (*Prunus avium* L.): Physiological and production variables. *Sci. Hortic.* **2019**, *251*, 108–117. [CrossRef]
16. Matteo, M.; Zoffoli, J.P.; Ayala, M. Calcium sprays and crop load reduction increase fruit quality and postharvest storage in sweet cherry (*Prunus avium* L.). *Agronomy* **2022**, *12*, 829. [CrossRef]
17. Rombolà, A.; Quartieri, M.; Rodríguez-Declet, A.; Minnocci, A.; Sebastiani, L.; Sorrenti, G. Canopy-applied silicon is an effective strategy for reducing sweet cherry cracking. *Hortic. Environ. Biotechnol.* **2023**, *64*, 371–378. [CrossRef]
18. Correia, S.; Oliveira, I.; Queirós, F.; Ribeiro, C.; Ferreira, L.; Luzio, A.; Silva, A.P.; Gonçalves, B. Preharvest application of seaweed based biostimulant reduced cherry (*Prunus avium* L.) cracking. *Procedia Environ. Sci.* **2015**, *29*, 251–252. [CrossRef]
19. Usenik, V.; Kastelec, D.; Štampar, F. Physicochemical changes of sweet cherry fruits related to application of gibberellic acid. *Food Chem.* **2005**, *90*, 663–671. [CrossRef]
20. Correia, S.; Queirós, F.; Ferreira, H.; Morais, M.C.; Afonso, S.; Silva, A.P.; Gonçalves, B. Foliar application of calcium and growth regulators modulate sweet cherry (*Prunus avium* L.) tree performance. *Plants* **2020**, *9*, 410. [CrossRef]
21. Balbontín, C.; Gutiérrez, C.; Wolff, M.; Figueroa, C.R. Effect of abscisic acid and methyl jasmonate preharvest applications on fruit quality and cracking tolerance of sweet cherry. *Chil. J. Agric. Res.* **2018**, *78*, 438–446. [CrossRef]
22. Kamiab, F.; Tavassolian, I.; Hosseinifarahi, M. Biologia futura: The role of polyamine in plant science. *Biol. Futur.* **2020**, *71*, 183–194. [CrossRef] [PubMed]
23. Alburquerque, N.; Egea, J.; Burgos, L.; Martinez-Romero, D.; Valero, D.; Serrano, M. The influence of polyamines on apricot ovary development and fruit set. *Ann. Appl. Biol.* **2006**, *149*, 27–33. [CrossRef]
24. Valero, D.; Romero, M.D.; Serrano, M. The role of polyamines in the improvement of shelf life of fruit. *Trends Food Sci. Technol.* **2002**, *13*, 228–234. [CrossRef]
25. Martinez-Romero, D.; Serrano, M.; Carbonell, A.; Burgos, L.; Riquelme, F.; Valero, D. Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. *J. Food Sci.* **2002**, *67*, 1706–1712. [CrossRef]
26. Sharma, S.; Pareek, S.; Sagar, N.A.; Valero, D.; Serrano, M. Modulatory effects of exogenously applied polyamines on postharvest physiology, antioxidant system and shelf life of fruits: A review. *Int. J. Mol. Sci.* **2017**, *18*, 1789. [CrossRef] [PubMed]
27. Mirdehghan, S.H.; Rahimi, S. Pre-harvest application of polyamines enhances antioxidants and table grape (*Vitis vinifera* L.) quality during postharvest period. *Food Chem.* **2016**, *196*, 1040–1047. [CrossRef] [PubMed]
28. Aghdam, M.S.; Jannatizadeh, A.; Luo, Z.; Paliyath, G. Ensuring sufficient intracellular ATP supplying and friendly extracellular ATP signaling attenuates stresses, delays senescence, and maintains quality in horticultural crops during postharvest life. *Trends Food Sci. Technol.* **2018**, *76*, 67–81. [CrossRef]
29. Malik, A.; Singh, Z. Pre-storage application of polyamines improves shelf-life and fruit quality of mango. *J. Hortic. Sci. Biotechnol.* **2005**, *80*, 363–369. [CrossRef]
30. Khan, A.S.; Singh, Z.; Abbasi, N.A. Pre-storage putrescine application suppresses ethylene biosynthesis and retards fruit softening during low temperature storage in 'Angelino' plum. *Postharvest Biol. Technol.* **2007**, *46*, 36–46. [CrossRef]
31. Shaaban, F.K.M.; El-Hadidy, G.A.M.; Mahmoud, T.S.M. Effects of salicylic acid, putrescine and moringa leaf extract application on storability, quality attributes and bioactive compounds of plum cv. 'Golden Japan'. *Future Food J.* **2020**, *8*, 1–14.
32. Shanbehpour, F.; Rastegar, S.; Ghasemi, M. Effect of preharvest application of calcium chloride, putrescine, and salicylic acid on antioxidant system and biochemical changes of two Indian jujube genotypes. *J. Food Biochem.* **2020**, *44*, e13474. [CrossRef] [PubMed]
33. Singh, V.; Jawandha, S.K.; Gill, P.S.; Singh, D. Preharvest putrescine application extends the shelf life and maintains the pear fruit quality. *Int. J. Fruit Sci.* **2022**, *22*, 514–524. [CrossRef]
34. Singh, V.; Jawandha, S.K.; Gill, P.S.; Kaur, R. Pre-storage putrescine treatment alleviates internal browning of stored pear fruit by regulating enzyme activity. *Erwerbs-Obstbau* **2023**, *65*, 1291–1301. [CrossRef]
35. Zhang, M.; Liu, W.; Li, C.; Shao, T.; Jiang, X.; Zhao, H.; Ai, W. Postharvest hot water dipping and hot water forced convection treatments alleviate chilling injury for zucchini fruit during cold storage. *Sci. Hortic.* **2019**, *249*, 219–227. [CrossRef]
36. Christensen, J.V. Rain-induced cracking of sweet cherries: Its causes and prevention. In *Cherries: Crop Physiology, Production and Uses*; Webster, A.D., Looney, N.E., Eds.; CAB International: Wallingford, UK, 2006; pp. 297–327.
37. Carrión-Antolí, A.; Martínez-Romero, D.; Guillén, F.; Zapata, P.J.; Serrano, M.; Valero, D. Melatonin pre-harvest treatments leads to maintenance of sweet cherry quality during storage by increasing antioxidant systems. *Front. Plant Sci.* **2022**, *13*, 984. [CrossRef] [PubMed]
38. Chen, G.-Q.; Ren, L.; Zhang, J.; Reed, B.M.; Zhang, D.; Shen, X.-H. Cryopreservation affects ROS-induced oxidative stress and antioxidant response in Arabidopsis seedlings. *Cryobiology* **2015**, *70*, 38–47. [CrossRef]
39. Mohammadrezakhani, S.; Rezanejad, F.; Hajilou, J. Effect of putrescine and proline on proflies of GABA, antioxidant activities in leaves of three *Citrus* species in response to low temperature stress. *J. Plant Biochem. Biotechnol.* **2021**, *30*, 545–553. [CrossRef]
40. Sachdev, S.; Ansari, S.A.; Ansari, M.I.; Fujita, M. Abiotic Stress and Reactive Oxygen Species: Generation. *Antioxidants* **2021**, *10*, 277. [CrossRef]

41. Giné-Bordonaba, J.; Echeverria, G.; Ubach, D.; Aguiló-Aguayo, I.; López, M.L.; Larrigaudière, C. Biochemical and physiological changes during fruit development and ripening of two sweet cherry varieties with different levels of cracking tolerance. *Plant Physiol. Biochem.* **2017**, *111*, 216–225. [CrossRef]
42. Matzneller, P.; Götz, K.P.; Chmielewski, F.M. Spring frost vulnerability of sweet cherries under controlled conditions. *Int. J. Biometeorol.* **2016**, *60*, 123–130. [CrossRef]
43. Beppu, K.; Kataoka, I. Studies on pistil doubling and fruit set of sweet cherry in warm climate. *J. Jpn. Soc. Hortic. Sci.* **2011**, *80*, 1–13. [CrossRef]
44. Cline, J.A.; Meland, M.; Sekse, L.; Webster, A.D. Rain cracking of sweet cherries: II. Influence of rain covers and rootstocks on cracking and fruit quality. *Acta Agric. Scand.* **1995**, *45*, 224–230. [CrossRef]
45. Imrak, B.; Sarier, A.; Kuden, A.; Kuden, A.B.; Comlekcioglu, S.; Tutuncu, M. Studies on shading system in sweet cherries (*Prunus avium* L.) to prevent double fruit formation under subtropical climatic conditions. *Acta Hortic.* **2014**, *1059*, 171–176. [CrossRef]
46. Rutkowski, K.; Łysiak, G.P. Thinning Methods to Regulate Sweet Cherry Crops—A Review. *Appl. Sci.* **2022**, *12*, 1280. [CrossRef]
47. Sabir, I.A.; Liu, X.; Jiu, S.; Whiting, M.; Zhang, C. Plant Growth regulators modify fruit set, fruit quality, and return bloom in sweet cherry. *HortScience* **2021**, *56*, 922–931. [CrossRef]
48. Sayyad-Amin, P.; Davarynejad, G.; Abedi, B. Effects of Preharvest Application of Chemical Compounds on Pear (*Pyrus communis* cv. 'Shekari') Fruit Traits. *Erwerbs-Obstbau* **2022**, *64*, 657–662. [CrossRef]
49. Wani, A.A.; Singh, P.; Gul, K.; Wani, M.H.; Langowski, H.C. Sweet cherry (*Prunus avium*): Critical factors affecting the composition and shelf life. *Food Packag. Shelf Life* **2014**, *1*, 86–99. [CrossRef]
50. Blažek, J.; Zelený, L.; Suran, P. Sweet cherry research world overview 2015–2017. *Hortic. Sci.* **2022**, *49*, 121–146. [CrossRef]
51. Quero-García, J.; Letourmy, P.; Campoy, J.A.; Branchereau, C.; Malchev, S.; Barreneche, T.; Dirlewanger, E. Multi-year analyses on three populations reveal the first stable QTLs for tolerance to rain-induced fruit cracking in sweet cherry (*Prunus avium* L.). *Hortic. Res.* **2021**, *8*, 136. [CrossRef]
52. Winkler, A.; Blumenberg, I.; Schürmann, L.; Knoche, M. Rain cracking in sweet cherries is caused by surface wetness, not by water uptake. *Sci. Hortic.* **2020**, *269*, 10940023. [CrossRef]
53. Faizy, A.H.; Ozturk, B.; Aglar, E.; Yıldız, K. Role of methyl jasmonate application regime on fruit quality and bioactive compounds of sweet cherry at harvest and during cold storage. *J. Food Process.* **2021**, *45*, e15882. [CrossRef]
54. Brijwal, M.; Dimri, D.C.; Mishra, D.S. Effect of foliar application of Ga3, putrescine and boric acid on yield and physical characteristics of litchi cultivars. *Ecol. Environ. Conserv.* **2017**, *23*, 7–12.
55. Torrigiani, P.; Bregoli, A.M.; Ziosi, V.; Scaranagli, S.; Ciriaci, T.; Rasori, A.; Biondi, S.; Costa, G. Pre-harvest polyamine and aminoethoxyvinylglycine (AVG) applications modulate fruit ripening in Stark Red Gold nectarines (*Prunus persica* L. Batsch). *Postharvest Biol. Technol.* **2004**, *33*, 293–308. [CrossRef]
56. Marzouk, H.A.; Kassem, H.A. Improving yield, quality, and shelf life of Thompson seedless grapevine by preharvest foliar applications. *Sci. Hortic.* **2011**, *130*, 425–430. [CrossRef]
57. Serrano, M.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Valero, D. Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *J. Agric. Food Chem.* **2005**, *53*, 2741–2745. [CrossRef] [PubMed]
58. Demirsoy, L.; Bilgener, S. The effects of preharvest calcium hydroxide applications on cracking in '0900 Ziraat', 'Lambert' and 'Van' sweet cherries. *Acta Hortic.* **1998**, *468*, 657–662. [CrossRef]
59. Pereira, S.; Silva, V.; Bacelar, E.; Guedes, F.; Silva, A.P.; Ribeiro, C.; Gonçalves, B. Cracking in sweet cherry cultivars Early Bigi and Lapins: Correlation with quality attributes. *Plants* **2020**, *9*, 1557. [CrossRef] [PubMed]
60. Brüggenwirth, M.; Knoche, M. Mechanical properties of skins of sweet cherry fruit of differing susceptibilities to cracking. *J. Am. Soc. Hortic. Sci.* **2016**, *141*, 162–168. [CrossRef]
61. Valero, D.; Serrano, M. *Postharvest Biology and Technology for Preserving Fruit Quality*; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2010; p. 138.
62. Du, H.; Liu, D.; Liu, G.; Liu, H.; Kurtenbach, R. Polyamines conjugated to the bio-membranes and membrane conformations are involved in the melatonin-mediated resistance of harvested plum fruit to cold stress. *Postharvest Biol. Technol.* **2023**, *204*, 112480. [CrossRef]
63. Ranjbar, S.; Rahemi, M.; Ramezanian, A. Comparison of nano-calcium and calcium chloride spray on postharvest quality and cell wall enzymes activity in apple cv. Red Delicious. *Sci. Hortic.* **2018**, *240*, 57–64. [CrossRef]
64. Beigbeder, A.; Vavadakis, M.; Navakoudis, E.; Kotzabasis, K. Influence of polyamine inhibitors on light-independent and light-dependent chlorophyll biosynthesis and on the photosynthetic rate. *J. Photochem. Photobiol. B* **1995**, *28*, 235–242. [CrossRef]
65. Serrano, M.; Zapata, P.J.; Martínez-Romero, D.; Díaz-Mula, H.M.; Valero, D. Polyamines as an ecofriendly postharvest tool to maintain fruit quality. In *Eco-Friendly Technology for Postharvest Produce Quality*; Siddiqui, M.W., Ed.; Academic Press-Elsevier: London, UK, 2016; pp. 219–242.
66. Singh, V.; Jawandha, S.K.; Gill, P.P.S.; Gill, M.S. Suppression of fruit softening and extension of shelf life of pear by putrescine application. *Sci. Hortic.* **2019**, *256*, 108623. [CrossRef]

4.2. Publicación II (Transcripción literal)

II

Enhancing sweet cherry resilience to spring frost and rain-induced cracking with pre-harvest melatonin treatments

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Enhancing sweet cherry resilience to spring frost and rain induced cracking with pre-harvest melatonin treatments

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A B S T R A C T

Sweet cherrySweet cherry producers annually confront climatic challenges such as spring frost and fruit cracking. This vulnerability arises primarily from spring frost during bloom or cracking at critical maturity stages during persistent rainfall. With changing climate patterns, innovative strategies are essential to mitigate these adversities. Foliar applications of melatonin (MT) at 0, 0.01, 0.05 and 0.1 mM were tested on the 'Prime Giant' and 'Sweetheart' cultivars over four different production cycles (2020–2023) to evaluate the effect on frost resilience on flower buds and fruit cracking reduction. MT-treated flower buds showed reduced malondialdehyde content and increased fruit set in most seasons, reducing their vulnerability to extreme weather events. In addition, MT consistently decreased sweet cherry cracking incidence across all studied seasons, indicating a strong effect between the fruit ripening stage and susceptibility to fruit cracking, which was cultivar dependent. Quality parameters at harvest, including fruit firmness, and colour at harvest, were either delayed or unaffected in MT-treated fruits compared with controls. However, other ripening parameters were stimulated by pre-harvest MT applications in several ~~growing~~ cycles, such as total soluble solids, which slightly reduced total acidity in MT-treated fruits. In summary, pre-harvest MT treatments can be a promising strategy for climate change adaptation and stress mitigation, potentially increasing sweet cherry production under extreme weather conditions.

1. Introduction

Frost events and fruit cracking negatively affect the productivity and quality of horticultural crops, such as sweet cherries and many other plant species. Crops are highly exposed to adverse and unpredictable weather events that become increasingly intense and frequent because of climate change [1]. In the Mediterranean area, these events already affect sweet cherries and other fruit species [2], and greater thermal fluctuations are expected in the future [3]. In this context, frost events disrupt cellular structures, leading to desiccation and cell death, resulting in substantial crop damage or loss. To increase frost tolerance, several strategies have been evaluated in sweet cherries, including traditional management (wind generators, sprinkler irrigation, and frost protection candles) and cover structures [4,5]. Conversely, breeding cultivars that adapt to local weather conditions [6] and the application of commercial substances that affect ethylene production, thus delaying phenotypical development, have been previously reported [3,7,8]. Plant-based substances such as cellulose nano-crystals [9] used for flower bud protection as a pre-harvest coating and putrescine have also been evaluated as having significant positive effects [10]. The lack of alternatives to control frost damage in sweet cherries is as extensive as the lack of methods to control fruit cracking. Fruit cracking in sweet cherries is influenced by several factors, including structural, climatic, nutritional and agronomic factors. Structurally, the elasticity and integrity of fruit skin and cuticles play a critical role, with low elasticity and micro-cracks increasing susceptibility [11]. For these reasons, susceptibility to cracking is not similar between different cultivars (different skin thicknesses, cuticular characteristics and physiological conditions), but this physiopathy can affect the entire crop, up to 100 % in cultivars such as 'Van' or 'Prime Giant'[12,13]. Climatic factors such as excessive rainfall and high humidity near harvest time significantly contribute to cracking, and sudden temperature changes can cause the fruit skin to shrink and crack [14,15]. In this context, fruit cracking is primarily induced by rapid water uptake, creating internal pressure

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within the fruit that leads to skin splitting. This phenomenon is stimulated by weather events such as rainfall and high humidity during the ripening period [14,15]. Calcium deficiency weakens cell walls, increasing cracking susceptibility, and calcium sprays can help in mitigating this, although they may result in smaller fruits and are sometimes ineffective when applied alone or combined with gibberellic acid (GA_3) [16,17]. In this context, regarding plant regulators such as GA3 and abscisic acid (ABA) applications, although some studies have shown their effectiveness [18–20], many others have not [21]. These contradictory results are probably due to the impact of application timing, the cultivar studied, and the applied concentrations. In addition, plant natural elicitors, such as methyl jasmonate and putrescine have shown to significantly reduce sweet cherry cracking in different cultivars [10,13,19]. Conversely, the choice of rootstock, pruning practices, affect water uptake. For this reason, irrigation and crop management are crucial factors to reduce fruit cracking [22]. In this context, larger, firmer fruits with deeper stem cavities are more susceptible to fruit cracking, and a high sugar content drives osmotic water uptake, increasing the fruit cracking risk [21]. Other mitigation methods include using plastic rain covers, although they may increase humidity and temperature [23–25]. For this reason, strategies such us the use of anti-transpirants to limit water uptake with edible coatings [26] and reducing rapidly the water contact on fruit surfaces after rainfall with helicopters have shown positive results reducing cracking [27]. New approaches such as developing genetically resistant cultivars, in-field sensing technologies for rainwater removal, and biostimulants, are promising strategies for reducing fruit cracking.

Melatonin (N-acetyl-5-methoxytryptamine, MT) is a natural elicitor and an endogenously produced indoleamine found in all plant species. MT is known for its health benefits as a dietary component provided by many fruits and vegetables, and its content is particularly high in cherries (***Prunus cerasus*** L.) but with a moderate content in sweet cherry cultivars (***Prunus avium*** L.) [28]. MT content is naturally reduced from the full bloom phenotypical stage until the end of sweet cherry fruit development [28,29]. MT acts as an elicitor that boosts the plant defence system against a wide range of biotic and abiotic stressors, and acts as a potent antioxidant, neutralising free radicals effectively [30,31]. Probably, some of these effects were related to the increase in quality attributes observed when MT was applied as a pre-harvest treatment for this plant species. In this context, MT-treated sweet cherry trees have shown increased production yields [32] and higher fruit quality at harvest [33] and during storage [34]. Although different technologies have attempted to mitigate fruit cracking or frost incidence with different effects, in this study we have evaluated the potential of MT as a common treatment to increase frost resilience and reduce sweet cherry cracking. Given the previously reported positive effects of MT on crop management and post-harvest storage, the aim of this study is to investigate whether MT can also serve as a control measure against adverse climatic conditions, which are becoming more frequent because of climate change.

2. Experimental design

2.1. Plant material and experimental design

From 2020–2023, a comprehensive four-year study was conducted across various field plots in Alcoy (Mas de Roc Coop. Agrícola, Alcoi, Spain). In this study, sweet cherry trees (***Prunus avium*** L.) of the 'Prime Giant' and 'Sweetheart' cultivars grafted onto SL-64 rootstock were evaluated. Different trees and orchard plots were used in 2020 and 2021. In 2022, a different plot was selected, which was then used again in 2023. The sweet cherry trees were subjected to uniform agronomic practices throughout the growing seasons. MT (Sigma-Aldrich, Germany, >98 % M5250) treatments (3 L per tree) were administered using a manual sprayer during the 2020–2023 growing seasons. Fresh MT solutions were prepared at concentrations of 0.01, 0.05 and 0.1 mM, with 1 mL L^{-1} of Tween 20 added as a surfactant. These concentrations were determined based on our previous studies [32]. The control trees were treated with 3 L of distilled water containing 1 mL L^{-1} Tween 20. For each treatment and cultivar, three sets of three trees were used as replicates. Each treatment involved four spray applications during the 2020 and 2021 growing seasons at critical phenological stages identified using the Biologische Bundesantalt, Bundessortenamt und Chemische Industrie (BBCH) scale: before the open cluster stage (BBCH 54), full bloom (BBCH 65), pit hardening (BBCH 77) and the onset of colour changes (BBCH 81). In 2022 and 2023, only three applications were performed, excluding the full bloom stage to optimise the number of applications. Only the lowest concentration was applied during the 2022 and 2023 periods.

For the evaluation of malondialdehyde (MDA) levels and fruit set, a total of 36 branch segments (equally distributed around the tree and on opposite sides of the canopy) were divided into three groups, with three trees of each cultivar. The assessment of cracking incidence on the tree was conducted on four specific branches of each tree, located on opposite sides, with 100 fruits evaluated for this disorder in each tree. These evaluations were performed only during two consecutive growing seasons (2021 and 2022).

1.1. 2.2. Sweet cherry frost tolerance evaluation

In March and April 2020, at the start of the COVID-19 pandemic, it was not possible to assess frost tolerance in different orchards. In the following growing seasons under study (2021–2023), the freezing temperatures recorded in April during the flowering stage were -0.4°C, -0.7°C, and -3.4°C, respectively. In 2023, when the 'Prime Giant' cultivar was at full bloom, the lowest temperature (-3.4°C) occurred, and this cultivar did not show resilience to this freezing condition. Consequently, the fruit set of this cultivar was significantly lower than that of all previous production cycles evaluated. In 2021 and 2022, MDA content was measured in flower bud tissues (one week after the first MT application). Then, flower buds at the BBCH 55 stage were collected from four equidistant branches on opposite sides of each tree. MDA was determined using the protocol described by Zhang et al. [35] with minor modifications. Fresh samples were homogenised in triplicate (n = 3) with three trees per replicate. A 1.0 g tissue sample was homogenised in 10 mL of 10 % trichloroacetic acid solution and centrifuged at 10,000 × g for 10 min. After centrifugation, 2 mL of the supernatant was mixed with 6 mL of 0.6 % thiobarbituric acid solution for each replicate and thoroughly mixed. The test tubes were then heated to 95°C for 20 min. After cooling to room temperature, absorbance was measured using a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan) at 450, 532 and 600 nm. MDA content was calculated using established equations and expressed as μmol kg^{-1} (n = 3).

Green pistil percentage was assessed in 2022 and 2023 because of significant impacts of low temperatures on pistils during the full blossom stage (BBCH 65). One hundred flowers from each tree (50 flowers evaluated from each side of the tree) were examined to determine whether the pistils were undamaged or affected by internal browning due to frost. Frost-damaged pistils showed signs of necrosis or internal damage. The percentage of green pistils was calculated based on three replicates of three trees (n = 3). Flower buds with green pistils were those that could potentially develop into commercial fruits. The fruit set percentage was determined by counting flower buds on four branch segments equally divided on both sides of the tree branches (n = 3). Flower buds that had not opened were counted in each labelled branch over the last three years 2021, 2022 and 2023. After fruit set (BBCH 75) and before the last pre-harvest application (BBCH 77), the quantity of mature sweet cherries for each branch segment was scored. The fruit set percentage was determined by calculating the ratio of fully developed sweet cherries to the initial count of flower buds on the studied branch segment.

2.3. Evaluation of sweet cherry cracking on trees and after water immersions

In 2020, we only assessed sweet cherry cracking at harvest time because the COVID-19 pandemic prevented evaluations during several critical months of fruit development. In the following production cycles (2021–2023), we reported the impact of MT treatments on sweet cherry cracking at specific developmental stages. The different ripening moments chosen for the different cracking evaluations were previously described by Ruiz-Aracil et al. [10] for the 'Prime Giant' and 'Sweetheart' cultivars. In this context, the different ripening stages (S) evaluated were identified as follows for the 'Prime Giant' cultivar: the onset of colour changes (S1), the appearance of a pink colour (S2), a bright red colour (S3) and a dark red colour (S4). For the 'Sweetheart' cultivar, evaluations were conducted for immature green–yellow fruit (S1), the beginning of fruit colour changes (S2), a pink coloured surface (S3), bright red colour (S4) and dark red colour (S5). In these specific development phases sweet cherry cracking on the tree was evaluated by studying four specific branches of each tree, with 100 fruits assessed for this disorder per tree, and the results were reported as percentages. In addition to these observations, we examined fruit cracking in intact and healthy fruits using the water-soaking method described by Christensen [36]. Each treatment group of trees had three replicates, with 50 fruits per replicate, which were soaked in distilled water to simulate heavy rainfall conditions, and the values obtained were recorded as a cracking index. The 'Prime Giant' cultivar was excluded from the 2023 report because its fruit production was not sufficiently high to be evaluated because of the frost damage experienced in early spring of that year.

2.4. Evaluation of fruit quality at harvest time

Over a four-year period from 2020 to 2023, an experiment at harvest was conducted. Sweet cherries were collected at their respective commercial ripening stages, which were determined primarily by the skin colour characteristic of each cultivar studied. For each treatment replicate consisting of three trees, the harvested cherries were combined into lots and quickly transported to the laboratory. At room temperature, three sets of 20 uniformly sized and coloured fruits from each field replicate and treatment without any visible defects were randomly selected for analytical assessments. The results were homogenised to show the impact of a 0.01 mM MT concentration, the common and effective pre-harvest treatment used across all seasons and cultivars in this study, which was also the lowest concentration tested. To measure fruit firmness, each sweet cherry was evaluated using a TX-XT2i texture analyser (Stable Microsystems, Godalming, UK) equipped with a flat-plate probe. The disc descent rate was set to 20 mm min^{-1} until achieving 5 % deformation. Firmness was calculated as the ratio of the applied force to the distance travelled (N mm^{-1}). Colour measurements for each fruit were conducted individually using a Minolta colourimeter (CR-C400, Konica Minolta Camera Co., Kantō, Tokyo, Japan). Two readings were taken for each fruit at two opposite and equidistant points on the equatorial zone, expressed as CIE *hue** (arctg b^*/a^*) based on the CIELab coordinates. To determine total soluble solids (TSS) and titratable acidity (TA), the flesh from 20 cherries in each replicate was homogenised to obtain a uniform sample. Approximately 50 g of this homogenate was filtered through two layers of cotton cloth. The resulting juice was used for TSS and TA measurements, which were performed in duplicate for each filtered juice sample from each replicate batch. TSS was measured at 20°C using an Atago PR-101 digital refractometer (Atago Co., Ltd., Tokyo, Japan), and the results were expressed as g per 100 g^{-1}. TA was determined through automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) and reported as g of malic acid equivalents per 100 g^{-1}.

2.5. Statistical analysis

Statistical analyses were carried out utilizing the SPSS software, version 22 (IBM Corp., Armonk, NY, USA). During 2020–2021, data were reported as the mean ± SE, and subjected to analysis of variance (ANOVA). To discern significant differences ($p < 0.05$) among treat- ments on identical sampling dates, mean comparisons were evaluated via Tukey's HSD test. These differences were reported by lowercase letters in each treatment for the same sampling date. For the 2022–2023 timeframe, data were evaluated using a one-way ANOVA, with results presented as mean ± standard error (n = 3). Significant differences between MT-treated and control samples were marked by (*) and identified using Student's unpaired *t*-test; * $p < 0.05$, ** $p < 0.01$.

3. Results

3.1. Impact of pre-harvest MT application on frost resistance in sweet cherries: MDA levels, ovary health and fruit set

Pre-harvest foliar application of MT at all tested concentrations reduced MDA medium levels in flower buds and was generally significant ($p < 0.05$) for most of the cultivars and concentrations evaluated, indicating a reduction in frost-induced oxidative stress (Fig. 1). These results were confirmed in two different growing seasons for the two evaluated cultivars. Although a dose-dependent trend was not generally observed, the highest MT concentration had a stronger effect but only in the 'Sweetheart' cultivar.

Significant frost events were reported in 2022 and 2023, occurring during periods near the full bloom stage. These spring frosts had a severe impact on sweet cherry trees, dramatically reducing the entire crop yield during these growing seasons. Because of the substantial impact on pistil browning (90 %–98 %), no significant differences ($p > 0.05$) were observed in relation to the exogenous pre-harvest MT treatment applied to flower buds evaluated during these two growing cycles (2022 and 2023) (Fig. 2). However, the fruit set results obtained in our study dis- played a general positive pattern in MT-treated trees in both cultivars, with a stronger effect for 'Sweetheart' (Fig. 3). For this cultivar, in all consecutive years studied, fruit set medium values were higher in MT- treated trees than in control trees, displaying significant differences in 2021 and 2022 ($p < 0.05$ and $p < 0.01$, respectively). Conversely, for the 'Prime Giant' cultivar, higher medium values were observed in MT- treated trees during 2021 and 2022, being only significant ($p < 0.05$) in 2022. Fruit set was reduced across the consecutive years studied. Nevertheless, the adverse effects of spring frost in 2023 significantly decreased the fruit set percentage in both cultivars, with a particularly pronounced impact on the 'Prime Giant' cultivar (Fig. 3E).

3.2. Impact of pre-harvest MT applications on sweet cherry cracking during on-tree ripening

Sweet cherry cracking can be directly related to rainfall events, but the 'Prime Giant' cultivar did not experience substantial rainfall in 2022 demonstrating the differences between production cycles due to the unpredictability of weather events, which are more frequent because of climate change. Similarly, the 'Sweetheart' cultivar was not affected by significant precipitation between 2020 and 2022 because it is unusual for heavy rain to occur in July, the typical harvest month for this cultivar, and 'Prime Giant' is an early season cultivar. The lack of heavy rainfall in 2022, particularly during the critical period of on-tree development when sweet cherries are most vulnerable, was crucial in preventing cracking issues in both cultivars (Fig. 4E, F). The evaluations of sweet cherry cracking on the tree in production cycles in which cracking was assessed during cherry ripening (2021–2023) displayed a stronger incidence from the phenotypic stage S2 for both cultivars. This cracking incidence remained constant from S2 until harvest time (Fig. 4C–G). MT applied at pre-harvest with a lower concentration

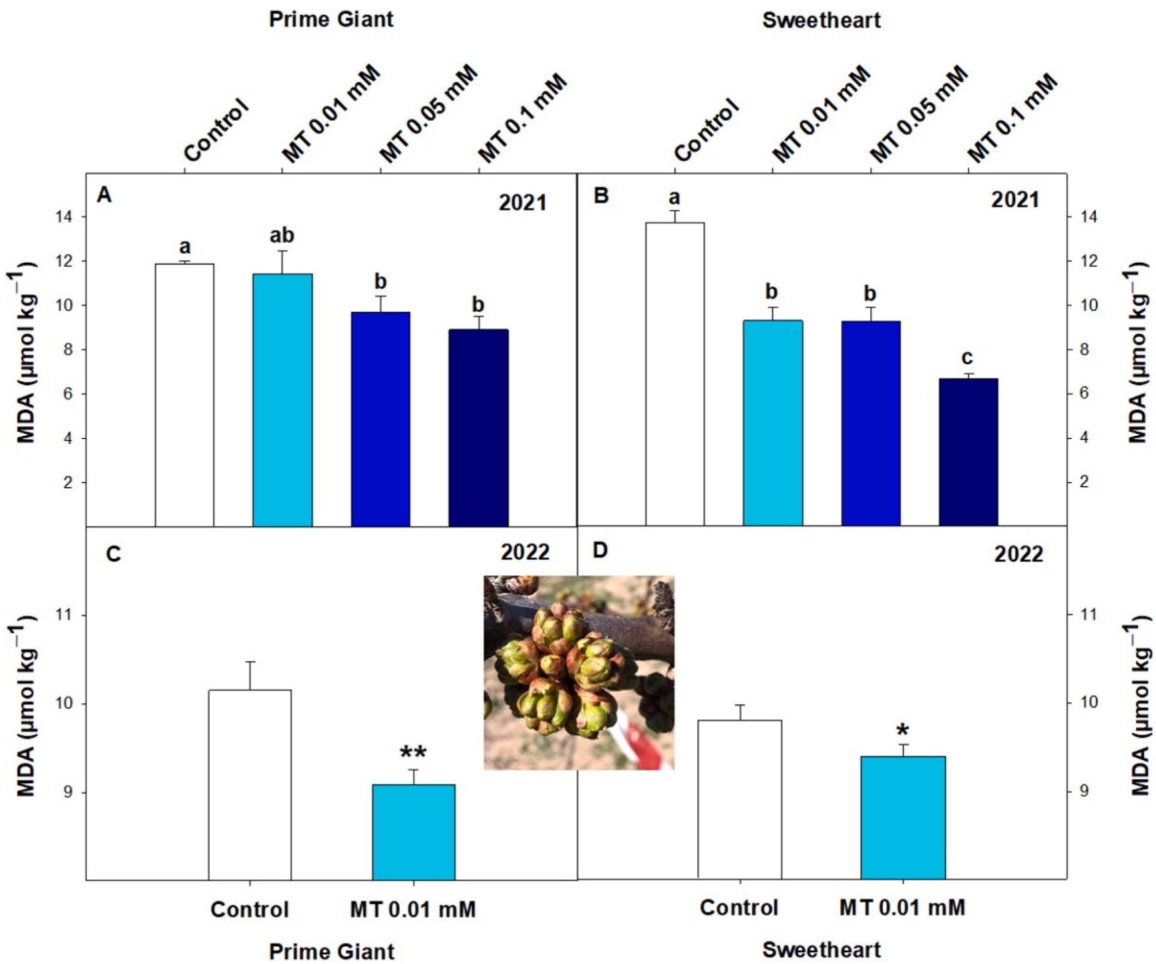

Fig. 1. (A, C) MDA content (μmol kg⁻¹) in the 'Prime Giant' cultivar and (B, D) in the 'Sweetheart' cultivar within flower buds treated with melatonin (0.01, 0.05, and 0.1 mM) compared to control samples in 2021 and 2022. The data represent the mean ± standard error (n=3). In 2021, different lowercase letters indicate significant differences ($p < 0.05$) among treatments within each cultivar. In the 2022 study, significant differences between melatonin-treated and control samples for each cultivar are marked with an asterisk (Student's unpaired *t*-test; *$p < 0.05$, **$p < 0.01$, *NS*: non-significant).

assayed (0.01 mM) significantly reduced the incidence of fruit cracking from the beginning of sweet cherry ripening on trees (Fig. 4) and increasing MT concentrations up to 0.1 mM did not show a general increased pattern for cracking resilience (Fig. 4A–D). For the 'Prime Giant' cultivar, the reductions in fruit cracking on the trees at harvest were 20.4 % and 92.2 % in 2020 and 2021, respectively, compared with the control trees (Fig. 4A, C). For the 'Sweetheart' cultivar, the re- ductions in fruit cracking were 69.7 %, 46.5 % and 88.4 % in MT-treated trees as observed in 2020, 2021 and 2023, respectively (Fig. 4B, D and G). Conversely, the 'Sweetheart' cultivar exhibited higher fruit cracking incidence during the later ripening stages but showed increased resilience closer to harvest time (Fig. 5). Pre-harvest MT applications were generally effective in mitigating sweet cherry cracking at all ripening stages and in both cultivars across nearly all consecutive years studied. One of the greatest effects of MT at the lowest concentration (0.01 mM) on reducing sweet cherry cracking at harvest was observed for both cultivars in 2020 (Fig. 5A and B), with reductions of 59.45 % and 81.5 % for the 'Prime Giant' and 'Sweetheart' cultivars, respectively. In 2023, similar tolerance was observed for 'Sweetheart' (Fig. 5G). Pre-harvest MT applications were reduced from four to three in 2022 and 2023. In this context, four applications in 2020 and 2021 (Fig. 5A and C) showed a stronger effect on the 'Prime Giant' cultivar at harvest than in the three applications in 2022 (Fig. 5E). However, in 'Sweetheart', the results were more consistent despite the number of applications because the resilience effect of MT remained similar across the consecutive years under study.

3.3. Impact of pre-harvest MT applications on sweet cherry quality traits at harvest

The effect of MT on sweet cherry quality characteristics at harvest, such as fruit firmness, was evaluated in the 'Prime Giant' and 'Sweetheart' cultivars over four consecutive years (Table 1). Our results indicated that the effect of MT on sweet cherry firmness might depend on specific factors related to the year and cultivar evaluated. MT treatments in some cases significantly ($p < 0.05$) promoted higher fruit firmness values, whereas in others, no significant effect was observed. In this context, we can conclude that in general, the MT treatment did not result in a significant ($p < 0.05$) decrease in firmness compared with the control fruit. Conversely, the colour of sweet cherry skin is an essential organoleptic parameter that significantly influences consumer purchase decisions. Our results indicated that the application of 0.01 mM MT had varying effects on fruit colour (CIE ***hue***°) across different years and cultivars. For the 'Prime Giant' and 'Sweetheart' cultivars, significant ($p < 0.05$) higher values compared with control fruits were found at harvest in 2021, but no significant changes were observed in other growing seasons. Overall, the effect of 0.01 mM MT on fruit colour either did not affect the colour or delayed its development.

Conversely, the MT effect on TSS and TA did not have a uniform impact on either total soluble solids or titratable acidity across the consecutive years studied for both cultivars. Overall, mean TSS values in MT-treated fruits showed a delay in most of the growing cycles studied in both cultivars, although significant accumulation of this parameter

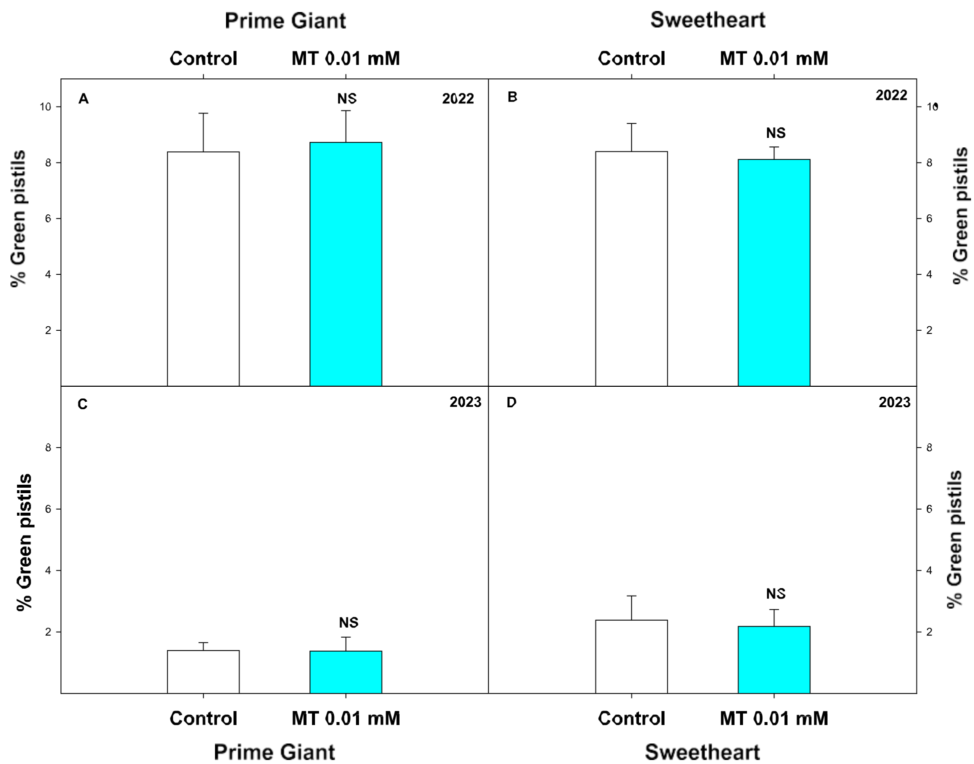

Fig. 2. (A, C) Percentage of green pistils in the 'Prime Giant' cultivar and (B, D) in the 'Sweetheart' cultivar within flower buds treated with melatonin (0.01, 0.05, and 0.1 mM) compared to control samples in 2022 and 2023 growing cycles. Significant differences between melatonin-treated and control samples for each cultivar are marked with an asterisk (Student's unpaired *t*-test; $*p < 0.05$, $**p < 0.01$, *NS*: non-significant).

was observed in 2020 for both the 'Prime Giant' and 'Sweetheart' cultivars. Conversely, with respect to total acidity, different effects were observed depending on the cultivar studied. Although TA accumulation in 'Prime Giant' was delayed by MT treatments compared with that in the control fruit, in the 'Sweetheart' cultivar, a higher TA level was observed in most of the growing cycles studied, although there was a delay in TA accumulation in 2020.

4. Discussion

During flower development, the frost tolerance of the flower buds continuously decreases [37]. The effect of spring frost on sweet cherry flower buds is associated with significant stress that triggers the synthesis of reactive oxygen species (ROS) [38]. In this context, MDA is one of the main reactive radicals, and MDA content is recognised as an indirect measure to evaluate the peroxidation of plasma membranes, which is directly related to the structural integrity of plant tissues. Cold stress dramatically increases ROS generation, leading to the formation of MDA as the main product when free radicals oxidise lipids in cell membranes [39]. These radicals can exert detrimental effects on cellular tissues [40]. Hence, variations in MDA content among different growing seasons can be directly associated with the abiotic conditions in each growing season. The observed reduction in MDA content in MT-treated trees may be associated with better control of the antioxidant balance in the evaluated tissues managed by MT, as we previously reported for sweet cherries [34], thereby protecting flower organs [31].

Spring frost also increases the percentage of damaged pistils, decreasing fruit set. A damaged pistil can be visually detected because the pistil is affected by browning [41]. As other authors reported, if cold damage affects more than 50 % of flower pistils, the tree cannot provide an optimal crop because, in general, only 20 %–30 % of sweet cherry flowers develop into fruits in some cultivars studied [37,42]. In this context, fruit set in sweet cherry trees depends on several key factors: flower bud density, the number of flowers per bud, the drop rate of flower buds, pollination success and climatic conditions during flowering [42,43].

The fruit set of sweet cherries is significantly affected by climatic conditions, which is a substantial physiological challenge affecting overall yield [10,44]. To achieve optimal production, it is crucial for sweet cherries to maintain a fruit-setting ratio within the range of 25 %–40 % [45]. This optimal fruit-setting range was only reached in 2021 for both cultivars studied (Fig. 3A, B). The increase in fruit set attributed to MT application was also documented in previous studies involving several fruit species, such as tomatoes and pomegranates [46,47]. This effect has been linked to the ability of MT to protect plant tissues, such as cotyledons, seedlings and reproductive organs, from environmental stressors [31]. In this context, these results were in agreement with those observed regarding the MDA content in flower buds, in which while in 2022 the MT effect was significant ($p < 0.05$) in 2021 was only significant ($p < 0.05$) for the 'Sweetheart' cultivar displaying a relation between both parameters.

Rain-induced cracking in sweet cherries is a significant global issue. Pre-harvest rainfall can cause cherries to crack, which severely reduces yield and leads to considerable economic losses for farmers.

This physiopathy is due to multi-factorial factors but is stimulated by two primary factors: excessive water uptake by the fruit's skin, which leads to internal pressure and cracking, and the adhesion of water on the fruit surface, which exacerbates micro-cracks into larger visible ones [26,48]. Different cultivars exhibit different levels of resilience against fruit cracking, which are influenced by their genetic and specific physical characteristics [49]. For instance, some cultivars are naturally more resistant because of thicker skin, better elasticity or more consistent cellular structures [21]. The present on-tree studies (2020–2022) of the 'Sweetheart' and 'Prime Giant' cultivars have shown different tolerance to cracking, with 'Prime Giant' being more vulnerable to environmental conditions each year. However, climatic conditions and cracking factors may act synergistically in unexpected combinations. Consequently, an optimal methodology for assessing cracking tolerance across different

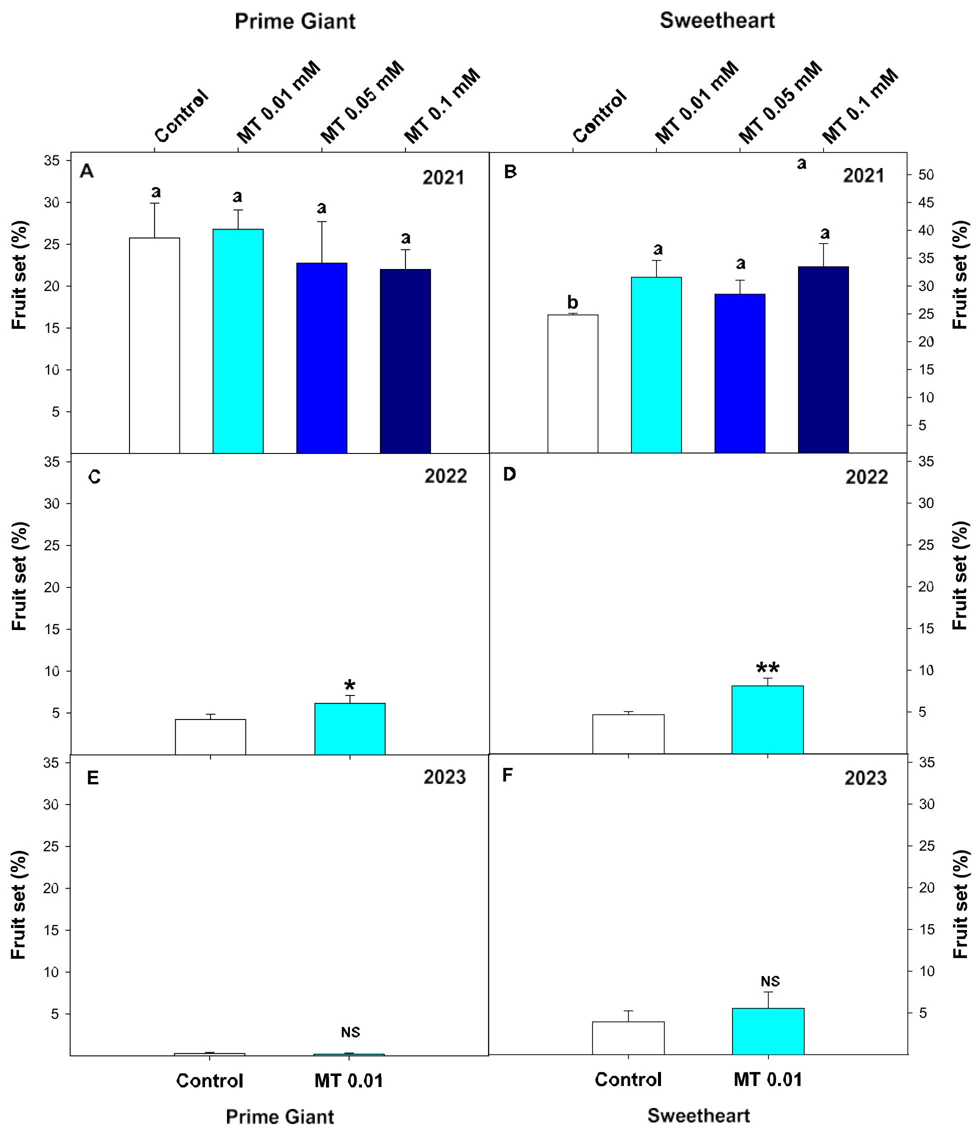

Fig. 3. (A, C, E) Fruit set percentage in the 'Prime Giant' cultivar and (B, D, F) in the 'Sweetheart' cultivar treated with melatonin (0.01, 0.05, and 0.1 mM) compared to control samples, evaluated in 2021, 2022, and 2023. In 2021, different lowercase letters indicate significant differences ($p < 0.05$) among treatments within each cultivar. For 2022 and 2023, an asterisk (*) denotes significant differences between melatonin-treated and control samples for each cultivar studied (Student's unpaired *t*-test; *$p < 0.05$, **$p < 0.01$, *NS*: non-significant).

cultivars and ripening stages involves simulating uniform water-soaking conditions, as we have assayed in this study, as was previously described by Christensen in 2006 [36]. Confirming these expectations, Winkler et al. [48] conducted a singular study and demonstrated that rain-induced cracking was less related to excessive water uptake through the roots and more correlated with the duration of water contact on the sweet cherry skin. In this context, regarding our results, when intact sweet cherries were water soaked, both cultivars exhibited reduced resilience to fruit cracking upon water immersions (Fig. 5) compared with the cracking results observed in trees under natural weather conditions (Fig. 4). We previously reported this pattern and the lower cracking resilience of the 'Prime Giant' cultivar at earlier ripening stages [10]. No previous studies have examined the effects of MT on the occurrence of cracking at harvest or during development in any fruit species. The effect of MT on sweet cherry quality are also affected by agronomic practices, adverse climatic events, plant species, cultivars [19,48] and the number of MT applications. Previous studies have reported that MT maintains chloroplast structure, stimulates photosynthesis, enhances energy status and reduces ROS directly or indirectly by increasing antioxidant activity [50]. However, these MT effects have never been directly linked to a reduction in cracking in sweet cherry, although it cannot be ruled out. In tomatoes, decreased ROS content and increased antioxidant activity are associated with fruit cracking resilience, which is related to the developmental stage [35]. In this context, the pre-harvest application of MT reduced MDA content in flower buds probably due to a higher antioxidant status because natural substances have improved the antioxidant status of pomegranates, reducing fruit cracking [51,52], and sweet cherry cultivars [53–55]. In addition, the coincidence of environmental factors, such as heavy rainfall, with specific fruit ripening stages is crucial and may vary yearly. In this context, intrinsic fruit attributes, such as the evolution of fruit firmness (along with fruit volume), and other quality traits, such as TSS or TA, seem to be closely linked to cracking tolerance in sweet cherries [15,56]. In this context, fruit firmness has been associated with sweet cherry susceptibility to cracking because a firmer fruit exhibits greater resistance to cracking due to the enhanced structural integrity of their skin and flesh [19]. This relationship is driven by the regulation of cell wall metabolism, where increased firmness is linked to a higher lignin content and reduced activity of enzymes that degrade the cell wall. In particular, MT pre-harvest applications maintain fruit firmness at harvest in climacteric

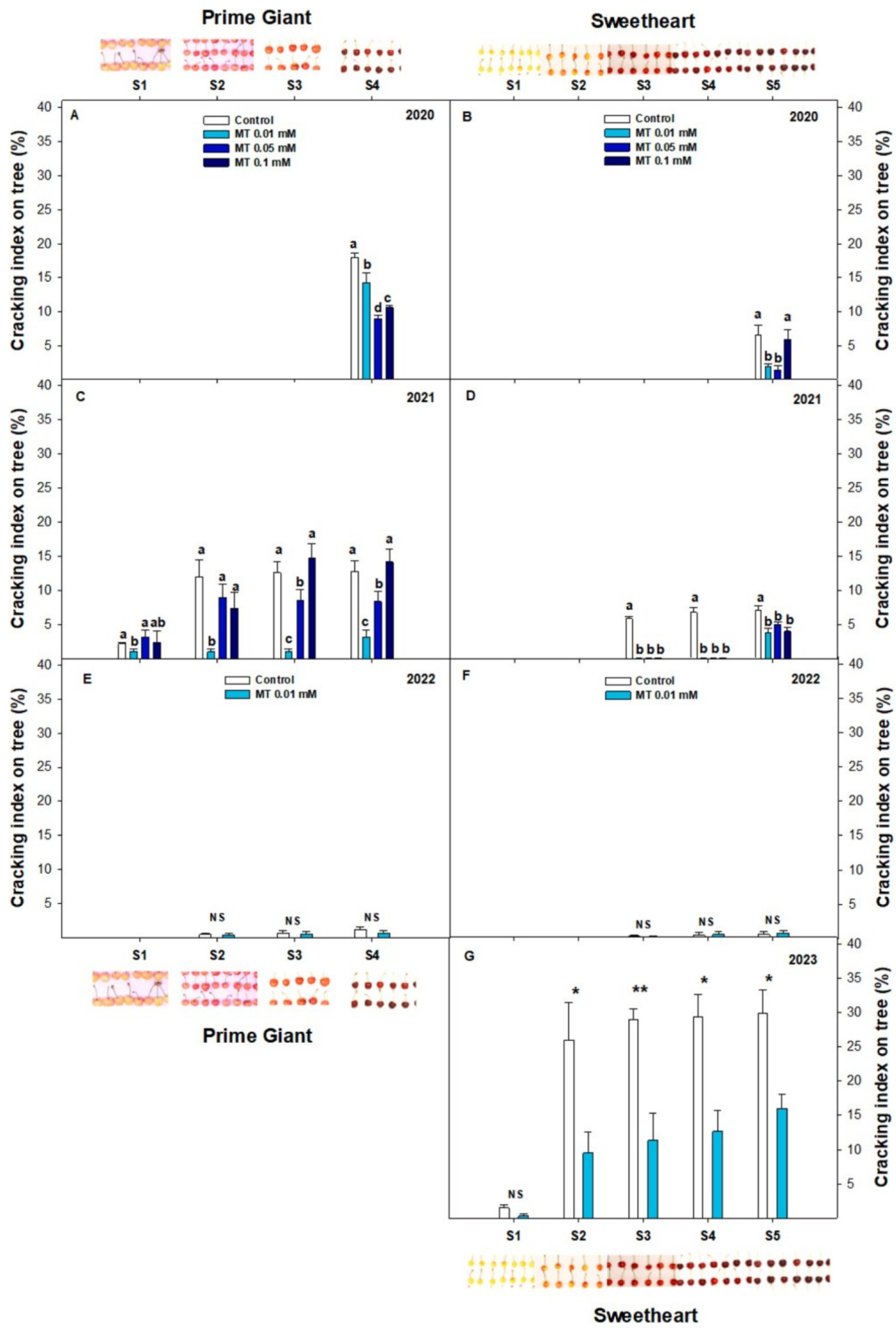

Fig. 4. (A, C, E) Cracking incidence during tree development (%) in the 'Prime Giant' cultivar and (B, D, F, G) in the 'Sweetheart' cultivar for fruits treated with melatonin (0.01, 0.05, and 0.1 mM) compared to control samples, evaluated from 2020 to 2023. Data represent the mean ± standard error (n=3). In 2020 and 2021, different lowercase letters indicate significant differences ($p < 0.05$) among treatments within each cultivar. For 2022 and 2023, an asterisk (*) denotes significant differences between melatonin-treated and control samples for each cultivar studied (Student's unpaired *t*-test; *$p < 0.05$, **$p < 0.01$, *NS*: non-significant).

fruits, such as apricot and plum, and this quality is maintained during storage [57,58]. For non-climacteric fruits, higher fruit firmness was observed in pomegranate fruits at harvest and during storage as a result of MT treatments during on-tree fruit development [59]. For the 'Prime Giant', 'Sweetheart' and 'Sandom Rose' cultivars, significantly higher fruit firmness values were observed at harvest or during storage with higher MT concentrations than those applied in this experiment [32,33]. These higher concentrations decreased fruit firmness at harvest of the 'Samba cultivar' [33], suggesting a cultivar-dependent effect. MT up-regulates genes involved in the maintenance and integrity of cell wall structures [60,61]. MT also exhibited antioxidant activity by itself and was directly related to delayed membrane peroxidation as we have observed when MDA was evaluated [62].

Regarding the sweet cherry colour at harvest, although we did not find a clear effect at harvest in this study, in previous studies on these cultivars and others, a higher MT concentration (0.1–0.5 mM) applied at pre-harvest increased colour intensity at harvest, leading to higher anthocyanin concentrations [34]. These differences can be attributed to

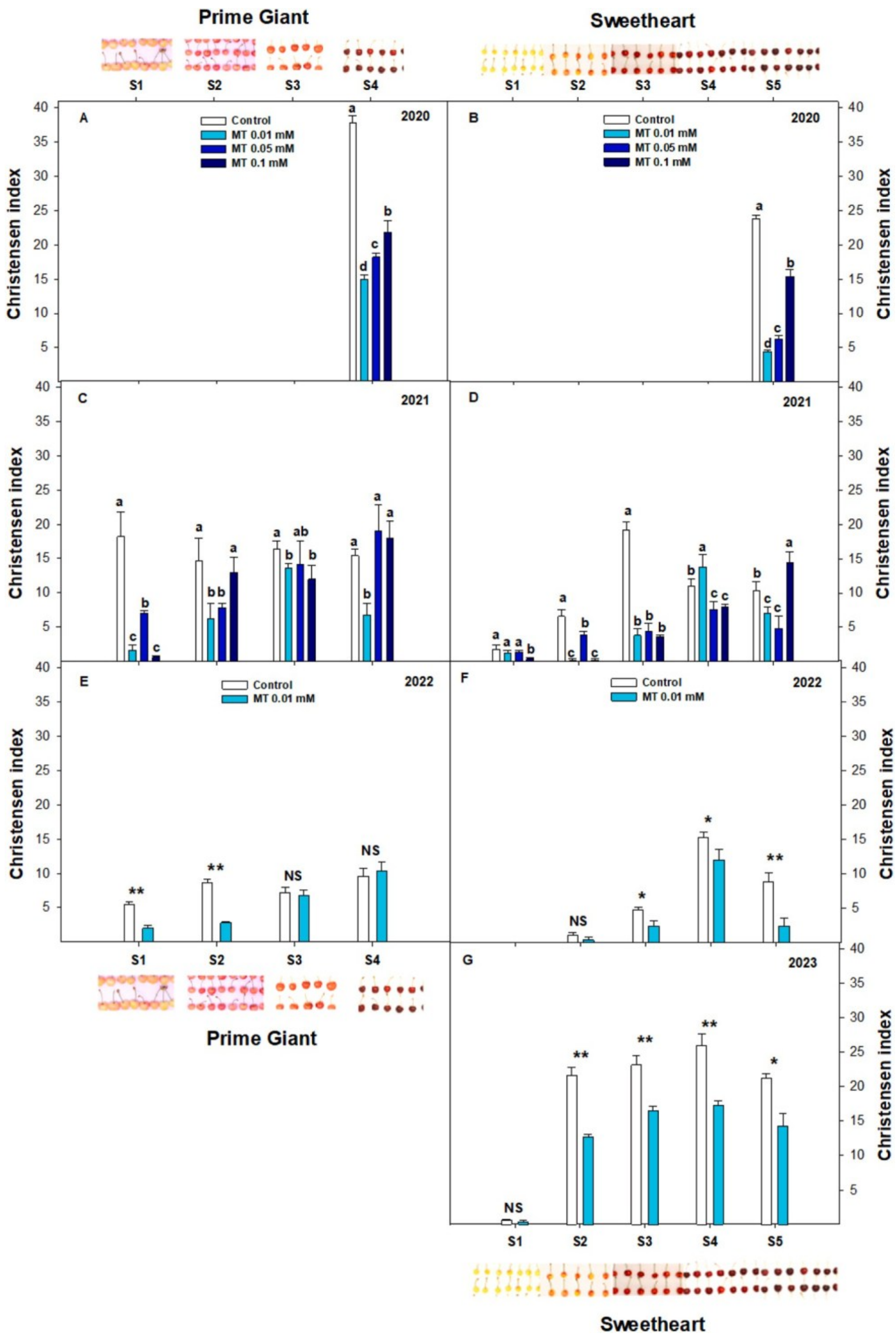

Fig. 5. (A, C, E) Christensen index during tree development in the 'Prime Giant' cultivar and (B, D, F, G) in the 'Sweetheart' cultivar for fruits treated with melatonin (0.01, 0.05, and 0.1 mM) compared to control samples, evaluated from 2020 to 2023. Data represent the mean ± standard error (n=3). In 2020 and 2021, different lowercase letters indicate significant differences ($p < 0.05$) among treatments within each cultivar. For 2022 and 2023, an asterisk (*) denotes significant differences between melatonin-treated and control samples for each cultivar studied (Student's unpaired *t*-test; *$p < 0.05$, **$p < 0.01$, *NS*: non-significant).

the timing of MT application, which differed from the application regimes in this study, as well as the different MT concentrations used. In this context, in other non-climacteric fruits, such as pomegranates and table grapes, it has been observed that other elicitors, such as oxalic acid and methyl jasmonate, can have opposite effects on fruit colour at harvest depending on the concentration applied [63–65].

In agreement with the overall results of this study, Michailidis et al. [66] also observed delayed TSS accumulation at harvest, but no differences were related to TA with higher concentrations than 0.01 mM used in this study. In addition, Tijero et al. [29] obtained different results of the concentrations applied, because higher MT concentrations than those studied in this research increased TSS and TA, in agreement with the results reported by Carrio´n-Antolí et al. [32]. In addition, 0.01 mM MT delayed TSS accumulation and mean TA values at harvest and during storage [29]. MT effects on delaying fruit ripening were previously confirmed through the inhibition of the transcription of genes related to senescence, and those involved in signal transduction and ABA synthesis [67]. The differences reported by various authors might be due

Table 1
The effect of the preharvest treatment with melatonin 0.01 mM on the physical and chemical quality of sweet cherries at harvest.

Fruit firmness (N mm −1)			
Cultivar	**Year**	**Control**	**MT 0.01 mM**
'Prime Giant'	2020	1.82 ± 0.09	1.99 ± 0.09NS
'Prime Giant'	2021	1.51 ± 0.07	1.64 ± 0.05*
'Prime Giant'	2022	1.18 ± 0.05	1.13 ± 0.06NS
'Sweetheart'	2020	2.08 ± 0.10	2.39 ± 0.09*
'Sweetheart'	2021	1.78 ± 0.09	1.99 ± 0.05*
'Sweetheart'	2022	1.69 ± 0.07	1.66 ± 0.08NS
'Sweetheart'	2023	1.89 ± 0.05	1.80 ± 0.06NS
Fruit colour (CIE *hue*°)			
Cultivar	**Year**	**Control**	**MT 0.01 mM**
'Prime Giant'	2020	21.15 ± 0.80	22.13 ± 0.85NS
'Prime Giant'	2021	13.36 ± 0.30	15.07 ± 0.27**
'Prime Giant'	2022	23.37 ± 0.31	23.63 ± 0.59NS
'Sweetheart'	2020	17.57 ± 0.58	16.79 ± 0.71NS
'Sweetheart'	2021	20.65 ± 0.19	21.10 ± 0.09*
'Sweetheart'	2022	19.27 ± 0.25	19.83 ±0.4NS
'Sweetheart'	2023	19.22 ± 0.47	19.39 ± 0.61NS
Total soluble solids (g 100 g −1)			
Cultivar	**Year**	**Control**	**MT 0.01 mM**
'Prime Giant'	2020	18.45 ± 0.12	17.87 ± 0.07NS
'Prime Giant'	2021	17.76 ± 0.18	18.46 ±0.44*
'Prime Giant'	2022	22.75 ± 0.41	22.28 ± 0.23NS
'Sweetheart'	2020	22.42 ± 0.07	24.54 ± 0.38**
'Sweetheart'	2021	18.90 ± 0.16	18.26 ± 0.20*
'Sweetheart'	2022	22.65 ± 0.44	21.75 ± 0.63NS
'Sweetheart'	2023	21.56 ± 0.20	20.35 ± 0.29**
Titratable acidity (g 100 g −1)			
Cultivar	**Year**	**Control**	**MT 0.01 mM**
'Prime Giant'	2020	1.00 ± 0.04	0.97 ± 0.01NS
'Prime Giant'	2021	1.09 ± 0.01	1.08 ± 0.02NS
'Prime Giant'	2022	1.58 ± 0.01	1.42 ± 0.05**
'Sweetheart'	2020	1.22 ± 0.02	1.12 ± 0.02**
'Sweetheart'	2021	1.33 ± 0.02	1.39 ± 0.01*
'Sweetheart'	2022	1.44 ± 0.03	1.61 ± 0.01**
'Sweetheart'	2023	1.98 ± 0.08	1.92 ± 0.02NS

(*) indicate significant differences among melatonin-treated and control samples (Student's unpaired t- test; *p < 0.05, **p < 0.01, NS: non-significant). Data are the mean ± standard error (n=3).

to the variations in the number of MT applications during the ripening of sweet cherries on trees, the concentrations of MT tested, the stage of ripeness at the time of harvest and cultivar-specific effects observed in the studies.

3. Conclusions

This study demonstrated that pre-harvest melatonin treatments effectively enhanced sweet cherry resilience against spring frost and rain-induced cracking, thereby providing a promising strategy for adapting to climate change and mitigating stress. Melatonin treatments reduced malondialdehyde levels in flower buds and resulted in a general increase in fruit set, particularly in the 'Sweetheart', which could potentially be related to an increase in crop production. However, the resilience against cracking on trees or water-soaked intact fruit varied in each growing season studied and for each melatonin concentration, although a significant effect was generally observed at low concentrations. Resilience to fruit cracking at different ripening stages was cultivar dependent and highlighted the importance of the ripening stage in cracking, indicated by delayed ripening evolution and higher fruit firmness values.

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CRediT authorship contribution statement

Maria Celeste Ruiz-Aracil: Investigation, Formal analysis, Data curation, Visualization, Writing-Original draft preparation, Validation. **Juan Miguel Valverde:** Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Resources, Funding acquisition, Visualization, Writing-Reviewing and Editing, Validation, Supervision. **Aleixandre Beltra`**: Investigation, Methodology, Funding acquisition, Project administration, Supervision. **Jose Manuel Lorente-Mento:** Investigation, Formal analysis, Data curation. **Alberto Carrio´n-Antolí**: Investigation, Methodology, Data curation. **Daniel Valero**: Investigation, Methodology, Resources, Funding acquisition, Project administration. **Fabia´n Guille´n**: Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Resources, Funding acquisition, Visualization, Writing-Reviewing and Editing, Validation, Supervision.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

References

[1] M.A. Manzoor, Y. Xu, Z. lv, J. Xu, I.H. Shah, I.A. Sabir, Y. Wang, W. Sun, X. Liu, L. Wang, R. Liu, S. Jiu, C. Zhang, Horticulture crop under pressure: unraveling the impact of climate change on nutrition and fruit cracking, J. Environ. Manag. 357 (2024) 120759, https://doi.org/10.1016/j.jenvman.2024.120759.

[2] E. Fernández, H. Mojahid, E. Fadón, J. Rodrigo, D. Ruiz, J.A. Egea, M.B. Mimoun, O. Kodad, A. El Yaacoubi, M. Ghrab, J. Egea, H. Benmoussa, N. Borgini, O. Elloumi, E. Luedeling, Climate change impacts on winter chill in Mediterranean temperate fruit orchards, Reg. Environ. Change 23 (2023) 7, https://doi.org/10.1007/s10113-022-02006-x.

[3] J.A. Yuri, M. Palma, Á. Sepúlveda, J. Sánchez-Contreras, M. Moya, Use of the biostimulant Retard Cherry® as a strategy to delay blooming period in sweet cherry trees, Adv. Hortic. Sci. 37 (2023) 427–432, https://doi.org/10.36253/ahsc-13659.

[4] M. Zago, E. Ropelato, M. Gobber, S. Franchini, Defence against late frost with frost protection candles, Laimburg J. 2 (2005) 58–61.

[5] V. Tudela, P. Sarricolea, R. Serrano-Notivoli, O. Meseguer-Ruiz, A pilot study for climate risk assessment in agriculture: a climate-based index for cherry trees, Nat. Hazards 115 (2023) 163–185, https://doi.org/10.1007/s11069-022-05549-8.

[6] K. Holušová, J. Čmejlová, I. Žďárská, P. Suran, R. Čmejla, J. Sedlák, L. Zelený, J. Bartoš, New markers for flowering-time selection in sweet cherry, Sci. Hortic. 332 (2024) 113226, https://doi.org/10.1016/j.scienta.2024.113226.

[7] S.A. Bound, D.C. Close, J.E. Jones, M.D. Whiting, Improving fruit set of 'Kordia' and 'Regina' sweet cherry with AVG, Acta Hortic. 1042 (2014) 285–292, https://doi.org/10.17660/ActaHortic.2014.1042.34.

[8] H. Xu, D. Ediger, M. Sharifi, Horticultural practices in early spring to mitigate the adverse effect of low temperature on fruit set in 'Lapins' sweet cherry, Plants 12 (2023) 468, https://doi.org/10.3390/plants12030468.

[9] J.O. Alhamid, C. Mo, X. Zhang, P. Wang, M.D. Whiting, Q. Zhang, Cellulose nanocrystals reduce cold damage to reproductive buds in fruit crops, Biosyst. Eng. 172 (2018) 124–133, https://doi.org/10.1016/j.biosystemseng.2018.06.006.

[10] M.C. Ruiz-Aracil, J.M. Valverde, A. Beltrà, A. Carrión-Antolí, J.M. Lorente-Mento, M. Nicolás-Almansa, F. Guillén, Putrescine increases frost tolerance and effectively mitigates sweet cherry (*Prunus avium* L.) cracking: a study of four different growing cycles, Agronomy 14 (2024) 23, https://doi.org/10.3390/agronomy14010023.

[11] M. Yamaguchi, I. Sato, M. Ishiguro, Influences of epidermal cell sizes and flesh firmness on cracking susceptibility in sweet cherry (*Prunus avium* L.) cultivars and selections, J. Jpn. Soc. Hortic. Sci. 71 (2002) 738–746, https://doi.org/10.2503/jjshs.71.738.

[12] A.D. Rombolà, M. Quartieri, A. Rodríguez-Declet, A. Minnocci, L. Sebastiani, G. Sorrenti, Canopy-applied silicon is an effective strategy for reducing sweet cherry cracking, Hortic. Environ. Biotechnol. 64 (2023) 371–378, https://doi.org/10.1007/s13580-022-00486-8.

[13] M.C. Ruiz-Aracil, J.M. Valverde, J.M. Lorente-Mento, A. Carrión-Antolí, S. Castillo, D. Martínez-Romero, F. Guillén, Sweet cherry (*Prunus avium* L.) cracking during

development on the tree and at harvest: the impact of methyl jasmonate on four different growing seasons, Agriculture 13 (2023) 1244, https://doi.org/10.3390/agriculture13061244.
[14] M. Knoche, S. Peschel, Water on the surface aggravates microscopic cracking of the sweet cherry fruit cuticle, J. Am. Soc. Hortic. Sci. 131 (2006) 192–200, https://doi.org/10.21273/JASHS.131.2.192.
[15] S. Pereira, V. Silva, E. Bacelar, F. Guedes, A.P. Silva, C. Ribeiro, B. Gonçalves, Cracking in sweet cherry cultivars early bigi and lapins: correlation with quality attributes, Plants 9 (2020) 1557, https://doi.org/10.3390/plants9111557.
[16] N.E. Looney, Benefits of calcium sprays below expectations in BC test, Good Fruit. Grow. 36 (10) (1986) 7–8.
[17] S. Horvitz, A.F. López Camelo, A. Yommi, C. Godoy, Application of gibberellic acid to 'Sweetheart' sweet cherries: effects on fruit quality at harvest and during cold storage, Acta Hortic. 628 (2003) 311–316, https://doi.org/10.17660/ActaHortic.2003.628.37.
[18] V. Usenik, D. Kastelec, F. Štampar, Physicochemical changes of sweet cherry fruits related to application of gibberellic acid, Food Chem. 90 (2005) 663–671, https://doi.org/10.1016/j.foodchem.2004.04.027.
[19] C. Balbontín, C. Gutiérrez, M. Wolff, C.R. Figueroa, Effect of abscisic acid and methyl jasmonate preharvest applications on fruit quality and cracking tolerance of sweet cherry, Chil. J. Agric. Res. 78 (2018) 438–446, https://doi.org/10.4067/S0718-58392018000300438.
[20] S. Correia, F. Queirós, H. Ferreira, M.C. Morais, S. Afonso, A.P. Silva, B. Gonçalves, Foliar application of calcium and growth regulators modulate sweet cherry (*Prunus avium* L.) tree performance, Plants 9 (2020) 410, https://doi.org/10.3390/plants9040410.
[21] S. Correia, R. Schouten, A.P. Silva, B. Gonçalves, Sweet cherry fruit cracking mechanisms and prevention strategies: a review, Sci. Hortic. 240 (2018) 369–377, https://doi.org/10.1016/j.scienta.2018.06.042.
[22] B. Gonçalves, V. Silva, E. Bacelar, F. Guedes, C. Ribeiro, A.P. Silva, S. Pereira, Orchard net covers improve resistance to cherry cracking disorder, Foods 12 (2023) 543, https://doi.org/10.3390/foods12030543.
[23] V. Blanco, J.P. Zoffoli, M. Ayala, High tunnel cultivation of sweet cherry (*Prunus avium* L.): physiological and production variables, Sci. Hortic. 251 (2019) 108–117, https://doi.org/10.1016/j.scienta.2019.02.023.
[24] M. Bustamante, A. Muñoz, I. Romero, P. Osorio, S. Mánquez, R. Arriola, M. Reyes-Díaz, A. Ribera-Fonseca, Impact of potassium pre-harvest applications on fruit quality and condition of sweet cherry (*Prunus avium* L.) cultivated under plastic covers in Southern chile orchards, Plants 10 (2021) 2778, https://doi.org/10.3390/plants10122778.
[25] S. Pino, M. Palma, Á. Sepúlveda, J. Sánchez-Contreras, M. Moya, J.A. Yuri, Effect of rain cover on tree physiology and fruit condition and quality of 'Rainier', 'Bing' and 'Sweetheart' sweet cherry trees, Horticulturae 9 (2023) 109, https://doi.org/10.3390/horticulturae9010109.
[26] C. Chen, S. Zhang, X. Cheng, Y. Ren, Y. Qian, C. Zhang, M. Chen, N. Sun, H. Liu, Reducing cherry rain-cracking: enhanced wetting and barrier properties of chitosan hydrochloride-based coating with dual nanoparticles, Int. J. Biol. Macromol. 268 (2024) 131660, https://doi.org/10.1016/j.ijbiomac.2024.131660.
[27] J. Zhou, L.R. Khot, R.T. Peters, M.D. Whiting, Q. Zhang, D.M. Granatstein, Efficacy of unmanned mid-sized helicopter in rainwater removal from cherry canopies, Comput. Electron. Agric. 124 (2016) 161–167, https://doi.org/10.1016/j.compag.2016.04.006.
[28] X. Feng, M. Wang, Y. Zhao, P. Han, Y. Dai, Melatonin from different fruit sources, functional roles, and analytical methods, Trends Food Sci. Technol. 37 (2014) 21–31, https://doi.org/10.1016/j.tifs.2014.02.001.
[29] V. Tijero, P. Munoz, S. Munné-Bosch, Melatonin as an inhibitor of sweet cherries ripening in orchard trees, Plant Physiol. Biochem. 140 (2019) 88–95, https://doi.org/10.1016/j.plaphy.2019.05.007.
[30] D.X. Tan, L.C. Manchester, E. Esteban-Zubero, Z. Zhou, R.J. Reiter, Melatonin as a potent and inducible endogenous antioxidant: synthesis and metabolism, Molecules 20 (2015) 18886–18906, https://doi.org/10.3390/molecules201018886.
[31] M.B. Arnao, J. Hernández-Ruiz, Melatonin as a regulatory hub of plant hormone levels and action in stress situations, Plant Biol. 23 (2020) 7–19, https://doi.org/10.1111/plb.13202.
[32] A. Carrión-Antolí, J.M. Lorente-Mento, J.M. Valverde, S. Castillo, D. Valero, M. Serrano, Effects of melatonin treatment on sweet cherry tree yield and fruit quality, Agronomy 12 (2022) 3, https://doi.org/10.3390/agronomy12010003.
[33] D. Cortés-Montaña, M.J. Bernalte-García, M. Palomino-Vasco, M.J. Serradilla, B. Velardo-Micharet, Effect of preharvest melatonin applications at dusk on quality and bioactive compounds content of early sweet cherries, J. Sci. Food Agric. 104 (2024) 1583–1590, https://doi.org/10.1002/jsfa.13040.
[34] A. Carrión-Antolí, D. Martínez-Romero, F. Guillén, P.J. Zapata, M. Serrano, D. Valero, Melatonin pre-harvest treatments lead to maintenance of sweet cherry quality during storage by increasing antioxidant systems, Front. Plant Sci. 13 (2022) 863467, https://doi.org/10.3389/fpls.2022.863467.
[35] C. Zhang, Y. Zhao, F. Jiang, Z. Wu, S. Cui, H. Lv, L. Yu, Differences of reactive oxygen species metabolism in top, middle and bottom part of epicarp and mesocarp influence tomato fruit cracking, J. Hortic. Sci. Biotech. 95 (2020) 746–756, https://doi.org/10.1080/14620316.2020.1748525.
[36] J.V. Christensen, Rain-induced cracking of sweet cherries: Its causes and prevention, in: A.D. Webster, N.E. Looney (Eds.), In Cherries: Crop Physiology, Production and Uses, CAB International, Wallingford, UK, 2006, pp. 297–327.
[37] F.M. Chmielewski, K.P. Götz, K.C. Weber, S. Moryson, Climate change and spring frost damages for sweet cherries in Germany, Int. J. Biometeorol. 62 (2018) 217–228, https://doi.org/10.1007/s00484-017-1443-9.
[38] R.L. Shewfelt, A. C. Purvis, Toward a comprehensive model for lipid peroxidation in plant tissue disorders, HortScience 30 (1995) 213–218, https://doi.org/10.21273/HORTSCI.28.5.471c.
[39] J. Giné-Bordonaba, G. Echeverria, D. Ubach, I. Aguiló-Aguayo, M.L. López, C. Larrigaudière, Biochemical and physiological changes during fruit development and ripening of two sweet cherry varieties with different levels of cracking tolerance, Plant Physiol. Biochem. 111 (2017) 216–225, https://doi.org/10.1016/j.plaphy.2016.12.002.
[40] S. Sachdev, S.A. Ansari, M.I. Ansari, M. Fujita, M. Hasanuzzaman, Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms, Antioxidants 10 (2021) 277, https://doi.org/10.3390/antiox10020277.
[41] J. Rodrigo, Spring frosts in deciduous fruit trees–morphological damage and flower hardiness, Sci. Hortic. 85 (2000) 155–173, https://doi.org/10.1016/S0304-4238(99)00150-8.
[42] F. Garcia-Montiel, M. Serrano, D. Martinez-Romero, N. Alburquerque, Factors influencing fruit set and quality in different sweet cherry cultivars, Span. J. Agric. Res. 8 (2010) 1118–1128, https://doi.org/10.5424/sjar/2010084-1238.
[43] K. Beppu, I. Kataoka, Studies on pistil doubling and fruit set of sweet cherry in warm climate, J. Jpn. Soc. Hortic. Sci. 80 (2011) 1–13, https://doi.org/10.2503/jjshs1.80.1.
[44] I.A. Sabir, X. Liu, S. Jiu, M. Whiting, C. Zhang, Plant growth regulators modify fruit set, fruit quality, and return bloom in sweet cherry, HortScience 56 (2021) 922–931, https://doi.org/10.21273/HORTSCI15835-21.
[45] K. Rutkowski, G.P. Łysiak, Thinning methods to regulate sweet cherry crops—a review, Appl. Sci. 12 (2022) 1280, https://doi.org/10.3390/app12031280.
[46] S. Usanmaz, M.A. Aşkın, I. Kahramanoğlu, Effects of foliar melatonin application on the flowering and fruit set of pomegranate (*Punica granatum* L. 'Wonderful'), Acta Hortic. (1349) (2022) 63–71, https://doi.org/10.17660/ActaHortic.2022.1349.10.
[47] A.K. Mumithrakamatchi, S. Alagarswamy, A. Kuppusamy, M. Djanaguiraman, M. K. Kalarani, R. Swarnapriya, S. Marimuthu, S. Vellaikumar, S. Kanagarajan, Melatonin imparts tolerance to combined drought and high-temperature stresses in tomato through osmotic adjustment and ABA accumulation, Front. Plant Sci. 15 (2024) 1382914, https://doi.org/10.3389/fpls.2024.1382914.
[48] A. Winkler, I. Blumenberg, L. Schürmann, M. Knoche, Rain cracking in sweet cherries is caused by surface wetness, not by water uptake, Sci. Hortic. 269 (2020) 10940023, https://doi.org/10.1016/j.scienta.2020.109400.
[49] J. Blažek, L. Zelený, P. Suran, Sweet cherry research world overview 2015–2017, Hortic. Sci. 49 (2022) 121–146, https://doi.org/10.17221/70/2021-hortsci.
[50] I. Muhammad, A. Shalmani, M. Ali, Q.H. Yang, H. Ahmad, F.B. Li, Mechanisms regulating the dynamics of photosynthesis under abiotic stresses, Front. Plant Sci. 11 (2021) 615942, https://doi.org/10.3389/fpls.2020.615942.
[51] P. Drogoudi, G.E. Pantelidis, S.A. Vekiari, Physiological disorders and fruit quality attributes in pomegranate: Effects of meteorological parameters, canopy position and acetylsalicylic acid foliar sprays, Front. Plant Sci. 12 (2021) 645547, https://doi.org/10.3389/fpls.2021.645547.
[52] S. Moradi, Z. Zamani, M.R.F. Moghadam, M.K. Saba, Combination effects of preharvest tree net-shading and postharvest fruit treatments with salicylic acid or hot water on attributes of pomegranate fruit, Sci. Hortic. 304 (2022) 111257, https://doi.org/10.1016/j.scienta.2022.111257.
[53] A.H. Faizy, B. Ozturk, E. Aglar, K. Yıldız, Role of methyl jasmonate application regime on fruit quality and bioactive compounds of sweet cherry at harvest and during cold storage, J. Food Process. 45 (2021) e15882, https://doi.org/10.1111/jfpp.15882.
[54] C. Palacios-Peralta, A. Ruiz, S. Ercoli, M. Reyes-Díaz, M. Bustamante, A. Muñoz, P. Osorio, A. Ribera-Fonseca, Plastic covers and potassium pre-harvest sprays and their influence on antioxidant properties, phenolic profile, and organic acids composition of sweet cherry fruits cultivated in Southern Chile, Plants 12 (2023) 50, https://doi.org/10.3390/plants12010050.
[55] A. Varaldo, F. Alchera, L. Brigante, G. Giacalone, Foliar applications of calcium and potassium increase cracking resistance and enhance fruit quality in sweet cherries, Italus Hortus 30 (2023) 25–36, https://doi.org/10.26353/j.itahort/2023.3.2536.
[56] L. Demirsoy, S. Bilgener, The effects of preharvest calcium hydroxide applications on cracking in '0900 Ziraat', 'Lambert' and 'Van' sweet cherries, Acta Hortic. 468 (1998) 657–662, https://doi.org/10.17660/ActaHortic.1998.468.82.
[57] J. Medina-Santamarina, M. Serrano, M.C. Ruiz-Aracil, M.I.M. Ilea, D. Martínez-Romero, F. Guillén, A synergistic effect based on the combination of melatonin with 1-methylcyclopropene as a new strategy to increase chilling tolerance and general quality in zucchini fruit, Foods 11 (2022) 2784, https://doi.org/10.3390/foods11182784.
[58] D. Cortés-Montaña, M.J. Bernalte-García, B. Velardo-Micharet, M. Serrano, M. J. Serradilla, Impact of pre-storage melatonin application on the standard, sensory, and bioactive quality of early sweet cherry, Foods 12 (2023) 1723, https://doi.org/10.3390/foods12081723.
[59] J.M. Lorente-Mento, F. Guillén, S. Castillo, D. Martínez-Romero, J.M. Valverde, D. Valero, M. Serrano, Melatonin treatment to pomegranate trees enhances fruit bioactive compounds and quality traits at harvest and during postharvest storage, Antioxidants 10 (2021) 820, https://doi.org/10.3390/antiox10060820.
[60] Q. Sun, N. Zhang, J. Wang, H. Zhang, D. Li, J. Shi, R. Li, S. Weeda, B. Zhao, S. Ren, Y.D. Guo, Melatonin promotes ripening and improves quality of tomato fruit during postharvest life, J. Exp. Bot. 66 (2015) 657–668, https://doi.org/10.1093/jxb/eru332.
[61] R. Zhai, J. Liu, F. Liu, Y. Zhao, L. Liu, C. Fang, H. Wang, X. Li, Z. Wang, F. Ma, L. Xu, Melatonin limited ethylene production, softening and reduced physiology disorder in pear (*Pyrus communis* L.) fruit during senescence, Postharvest Biol. Technol. 139 (2018) 38–46, https://doi.org/10.1016/j.postharvbio.2018.01.017.

[62] P. Sharma, N. Thakur, N.A. Mann, A. Umar, Melatonin as plant growth regulator in sustainable agriculture, Sci. Hortic. 323 (2024) 112421, https://doi.org/10.1016/j.scienta.2023.112421.

[63] M.E. García-Pastor, M. Serrano, F. Guillén, S. Castillo, D. Martínez-Romero, D. Valero, P.J. Zapata, Methyl jasmonate effects on table grape ripening, vine yield, berry quality and bioactive compounds depend on applied concentration, Sci. Hortic. 247 (2019) 380–389, https://doi.org/10.1016/j.scienta.2018.12.043.

[64] M.E. García-Pastor, M.J. Giménez, J.M. Valverde, F. Guillén, S. Castillo, D. Martínez-Romero, M. Serrano, D. Valero, P.J. Zapata, Preharvest application of oxalic acid improved pomegranate fruit yield, quality, and bioactive compounds at harvest in a concentration-dependent manner, Agronomy 10 (2020) 1522, https://doi.org/10.3390/agronomy10101522.

[65] M.E. García-Pastor, M. Serrano, F. Guillén, M.J. Giménez, D. Martínez-Romero, D. Valero, P.J. Zapata, Preharvest application of methyl jasmonate increases crop yield, fruit quality and bioactive compounds in pomegranate 'Mollar de Elche' at harvest and during postharvest storage, J. Sci. Food Agric. 100 (2020) 145–153, https://doi.org/10.1002/jsfa.10007.

[66] M. Michailidis, G. Tanou, E. Sarrou, E. Karagiannis, I. Ganopoulos, S. Martens, A. Molassiotis, Pre-and post-harvest melatonin application boosted phenolic compounds accumulation and altered respiratory characters in sweet cherry fruit, Front. Nutr. 8 (2021) 695061, https://doi.org/10.3389/fnut.2021.695061.

[67] J. Zhang, Y. Shi, X. Zhang, H. Du, B. Xu, B. Huang, Melatonin suppression of heat-induced leaf senescence involves changes in abscisic acid and cytokinin biosynthesis and signaling pathways in perennial ryegrass (*Lolium perenne* L, Environ. Exp. Bot. 138 (2017) 36–45, https://doi.org/10.1016/j.envexpbot.2017.02.012.

4.3. Publicación III (Transcripción literal)

III

Sweet cherry (*Prunus avium* L.) cracking during development on the tree and at harvest: the impact of methyl jasmonate on four different growing seasons

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Article

Sweet Cherry (*Prunus avium* L.) Cracking during Development on the Tree and at Harvest: The Impact of Methyl Jasmonate on Four Different Growing Seasons

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Abstract: Rainfall occurring during the developmental stages of sweet cherries on the tree can lead to significant preharvest losses, primarily due to fruit cracking. Certain cultivars exhibit a higher susceptibility to such losses, particularly when persistent rains coincide with advanced phenological stages. The current study aims to investigate the efficacy of preharvest methyl jasmonate (MeJA) applications at harvest and during distinct developmental ripening stages in mitigating sweet cherry cracking at harvest and on-tree ripening. Preharvest foliar applications of 0.5 mM MeJA were applied across various sweet cherry cultivars, including 'Prime Giant', 'Early Lory', 'Sweetheart', and 'Staccato'. By conducting this experiment over four growing seasons, we evaluated the impact of this natural elicitor on the cracking tolerance of these cultivars. The results of our analysis indicate that MeJA preharvest treatments effectively reduce fruit cracking, enhancing abiotic stress tolerance. Additionally, these treatments induce a general delay in fruit ripening on the tree across the examined cultivars. This delayed ripening effect is reflected in several quality parameters at harvest, such as the fruit firmness, external colour, total soluble solids, and total acidity. These parameters in the MeJA-treated fruit were delayed compared to the control fruit or remained unaffected for the total acidity. Conversely, the MeJA treatments delayed the accumulation of total polyphenols, exhibiting a minimal impact on reducing pedicel browning. The enhanced tolerance to cracking and delayed ripening attributed to the MeJA preharvest treatments could be helpful for plot management. Consequently, these MeJA-based preharvest treatments hold potential as valuable tools in adapting to climate change and mitigating abiotic stress in sweet cherry.

Keywords: *Prunus avium*; ripening stage; preharvest; cracking; methyl jasmonate; climate change

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1. Introduction

The increasing occurrence of extreme weather conditions associated with climate change can have a significant impact on fruit cracking. In this sense, climate change affects environmental factors, such as excessive rainfall, high humidity, and rapid changes in temperature [1]. All of these factors are involved in fruit cracking of different fruit. Thinner-skinned fruit, such as sweet cherries, tend to be more susceptible to cracking than thicker-skinned fruit, such as other stone fruit [2,3]. There are many factors involved in sweet cherry cracking, such as the fruit size and shape, growing conditions, genetic factors, and sugar content [4–6]. According to these authors, the ripening stage of the fruit is very relevant and can determine the incidence of cracking in sweet cherries [7]. In fact, Giné-Bordonada et al. [8] have observed that the different growth and ripening stages displayed differences in several parameters including the sweet cherry texture and ROS content, which are directly related to the membrane integrity, thus affecting fruit cracking, especially at the latter ripening stages. According to Yamaguchi et al. [9], the more crack-tolerant cultivars have longer periods of cell division, resulting in a larger mesocarp. Although sweet cherry cracking is a multifactorial process, some factors are decisive, such as sudden rains or high humidity, during the development on the tree, which can result in significant losses for producers [4,10].

To prevent sweet cherry cracking, several management strategies have been evaluated, including rain cover protection [11,12] and preharvest sprays based on calcium applications [4,13] and seaweed extracts [14]. Additionally, growth regulators, such as gibberellic acid [15], glycine betaine [16], and methyl jasmonate (MeJA) [17], have been applied at preharvest to reduce sweet cherry cracking. In this sense, MeJA, as an endogenous signalling molecule, plays a vital role in the growth, development, and mechanism systems in plants to effectively respond to challenging environmental conditions [18].

In Spain, there is a specific region in which sweet cherries are grown under the Protected Geographical Indication (PGI) "Cerezas de la Montaña de Alicante". The PGI emphasizes the relationship between the specific geographic region and the name of the product, where a particular quality, reputation, or other characteristic is essentially attributed to its geographical origin. These sweet cherries are produced in mountainous and steep areas and are highly appreciated by consumers for their aroma, flavour, and earlier-harvested cultivars. However, due to this topography, this crop constantly suffers losses from the incidence of rainfall year after year. In a recent study, the authors found that if the harvest time is delayed by one week more than the commercial harvest, the impact of sweet cherry cracking is increased [19]. However, as far as we know, preharvest technologies regarding sweet cherry cracking have focused on evaluating the effect of immersion or applying artificial rain on sweet cherries to evaluate the cracking impact at commercial harvest time. Therefore, this study aimed to evaluate the incidence of MeJA in reducing the cracking incidence during fruit development on the tree as well as under controlled conditions at different ripening stages in four different seasons.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Experiments were performed in different field plots located at Planes and Alcoy (Alicante, Spain) with sweet cherry trees (*Prunus avium* L.) of the cultivars 'Early Lory', 'Prime Giant', 'Sweetheart', and 'Staccato' between 2019 and 2022, which were grafted onto SL-64 rootstock. The sweet cherry trees were all grown under similar agronomic practices for the different growing seasons. The MeJA treatments (Sigma-Aldrich, Madrid, Spain) were performed by applying 3 L per tree (with a manual sprayer machine) of freshly prepared MeJA solutions at 0.5 mM containing 1 mL L^{-1} Tween 20 as a surfactant. Similarly, 3 L of distilled water with 1 mL L^{-1} Tween 20 was applied to the control trees. For each treatment and cultivar, three replicates of three trees were used. Each treatment was applied two times, at pit hardening and at the beginning of colour changes. The selection of the number of preharvest applications was based on the results observed in three different tree groups studied in the 2019 season for the 'Prime Giant' and 'Early Lory' cultivars. These tree groups were treated separately with 1, 2, and 3 applications. However, the third and final application (days before harvest) did not significantly increase, in general, the preharvest and postharvest potential of the MeJA treatments in both cultivars for different parameters.

2.2. Sweet Cherry Cracking Evaluation

A sweet cherry cracking evaluation was performed at harvest for the 'Early Lory' and 'Staccato' cultivars. On the other hand, the impact of the MeJA treatments on sweet cherry cracking during fruit growth and development at different ripening stages on the tree for 'Prime Giant' and 'Sweetheart' was investigated. The different ripening stages selected were chosen as follows: for 'Prime Giant', coincident with the beginning of the colour changes (S1), a pink colour (S2), a bright red colour (S3), and a dark red colour (S4); for 'Sweetheart', the cultivar ripening stages in which sweet cherry cracking was evalu- ated were coincident with an immature green-yellow colour (S1), the beginning of colour changes (S2), a pink colour (S3), a bright red colour (S4), and dark red colour (S5). The cracking incidence on the tree was evaluated in 4 opposite labelled branches in each tree in which this disorder was evaluated in 100 fruits per tree, and the results were expressed as percentages. On the other hand, at the same time, sweet cherry cracking was evaluated in healthy fruits according to the method described by Christensen [20] in three replicates of 50 fruits for each treatment batch and expressed as the cracking index.

2.3. Fruit Quality Parameters at Harvest

Regarding the fruit quality measurements at harvest, sweet cherries were harvested at the commercial ripening stage, according to commercial practices, based on the characteristic skin colour of each cultivar. Lots for each replicate were mixed and immediately transferred to the laboratory. Then, at room temperature, 3 lots of 20 fruits that were 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 measurements at harvest.

CO_2 was determined in triplicate by placing 20 fruits from each replicate in a 0.5 L plastic container that was hermetically sealed with a rubber stopper for 60 min using the static method [21]. After that, a 1 mL gas sample was taken in duplicate from the headspace, and carbon dioxide was quantified using a Shimadzu 14B (Shimadzu Europa GmbH, Duisburg, Germany) and expressed as mg of CO_2 kg^{-1} h^{-1}.

The colour was measured at harvest in each fruit individually with a Minolta colorimeter (CR-400, Konica Minolta Camera Co., Kantō, Tokyo, Japan), and three colour measurements were made for each fruit at two opposite and equidistant points at the equatorial zone and expressed as CIE h° (arctg b*/a*) according to CIELab coordinates.

The firmness was determined individually using a TX-XT2i texture analyser (Stable Microsystems, Godalming, UK) equipped with a flat plate probe. The rate of descent of the disc was 20 mm min^{-1} until a deformation of 5% was reached. The fruit firmness was expressed as the ratio of the applied force to the distance travelled (N mm^{-1}).

After that, the flesh of 20 fruit of each replicate was cut in small pieces to obtain a homogeneous sample, and about 50 g was 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). These two parameters were determined by duplicate in the filtered juice extracted, as previously described [22], in each replicate per batch. The TSS in the sweet cherry juice was measured using an Atago PR-101 digital refractometer (Atago Co., Ltd., Tokyo, Japan) at 20 °C and the TA was determined in each sample by automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland). The TSS was expressed as g 100 g^{-1} and the TA was expressed as g of malic acid equivalent 100 g^{-1}.

The total phenolics were extracted by homogenizing 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 an Ultraturrax homogeniser (T18 basic, IKA, Berlin, Germany). Then, the extracts were centrifuged at 10,000× *g* for 10 min at 4 °C, and the total phenolics were quantified in duplicate in the supernatant using the Folin-Ciocalteu reagent as previously described for different plant organs [23]. The results were expressed as mg gallic acid equivalent 100 g^{-1} and are the mean ± SE.

Sweet cherry fruits with visible symptoms of pedicel browning were reported in accordance with the following scale: stems with no visible symptoms (0), stems affected by browning <25% (1), 26–50% (2), 51–75% (3), and >75% of the pedicel area affected by browning (4).

2.4. Statistical Analysis

The experiments were performed using a completely randomized design. All the statistical analyses were performed with the SPSS package program, version 22 (IBM Corp., Armonk, NY, USA). The data were analysed by one-way analysis of variance and are the mean ± standard error ($n = 3$). (*) indicates significant differences among the MeJA-treated and control samples (Student's unpaired *t*-test; * $p < 0.05$, ** $p < 0.01$).

3. Results and Discussion

3.1. Effect of Exogenous MeJA on Sweet Cherry Cracking at Harvest

Cracking represents a major challenge for sweet cherry producers as it can lead to substantial economic losses. Once the fruit cracks, it becomes more susceptible to pests and diseases, which can further undermine its quality and value in the marketplace [24].

Among the cultivars, 'Prime Giant' exhibited the highest incidence of cracking on the tree compared to the other cultivars, such as the 'Staccato' and 'Sweetheart' cultivars, which displayed the lowest incidence of fruit cracking under ambient uncontrolled conditions (Figure 1A,B). The factors contributing to the differential tolerance among the cultivars remain unknown, but rain cracking, as a localized phenomenon, has a significant impact on fruit cracking [25,26]. In this sense, the 'Sweetheart' and 'Staccato' cultivars were not exposed to heavy rainfall since it is not common to have significant rainfalls towards the end of July, which is the typical harvesting time for these cultivars in the southeast of Spain. However, when healthy sweet cherries from all the studied cultivars were exposed right after the harvest time to controlled conditions using water immersions [20], creating an additional artificial heavy rainfall for 6 h at harvest (Figure 1C,D), the results were different. The highest incidence of sweet cherry cracking was observed in the 'Sweetheart' cultivar, with a similar incidence of cracking in the 'Prime Giant' and 'Staccato' cultivars.

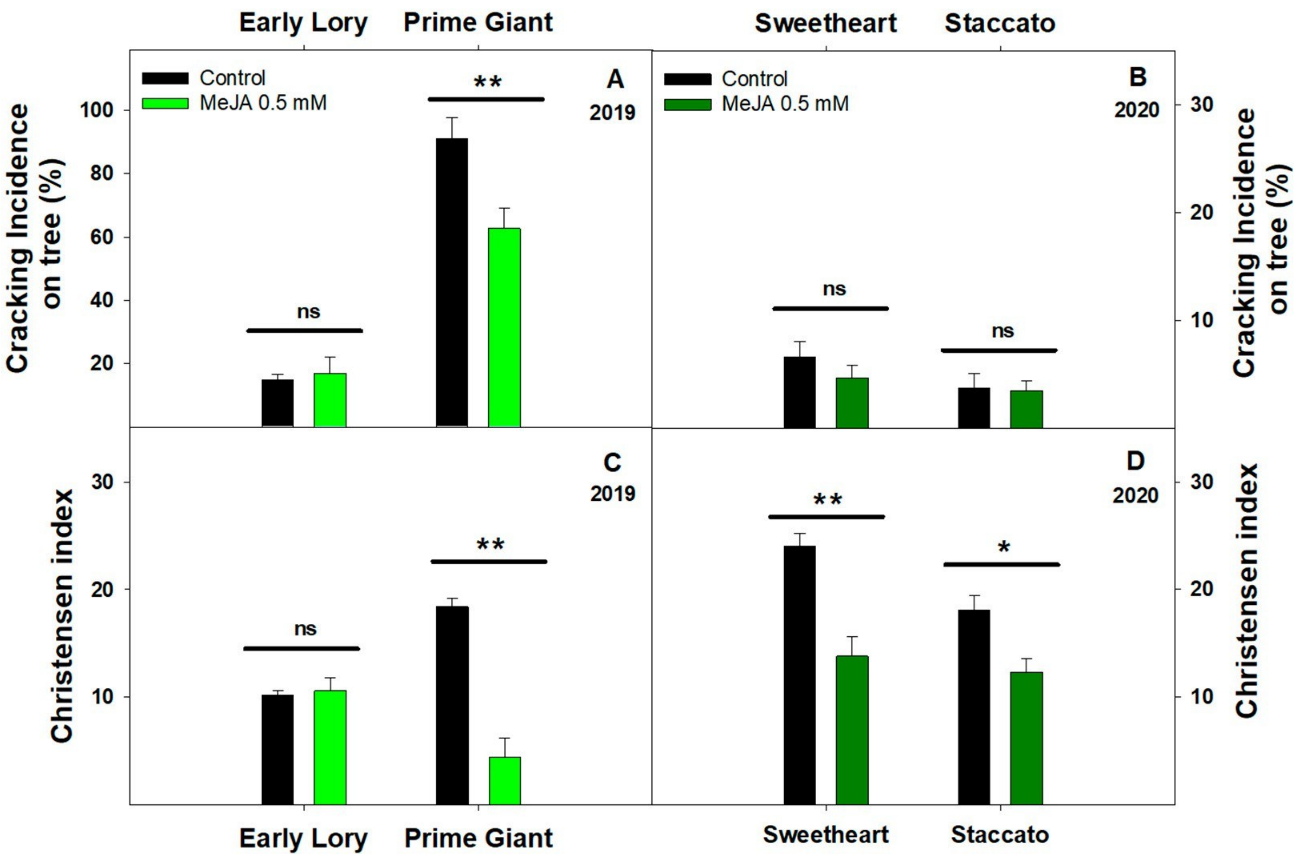

Figure 1. (**A**,**B**) Cracking incidence on tree at harvest (%) and (**C**,**D**) Christensen index at harvest (%) of four sweet cherry cultivars evaluated in 2019 (Early Lory and Prime Giant) and 2020 (Sweetheart and Staccato). Data are the mean ± standard error ($n = 3$). (*) indicates significant differences among MeJA-treated and control samples (Student's unpaired *t*-test; * $p < 0.05$, ** $p < 0.01$, ns non significant).

On the other hand, fruit cracking was reduced significantly ($p < 0.01$) in the MeJA-treated 'Prime Giant' cultivar under ambient conditions although a non-significant effect ($p > 0.05$) was observed in general for the rest of the sweet cherry cultivars studied under uncontrolled ambient conditions at harvest time. In this sense, also no significant differences ($p > 0.05$) regarding the effect of MeJA were observed after water immersions in the 'Early

Lory' cultivar, but the effect of reducing the cracking index in the rest of the cultivars studied was clear (Figure 1C,D). According to our results, a greater effect was observed for the cultivars 'Prime Giant' and 'Sweetheart', with the highest fruit cracking reduction (76.08 and 42.05%, respectively) observed after the MeJA was applied compared with the control fruit at harvest time. In previous studies, the effect on crack reduction by MeJA has been observed at similar percentages when its action was evaluated at harvest [17–19]. In this regard, Faizy et al. [19] observed higher cracking when the commercial harvest was delayed by one week for the '0900 Ziraat' cultivar in both the MeJA-treated and control fruit. For this reason, although the MeJA effect on cracking reduction was positive for three cultivars in different growing seasons at commercial harvest in our study, the fruits' degree of ripeness and coincident abiotic stress conditions before the commercial harvest time could also affect these results. In fact, during sweet cherry development on the tree, Serrano et al. [27] described the different physiological characteristics in several parameters related to cracking, such as firmness, soluble solids content, or fruit volume [4,15,28]. On the other hand, the susceptibility to cracking in sweet cherries is dependent on the cultivar [26], and the effect of MeJA on different ripening parameters is also dependent on the species, cultivar, agronomic and environmental conditions, MeJA concentrations, and the number of applications. [29]

3.2. Effect of Exogenous MeJA on Sweet Cherry Cracking during Ripening on Tree

The ripening evolution directly affects the sweet cherry skin thickness and integrity [4,30], which can influence the cracking incidence. To elucidate the general effect of the ripening stage on the cracking incidence, this relationship was studied for the 'Prime Giant' and 'Sweetheart' cultivars during their development on the tree and when exposed to additional water immersion conditions [20] at different developmental stages with distilled water. The results demonstrated that MeJA treatment can significantly reduce cherry cracking at different ripening stages on the tree, but the effect on the cracking incidence showed a different trend among the cultivars studied. In general, during sweet cherry ripening on the tree, an increase in the cracking incidence was observed, which was effectively reduced by preharvest applications of methyl jasmonate coinciding with pit hardening (before S1) in both cultivars and with the colour change of the cherries (S1 for the 'Prime Giant' cultivar and S2 for the 'Sweetheart' cultivar), as shown in Figure 2A,B. However, this effect decreased as the fruit ripening progressed, particularly for the 'Prime Giant' cultivar, possibly because it is an earlier variety than 'Sweetheart', and more advanced ripening stages usually coincide with more intense rainfalls compared with those occurring during the ripening of the 'Sweetheart' cultivar.

Previous research [31] suggests that rain cracking in sweet cherries may not only be caused by excessive water uptake and skin phenomena. Instead, it is likely a localized phenomenon that is caused by the direct exposure of the fruit skin to liquid water. These findings indicate that rain cracking is not related to the net fruit water balance. For this reason, new rain-protection systems have demonstrated their capability to ensure good commercial yields by reducing sweet cherry cracking, even during seasons of heavy rainfall, which could result in the loss of the entire crop for unprotected orchards [11,32–34].

On the other hand, when we evaluated the susceptibility to cracking under controlled conditions, we observed that although in the 'Prime Giant' cultivar, the fruit susceptibility to cracking was similar at all the ripening stages after the colour change, the 'Sweetheart' cultivar showed a different cracking sensitivity depending on the ripening stage coinciding with high humidity or persistent rain (Figure 2C,D). In fact, in the 'Sweetheart' cultivar, the highest incidence of cracking occurred at stage 3, which coincides with the first ripening stage when the fruit acquires a homogeneous reddish coloration. Thus, both the earlier and later stages of ripening showed a lower incidence of cracking under controlled conditions (Figure 2D). In this regard, preharvest applications of MeJA were significantly effective ($p < 0.01$), especially in maturity stage 3 for both the 'Prime Giant' and 'Sweetheart' cultivars, reducing the incidence of cracking by 75.67% and 68.75%, respectively.

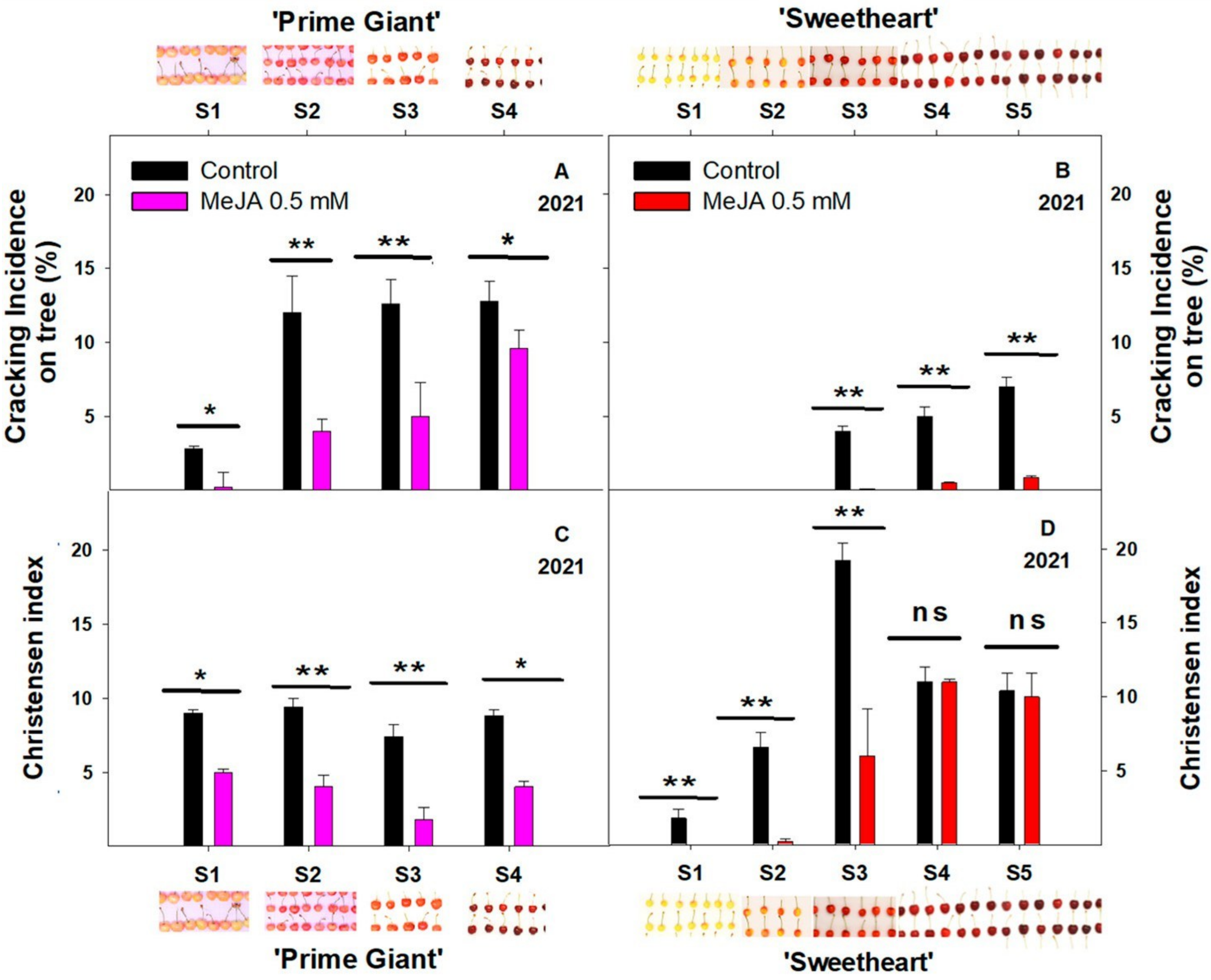

Figure 2. (**A**,**B**) Cracking incidence during development on tree (%) and (**C**,**D**) Christensen index during development on tree (%) of two sweet cherry cultivars evaluated in 2021 (Prime Giant and Sweetheart). Data are the mean ± standard error (n = 3). (*) indicates significant differences among MeJA-treated and control samples (Student's unpaired t-test; * $p < 0.05$, ** $p < 0.01$, ns non significant).

A similar pattern in the evolution of cracking under controlled conditions was observed when the experiment was repeated in both cultivars under similar conditions in the last productive cycle studied in 2022 (Figure 3A,B).

In the case of the 'Prime Giant' cultivar, the cracking showed a small increase as the fruit matured on the tree, while, again, 'Sweetheart' showed a higher incidence of cracking after full fruit coloration than that shown in the other ripening stages studied. This trend was also observed in the previous growing cycles (2021) since the 'Sweetheart' cultivar also showed lower resilience to fruit cracking under controlled conditions. Similar to previous crop cycles, MeJA significantly reduced the incidence of cracking for both the 'Prime Giant' (Figure 3A) and 'Sweetheart' cultivars (Figure 3B). The observed differences between the studied production cycles of the 'Sweetheart' cultivar could be due to a less advanced stage of maturity at the time of sampling, as shown below regarding the fruit quality parameters at harvest. In this sense, a different ethylene production on the tree [10] and the fruit firmness [17], as well as a lower soluble solid content and acidity, could reduce the susceptibility of the fruit to cracking [7]. In fact, a lower incidence of cracking is observed in sweet cherries when the epidermis, hypodermis, and parenchyma cells exhibit larger cell sizes at the latter ripening stages, highlighting the significance of the flexibility and elasticity of the epidermis to reduce this disorder [4,35]. For this reason, if the fruit evolution is delayed, the incidence of the impact of cracking on cherries subjected to different environmental stresses, especially persistent rainfalls, would also be delayed, as observed when comparing Figures 2D and 3B.

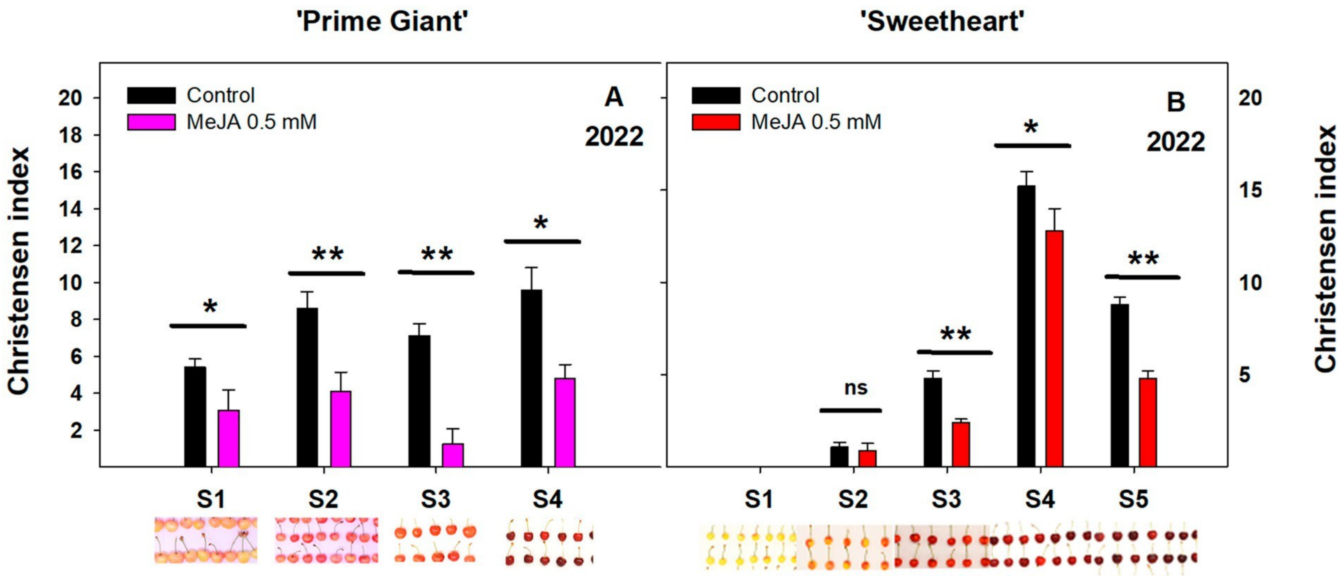

Figure 3. (**A**,**B**) Cracking incidence (Christensen index) during development on tree (%) of two sweet cherry cultivars evaluated in 2022 (Prime Giant and Sweetheart). Data are the mean ± standard error (n = 3). (*) indicates significant differences among MeJA-treated and control samples (Student's unpaired t-test; * $p < 0.05$, ** $p < 0.01$, ns non significant).

3.3. Effect of Exogenous MeJA Preharvest Treatments on Quality Parameters at Harvest

Sweet cherries that are harvested at the right stage of maturity, with the appropriate firmness, colour, and sweetness, have a longer shelf life and can reach a higher price in the market [36]. MeJA preharvest treatments resulted in higher fruit firmness values at harvest for all the evaluated cultivars, including 'Early Lory', 'Prime Giant', and 'Sweetheart', across multiple growing cycles (Table 1). However, the 'Staccato' cultivar, as well as 'Prime Giant' and 'Sweetheart', during the 2022 and 2020 growing cycles, respectively, did not exhibit significant differences ($p > 0.05$) in the fruit firmness following the preharvest treatments with MeJA. A similar positive effect, displaying a higher fruit firmness after preharvest MeJA treatments, was reported [17,37] in most of the sweet cherry cultivars evaluated, including 'Sweetheart', while in other cultivars, such as '0900 Ziraat', the fruit firmness was unaffected or showed lower values in MeJA-treated fruit at harvest after different preharvest MeJA applications [19]. In this study, it appears that an increase in firmness is correlated with a reduction in the susceptibility to cracking in most of the evaluated cultivars. This relationship has also been reported by Erogul [38] and Yildirim et al. [39], who investigated the impact of preharvest treatments involving calcium or gibberellins, respectively, on cracking susceptibility. However, it is worth noting that this association may be influenced by specific cultivars and species, as suggested by Balbotin et al. [17] and Erogul [38].

Colour is one of the most important characteristics that buyers and consumers use to determine the maturity and ripeness of fruit [40]. Sweet cherry cultivars differ in their optimal colour at harvest, but, in general, sweet cherries with a deep red colour are preferred because they are associated with sweetness, flavour, and a high nutritional value [41]. In general, preharvest treatments with MeJA delayed the colour evolution in most of the cultivars studied in this research at harvest time (Table 1). Different sweet cherry cultivars, such as 'Prime Giant', 'Sweetheart', and 'Staccato', showed a significant delay ($p < 0.05$) in the evolution of CIE h° compared to the control fruit. However, similar values were observed in the 'Early Lory' cultivar treated with MeJA compared to the control batches at harvest. On the other hand, it is important to highlight that the cultivar 'Sweetheart' was harvested in 2020 and 2022 with a very similar colour, indicating a similar effect of reducing the cracking incidence in both growing seasons (Figures 1D and 3B). Supporting these results, it is easy to see in Figure 3B that the highest cracking was observed at ripening stage S4 (2022), while in 2021, sweet cherries harvested at that stage (S4) already showed increased cracking resilience (Figure 2D). This was probably because a later stage of ripening could induce cracking tolerance in different cultivars, as has already been described in this study and which is in agreement with other preharvest sweet cherry studies, which indicated that a later ripening stage shows lower turgor pressure after stage III, inducing resilience to rainfall cracking [42]. In this sense, the TSS in the 'Sweetheart' cultivar were also higher in the control fruit in 2021 compared to the 2022 harvest. This indicates that the 'Sweetheart' cherries in 2021 had advanced maturity compared with that observed in 2022 at a similar colour stage, which is in consonance with the lower TA level found in 2021 compared to 2022 (Table 1).

The effect of the preharvest MeJA treatments, in general, had a greater impact on delaying sugar accumulation than affecting the TA parameter compared to the control fruits. In fact, with respect to the TA, no significant differences were found in most of the cultivars and growing seasons studied (Table 1). However, a significant effect was observed in most of the MeJA-treated batches studied during the four growing seasons, delaying the TSS accumulation compared to the control fruit. Balbotín et al. [17] and Saracoglu et al. [37] conducted studies on the 'Regina', 'Sweetheart', '0900 Ziraat', and 'Bing' sweet cherry cultivars and found that MeJA-treated fruit exhibited lower levels of total soluble solids (TSS) compared to the control group. However, there were no significant differences in terms of the titratable acidity (TA) content in the MeJA-treated fruit. These observations were in consonance with the results obtained from our analysis in the present study. On the other hand, Faizy et al. [19] observed a distinct pattern in the '0900 Ziraat' cultivar, where the application of MeJA not only delayed the accumulation of TSS but also postponed the accumulation of TA at two different harvest times. We observed the same effect in 2021, but only for the 'Sweetheart' cultivar, where a significant delay ($p < 0.05$) in the TA was recorded. It is important to note that preharvest treatments with MeJA have been demonstrated to impact the expression of multiple genes associated with fruit ripening, resulting in a delayed ripening process in peaches, as demonstrated by [43]. In contrast, Shafiq et al. [44] found that the preharvest MeJA treatment did not have a significant effect on the TSS and TA in apples. Conversely, various authors have reported an increase in the TSS content following the application of MeJA in peaches, blackberries, and raspberries [45–47]. Therefore, the effect on the TSS and TA in MeJA-treated fruit may vary depending on the ripening stage at which the fruit is evaluated and the species being tested.

Polyphenol accumulation is related to a decrease in the CIE h°, as reported by different authors [41,48]. The CIE h° values of the MeJA-treated sweet cherries from all four cultivars

were higher compared to the control fruit (Table 1). These findings suggest that the MeJA treatments generally delayed the fruit skin colour development in all the cultivars, although the observed impact on polyphenols was not statistically significant ($p > 0.05$). However, some specific cultivars in some of the growing cycles studied also showed a significant delay in total polyphenol accumulation (Table 2). Balbotín et al. [17] reported that the application of 0.4 mM MeJA on sweet cherries resulted in a decrease in the CIE h° after preharvest treatments, while Faizy et al. [19] observed redder sweet cherries after treatments with 2 mM MeJA on the '0900 Ziraat' cultivar. However, our data showed the opposite effect

with a 0.5 mM MeJA treatment on the total polyphenols. Several other authors did not observe an increase in polyphenols with different MeJA concentrations or a decrease in the CIE h° [38,49,50].

Table 1. Effects of preharvest methyl jasmonate treatments at a 0.5 mM concentration on fruit colour, total polyphenols, firmness, total soluble solids, and titratable acidity at harvest time.

Fruit Firmness (N mm^{-1})			
Cultivar	**Year**	**Control**	**MeJA 0.5 mM**
'Early Lory'	2019	1.10 ± 0.04	1.25 ± 0.04 *
'Prime Giant'	2019	1.77 ± 0.07	1.91 ± 0.08 *
'Prime Giant'	2021	1.31 ± 0.04	1.51 ± 0.07 *
'Prime Giant'	2022	1.19 ± 0.05	1.21 ± 0.05 ^{ns}
'Staccato'	2020	2.84 ± 0.11	2.88 ± 0.12 ^{ns}
'Sweetheart'	2020	1.94 ± 0.07	1.99 ± 0.07 ^{ns}
'Sweetheart'	2021	1.78 ± 0.09	2.08 ± 0.08 *
'Sweetheart'	2022	1.73 ± 0.08	2.17 ± 0.09 **
Fruit Colour (CIE *h*°)			
Cultivar	**Year**	**Control**	**MeJA 0.5 mM**
'Early Lory'	2019	22.50 ± 0.40	21.93 ± 0.8 ^{ns}
'Prime Giant'	2019	15.65 ± 0.65	18.61 ± 0.49 *
'Prime Giant'	2021	13.37 ± 0.31	14.32 ± 0.43 **
'Prime Giant'	2022	21.50 ± 0.52	23.92 ± 0.59 *
'Staccato'	2020	16.24 ± 0.55	18.73 ± 0.72 *
'Sweetheart'	2020	16.58 ± 0.58	18.19 ± 0.70 *
'Sweetheart'	2021	20.64 ± 0.20	22.48 ± 0.32 *
'Sweetheart'	2022	17.49 ± 0.34	19.67 ± 0.25 **
Total Soluble Solids (g 100 g^{-1})			
Cultivar	**Year**	**Control**	**MeJA 0.5 mM**
'Early Lory'	2019	13.05 ± 0.19	11.88 ± 0.04 *
'Prime Giant'	2019	22.96 ± 0.59	18.50 ± 0.45 **
'Prime Giant'	2021	17.56 ± 0.18	17.83 ± 0.11 ^{ns}
'Prime Giant'	2022	22.75 ± 0.41	22.06 ± 0.45 ^{ns}
'Staccato'	2020	21.05 ± 0.05	20.17 ± 0.26 *
'Sweetheart'	2020	22.42 ± 0.07	22.37 ± 0.10 ^{ns}
'Sweetheart'	2021	19.61 ± 0.35	18.90 ± 0.16 *
'Sweetheart'	2022	19.03 ± 0.18	18.21 ± 0.18 *
Titratable Acidity (g 100 g^{-1})			
Cultivar	**Year**	**Control**	**MeJA 0.5 mM**
'Early Lory'	2019	1.12 ± 0.01	1.07 ± 0.01 ^{ns}
'Prime Giant'	2019	1.20 ± 0.05	1.31 ± 0.03 ^{ns}
'Prime Giant'	2021	1.09 ± 0.01	1.11 ± 0.01 ^{ns}
'Prime Giant'	2022	1.52 ± 0.01	1.53 ± 0.02 ^{ns}
'Staccato'	2020	1.21 ± 0.01	1.28 ± 0.01 *
'Sweetheart'	2020	1.23 ± 0.02	1.24 ± 0.01 ^{ns}
'Sweetheart'	2021	1.33 ± 0.02	1.24 ± 0.03 *
'Sweetheart'	2022	1.44 ± 0.03	1.48 ± 6.12 × 10$^{-3\ ns}$

Data are the mean ± standard error (n = 3). (*) indicate significant differences among MeJA-treated and control samples (Student's unpaired *t*-test; * p < 0.05, ** p < 0.01, ^{ns} non significant).

MeJA-induced anthocyanin accumulation has been demonstrated in many species, such as strawberries [51], apples [52], loquat [53,54], mango [55], and pomegranate arils [56]. However, all the studies regarding sweet cherries have a different number of applications and different key moments of application. Therefore, the difference observed by the different authors could be due to different moments of application during sweet cherry development on the tree and a cultivar-dependent effect. However, it could also be due to the different concentrations of MeJA applied. In this sense, our research team already demonstrated that a higher concentration of MeJA could delay and even stop table grape colour evolution, while lower concentrations of MeJA were capable of increasing the anthocyanin content, reducing the CIE *h*° [57].

The pedicel, which attaches the cherry to the tree, serves as a vital connection point for nutrient uptake and water transport, influencing fruit development [58]. Pedicel browning in sweet cherries has been linked to fruit ripening and dehydration, as reported by [59]. Additionally, environmental factors, such as the use of plastic cover trees, have been suggested to affect the condition of sweet cherry pedicels, as indicated by [33]. These authors compared fruit obtained from uncovered trees and those from covered trees. Consistently, the fruit from the uncovered trees exhibited higher levels of pedicel browning, which serves as an indicator of condition defects. These findings highlight the importance of providing adequate protection to minimize stress on the pedicels and maintain their freshness. The MeJA-treated fruits generally displayed lower values of pedicel browning at harvest, as shown in (Table 2). However, no significant differences ($p > 0.05$) were observed among all the studied cultivars, except for 'Prime Giant' in the 2019 season. The overall incidence of pedicel browning was not high at harvest, as this condition typically develops during the postharvest storage of sweet cherries [59]. However, a recent review described the effect of MeJA in maintaining the chlorophyll content and reducing the oxidation of this molecule, thus preserving the greenness of the tissues [60]. In this sense, several authors of studies on different horticulture products also described that preharvest [61] and postharvest [62] MeJA treatments positively impacted plant tolerance by modifying the antioxidant defence mechanism and reducing chlorophyll loss.

Table 2. Effects of preharvest methyl jasmonate treatments at a 0.5 mM concentration on fruit total polyphenols and stem quality at harvest time.

Total Polyphenols (mg 100 g^{-1})			
Cultivar	**Year**	**Control**	**Meja 0.5 mM**
'Early Lory'	2019	99.48 ± 3.91	90.75 ± 2.77 *
'Prime Giant'	2019	87.15 ± 4.16	78.42 ± 7.65 *
'Prime Giant'	2021	98.39 ± 6.10	102.57 ± 2.55 ^{ns}
'Prime Giant'	2022	91.45 ± 5.67	84.56 ± 3.45 ^{ns}
'Staccato'	2020	81.66 ± 2.98	73.46 ± 1.44 *
'Sweetheart'	2020	75.07 ± 2.98	73.60 ± 3.44 ^{ns}
'Sweetheart'	2021	109.73 ± 9.74	100.64 ± 9.77 ^{ns}
'Sweetheart'	2022	85.97 ± 3.21	83.56 ± 2.97 ^{ns}
Pedicel Browning (Scale 0–4)			
Cultivar	**Year**	**Control**	**Meja 0.5 mM**
'Early Lory'	2019	0.75 ± 0.20	0.72 ± 0.12 ^{ns}
'Prime Giant'	2019	0.81 ± 0.17	0.39 ± 0.16 **
'Prime Giant'	2021	0.83 ± 0.09	0.70 ± 0.10 ^{ns}
'Prime Giant'	2022	0.07 ± 0.02	0.06 ± 0.02 ^{ns}
'Staccato'	2020	0.20 ± 0.03	0.16 ± 0.02 ^{ns}
'Sweetheart'	2020	0.18 ± 0.01	0.19 ± 0.01 ^{ns}
'Sweetheart'	2021	0.62 ± 0.09	0.66 ± 0.07 ^{ns}
'Sweetheart'	2022	0.06 ± 0.02	0.05 ± 0.02 ^{ns}

Data are the mean ± standard error ($n = 3$). (*) indicate significant differences among MeJA-treated and control samples (Student's unpaired t-test; * $p < 0.05$, ** $p < 0.01$, ^{ns} non significant).

4. Conclusions

MeJA could effectively reduce sweet cherry cracking during ripening on the tree and at harvest time. Medium and advanced ripening stages before harvest time were more susceptible to fruit cracking. MeJA preharvest treatments delayed fruit ripening, increasing the fruit firmness and delaying the colour, soluble solids, and total acidity evolution compared to the control fruit at harvest. For this reason, MeJA, as a preharvest treatment, could be an efficient tool to reduce abiotic stress and delay ripening during fruit development on the tree, improving the fruit quality at harvest.

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References

1. Huang, W.; Gao, Z.; Wang, L.; Zhang, Y.; Fu, X. Advances in Research on Cherry Fruit Cracking: Causes, Prevention and Control. *Front. Plant Sci.* **2019**, *10*, 986.
2. Moing, A.; Renaud, C.; Christmann, H.; Fouilhaux, L.; Tauzin, Y.; Zanetto, A. Is there a relation between changes in osmolarity of cherry fruit flesh or skin and fruit cracking susceptibility? *J. Am. Soc. Hortic. Sci.* **2004**, *129*, 635–641. [CrossRef]
3. Pantelidis, G.; Mavromatis, T.; Drogoudi, P. Consecutive wet days may impede fruit quality of peach and nectarine and cause fruit drop. *Sci. Hortic.* **2021**, *282*, 110011. [CrossRef]
4. Correia, S.; Schouten, R.; Silva, A.P.; Gonçalves, B. Sweet cherry fruit cracking mechanisms and prevention strategies: A review. *Sci. Hortic.* **2018**, *240*, 369–377. [CrossRef]
5. Measham, P.F.; Bound, S.A.; Gracie, A.J.; Wilson, S.J. Crop load manipulation and fruit cracking in sweet cherry (*Prunus avium* L.). *Adv. Hortic. Sci.* **2002**, *26*, 25–31.
6. Blanco, V.; Blaya-Ros, P.J.; Torres-Sánchez, R.; Domingo, R. Irrigation and Crop Load Management Lessen Rain-Induced Cherry Cracking. *Plants* **2022**, *11*, 3249. [CrossRef]
7. Pereira, S.; Silva, V.; Bacelar, E.; Guedes, F.; Silva, A.P.; Ribeiro, C.; Gonçalves, B. Cracking in sweet cherry cultivars Early Bigi and Lapins: Correlation with quality attributes. *Plants* **2020**, *9*, 1557. [CrossRef]
8. Giné-Bordonaba, J.; Echeverria, G.; Ubach, D.; Aguiló-Aguayo, I.; López, M.L.; Larrigaudière, C. Biochemical and physiological changes during fruit development and ripening of two sweet cherry varieties with different levels of cracking tolerance. *Plant Physiol. Biochem.* **2017**, *111*, 216–225. [CrossRef]
9. Yamaguchi, M.; Sato, I.; Ishiguro, M. Influences of epidermal cell sizes and flesh firmness on cracking susceptibility in sweet cherry (*Prunus avium* L.) cultivars and selections. *J. Jpn. Soc. Hortic. Sci.* **2002**, *71*, 738–746.
10. Knoche, M.; Peschel, S. Water on the surface aggravates microscopic cracking of the sweet cherry fruit cuticle. *J. Am. Soc. Hortic. Sci.* **2006**, *131*, 192–200. [CrossRef]
11. Pino, S.; Palma, M.; Sepúlveda, Á.; Sánchez-Contreras, J.; Moya, M.; Yuri, J.A. Effect of Rain Cover on Tree Physiology and Fruit Condition and Quality of 'Rainier', 'Bing' and 'Sweetheart' Sweet Cherry Trees. *Horticulturae* **2023**, *9*, 109. [CrossRef]
12. Blanco, V.; Zoffoli, J.P.; Ayala, M. High tunnel cultivation of sweet cherry (*Prunus avium* L.): Physiological and production variables. *Sci. Hortic.* **2019**, *251*, 108–117. [CrossRef]
13. Matteo, M.; Zoffoli, J.P.; Ayala, M. Calcium Sprays and Crop Load Reduction Increase Fruit Quality and Postharvest Storage in Sweet Cherry (*Prunus avium* L.). *Agronomy* **2022**, *12*, 829. [CrossRef]
14. Correia, S.; Oliveira, I.; Queirós, F.; Ribeiro, C.; Ferreira, L.; Luzio, A.; Silva, A.P.; Gonçalves, B. Preharvest application of seaweed based biostimulant reduced cherry (*Prunus avium* L.) cracking. *Procedia Environ. Sci.* **2015**, *29*, 251–252. [CrossRef]
15. Usenik, V.; Kastelec, D.; Štampar, F. Physicochemical changes of sweet cherry fruits related to application of gibberellic acid. *Food Chem.* **2005**, *90*, 663–671. [CrossRef]
16. Correia, S.; Queirós, F.; Ferreira, H.; Morais, M.C.; Afonso, S.; Silva, A.P.; Gonçalves, B. Foliar application of calcium and growth regulators modulate sweet cherry (*Prunus avium* L.) tree performance. *Plants* **2020**, *9*, 410. [CrossRef]
17. Balbontín, C.; Gutiérrez, C.; Wolff, M.; Figueroa, C.R. Effect of abscisic acid and methyl jasmonate preharvest applications on fruit quality and cracking tolerance of sweet cherry. *Chil. J. Agric. Res.* **2018**, *78*, 438–446. [CrossRef]
18. Yao, H.; Tian, S. Effects of pre-and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. *Postharvest Biol. Technol.* **2005**, *35*, 253–262. [CrossRef]

19. Faizy, A.H.; Ozturk, B.; Aglar, E.; Yıldız, K. Role of methyl jasmonate application regime on fruit quality and bioactive compounds of sweet cherry at harvest and during cold storage. *J. Food Process.* **2021**, *45*, e15882. [CrossRef]
20. Christensen, J.V. Rain-induced cracking of sweet cherries: Its causes and prevention. In *Cherries: Crop Physiology, Production and Uses*; Webster, A.D., Looney, N.E., Eds.; CAB International: Wallingford, UK, 2006; pp. 297–327.
21. Kader, A.A. Methods of gas mixing, sampling and analysis. In *Postharvest Technology of Horticultural Crops*; Kader, A.A., Ed.; University of California, Division of Agriculture and Natural Resources: Oakland, CA, USA, 1992; pp. 93–95.
22. Carrión-Antolí, A.; Martínez-Romero, D.; Guillén, F.; Zapata, P.J.; Serrano, M.; Valero, D. Melatonin pre-harvest treatments leads to maintenance of sweet cherry quality during storage by increasing antioxidant systems. *Front. Plant Sci.* **2022**, *13*, 984.
23. Lezoul, N.E.H.; Belkadi, M.; Habibi, F.; Guillén, F. Extraction processes withseveral solvents on total bioactive compounds in different organs of three medicinal plants. *Molecules* **2020**, *25*, 4672. [CrossRef] [PubMed]
24. Wani, A.A.; Singh, P.; Gul, K.; Wani, M.H.; Langowski, H.C. Sweet cherry (*Prunus avium*): Critical factors affecting the composition and shelf life. *Food Packag. Shelf Life* **2014**, *1*, 86–99. [CrossRef]
25. Blažek, J.; Zelený, L.; Suran, P. Sweet cherry research world overview 2015–2017. *Hort. Sci.* **2022**, *49*, 121–146. [CrossRef]
26. Quero-García, J.; Letourmy, P.; Campoy, J.A.; Branchereau, C.; Malchev, S.; Barreneche, T.; Dirlewanger, E. Multi-year analyses on three populations reveal the first stable QTLs for tolerance to rain-induced fruit cracking in sweet cherry (*Prunus avium* L.). *Hort. Res.* **2021**, *8*, 136. [CrossRef]
27. Serrano, M.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Valero, D. Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *J. Agric. Food Chem.* **2005**, *53*, 2741–2745. [CrossRef] [PubMed]
28. Demirsoy, L.; Bilgener, S. The effects of preharvest calcium hydroxide applications on cracking in '0900 Ziraat', 'Lambert' and 'Van' sweet cherries. *Acta Hortic.* **1998**, *468*, 657–662. [CrossRef]
29. Rohwer, C.L.; Erwin, J.E. Horticultural applications of jasmonates: A review. *J. Hort. Sci. Biotech.* **2008**, *83*, 283–304. [CrossRef]
30. Demirsoy, L.; Demirsoy, H. The epidermal characteristics of fruit skin of some sweet cherry cultivars in relation to fruit cracking. *Pak. J. Bot.* **2004**, *34*, 725–731.
31. Winkler, A.; Blumenberg, I.; Schürmann, L.; Knoche, M. Rain cracking in sweet cherries is caused by surface wetness, not by water uptake. *Sci. Hortic.* **2020**, *269*, 10940023. [CrossRef]
32. Etiopi, C.; Monari, W. Effectiveness of new rainprotection systems on cracking, ripening date and fruit quality of sweet cherry cultivars. *Acta Hortic.* **2017**, *1161*, 213–220.
33. Bustamante, M.; Muñoz, A.; Romero, I.; Osorio, P.; Mánquez, S.; Arriola, R.; Reyes-Díaz, M.; Ribera-Fonseca, A. Impact of potassium pre-harvest applications on fruit quality and condition of sweet cherry (*Prunus avium* L.) cultivated under plastic covers in southern chile orchards. *Plants* **2021**, *10*, 2778. [CrossRef] [PubMed]
34. Gonçalves, B.; Silva, V.; Bacelar, E.; Guedes, F.; Ribeiro, C.; Silva, A.P.; Pereira, S. Orchard net covers improve resistance to cherry cracking disorder. *Foods* **2023**, *12*, 543. [CrossRef] [PubMed]
35. Brüggenwirth, M.; Knoche, M. Mechanical properties of skins of sweet cherry fruit of differing susceptibilities to cracking. *J. Am. Soc. Hortic. Sci.* **2016**, *141*, 162–168. [CrossRef]
36. Valero, D.; Serrano, M. *Postharvest Biology and Technology for Preserving Fruit Quality*; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2010.
37. Saracoglu, O.; Ozturk, B.; Yildiz, K.; Kucuker, E. Pre-harvest methyl jasmonate treatments delayed ripening and improved quality of sweet cherry fruits. *Sci. Hortic.* **2017**, *226*, 19–23. [CrossRef]
38. Erogul, D. Effect of preharvest calcium treatments on sweet cherry fruit quality. *Not. Bot. Horti Agrobot. Cluj Napoca* **2014**, *42*, 150–153. [CrossRef]
39. Yildirim, A.N.; Koyuncu, F. The effect of gibberellic acid applications on the cracking rate and fruit quality in the '0900 Ziraat'sweet cherry cultivar. *Afr. J. Biotechnol.* **2010**, *9*, 6307–6311.
40. Díaz-Mula, H.M.; Serrano, M.; Valero, D. Alginate coatings preserve fruit quality and bioactive compounds during storage of sweet cherry fruit. *Food Bioproc. Tech.* **2012**, *5*, 2990–2997. [CrossRef]
41. Serrano, M.; Díaz-Mula, H.M.; Zapata, P.J.; Castillo, S.; Guillén, F.; Martínez-Romero, D.; Valverde, J.M.; Valero, D. Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. *J. Agric. Food Chem.* **2009**, *57*, 3240–3246. [CrossRef]
42. Knoche, M.; Grimm, E.; Schlegel, H.J. Mature sweet cherries have low turgor. *J. Am. Soc. Hortic. Sci.* **2014**, *139*, 3–12. [CrossRef]
43. Ruiz, K.B.; Trainotti, L.; Bonghi, C.; Ziosi, V.; Costa, G.; Torrigiani, P. Early methyl jasmonate application to peach delays fruit/seed development by altering the expression of multiple hormone-related genes. *J. Plant Growth Regul.* **2013**, *32*, 852–864. [CrossRef]
44. Shafiq, M.; Singh, Z.; Ahmad, S.K. Pre-harvest spray application of methyl jasmonate improves red blush and flavonoid content in 'Cripps Pink' apple. *J. Hortic. Sci. Biotechnol.* **2011**, *86*, 422–430. [CrossRef]
45. Meng, X.; Han, J.; Wang, Q.; Tian, S. Changes in physiology and quality of peach fruits treated by methyl jasmonate under low temperature stress. *Food Chem.* **2009**, *114*, 1028–1035. [CrossRef]
46. Wang, S.Y.; Bowman, L.; Ding, M. Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (*Rubus* sp.) and promotes antiproliferation of human cancer cells. *Food Chem.* **2008**, *107*, 1261–1269. [CrossRef]
47. Wang, S.Y.; Zheng, W. Preharvest application of methyl jasmonate increases fruit quality and antioxidant capacity in raspberries. *Int. J. Food Sci. Technol.* **2005**, *40*, 187–195. [CrossRef]

48. Gonçalves, B.; Silva, A.P.; Moutinho-Pereira, J.; Bacelar, E.; Rosa, E.; Meyer, A.S. Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.). *Food Chem.* **2007**, *103*, 976–984. [CrossRef]
49. Kondo, S. Roles of jasmonates in fruit ripening and environmental stress. In Proceedings of the XI International Symposium on Plant Bioregulators in Fruit Production. *Acta Hortic.* **2009**, *884*, 711–716.
50. Kucuker, E.; Ozturk, B. The effects of aminoethoxyvinylglycine and methyl jasmonate on bioactive compounds and fruit quality of 'North Wonder' sweet cherry. *Afr. J. Tradit. Complement. Altern. Med.* **2015**, *12*, 114–119. [CrossRef]
51. Concha, C.M.; Figueroa, N.E.; Poblete, L.A.; Oñate, F.A.; Schwab, W.; Figueroa, C.R. Methyl jasmonate treatment induces changes in fruit ripening by modifying the expression of several ripening genes in *Fragaria chiloensis* fruit. *Plant Physiol. Biochem.* **2013**, *70*, 433–444. [CrossRef]
52. Öztürk, B.; Özkan, Y.; Yildiz, K. Methyl jasmonate treatments influence bioactive compounds and red peel color development of Braeburn apple. *Turk. J. Agric. For.* **2014**, *38*, 688–699. [CrossRef]
53. Khan, A.S.; Singh, Z. Methyl jasmonate promotes fruit ripening and improves fruit quality in Japanese plum. *J. Hortic. Sci. Biotechnol.* **2007**, *82*, 695–706. [CrossRef]
54. Kucuker, E.; Ozturk, B.; Celik, S.M.; Aksit, H. Pre-harvest spray application of methyl jasmonate plays an important role in fruit ripening, fruit quality and bioactive compounds of Japanese plums. *Sci. Hortic.* **2014**, *176*, 162–169. [CrossRef]
55. Sudheeran, P.K.; Love, C.; Feygenberg, O.; Maurer, D.; Ovadia, R.; Oren-Shamir, M.; Alkan, N. Induction of red skin and improvement of fruit quality in 'Kent', 'Shelly' and 'Maya' mangoes by preharvest spraying of prohydrojasmon at the orchard. *Postharvest Biol. Technol.* **2019**, *149*, 18–26. [CrossRef]
56. García-Pastor, M.E.; Serrano, M.; Guillén, F.; Zapata, P.J.; Valero, D. Preharvest or a combination of preharvest and postharvest treatments with methyl jasmonate reduced chilling injury, by maintaining higher unsaturated fatty acids, and increased aril colour and phenolics content in pomegranate. *Postharvest Biol. Technol.* **2020**, *167*, 111226. [CrossRef]
57. García-Pastor, M.E.; Serrano, M.; Guillén, F.; Castillo, S.; Martínez-Romero, D.; Valero, D.; Zapata, P.J. Methyl jasmonate effects on table grape ripening, vine yield, berry quality and bioactive compounds depend on applied concentration. *Sci. Hortic.* **2019**, *247*, 380–389. [CrossRef]
58. Blanusa, T.; Else, M.A.; Atkinson, C.J.; Davies, W.J. The regulation of sweet cherry fruit abscission by polar auxin transport. *J. Plant Growth Regul.* **2005**, *45*, 189–198. [CrossRef]
59. Martínez-Romero, D.; Alburquerque, N.; Valverde, J.M.; Guillén, F.; Castillo, S.; Valero, D.; Serrano, M. Postharvest sweet cherry quality and safety maintenance by *Aloe vera* treatment: A new edible coating. *Postharvest Biol. Technol.* **2006**, *39*, 93–100. [CrossRef]
60. Hewedy, O.A.; Elsheery, N.I.; Karkour, A.M.; Elhamouly, N.; Arafa, R.A.; Mahmoud, G.A.E.; Dawood, M.F.-A.; Hussein, W.E.; Mansour, A.; Amin, D.H.; et al. Jasmonic acid regulates plant development and orchestrates stress response during tough times. *Environ. Exp. Bot.* **2023**, *208*, 105260. [CrossRef]
61. Swain, R.; Sahoo, S.; Behera, M.; Rout, G.R. Instigating prevalent abiotic stress resilience in crop by exogenous application of phytohormones and nutrient. *Front. Plant Sci.* **2023**, *14*, 1104874. [CrossRef] [PubMed]
62. Duarte-Sierra, A.; Thomas, M.; Arul, J. Ethanol and Methyl Jasmonate Fumigation Impact on Quality, Antioxidant Capacity, and Phytochemical Content of Broccoli Florets during Storage. *Horticulturae* **2023**, *9*, 465. [CrossRef]

4.4. Publicación IV (Transcripción literal)

IV

Melatonin as a new postharvest treatment for increasing cut carnation (*Dianthus caryophyllus* L.) vase life

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Melatonin as a new postharvest treatment for increasing cut carnation (*Dianthus caryophyllus* L.) vase life

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A R T I C L E I N F O

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Melatonin

A B S T R A C T

The marketability of cut flowers is directly affected by their vase life, which determines acceptability for commercial purposes. In carnations and other species of cut flowers, corolla is one of the most affected parts during flower senescence due to the petal withering which is accelerated by metabolic processes occurring after separation from the mother plant. Melatonin (MT) is a compound with antioxidant properties, naturally present in plant tissues that plays important roles in the regulation of different metabolic processes. In this research work the effect of different MT concentrations (0.01, 0.1 and 1 mM) on the vase life of cut carnations flowers cv. Baltico was evaluated. The greatest delay in senescence was observed with 0.1 mM MT concentration, increasing vase life up to 10 days more as compared to control carnations. Although all MT concentrations assayed significantly ($P < 0.05$) maintained initial levels of fresh weight, membrane stability index, bioactive compounds and antioxidant activity for longer time, the lowest concentrations were those that had the most relevant impact on vase life. The highest dose evaluated (1 mM) maintained all the parameters evaluated but showed the wilting symptoms earlier. For this reason, 0.1 MT concentration could be a tool capable of improving carnation vase life for longer time, increasing the commercial potential of this cut flower.

5. Introduction

Carnation (*Dianthus caryophyllus* L.) is a flower with important economic and ornamental values in Mediterranean countries and worldwide (Ranjbar and Ahmadi, 2015). In addition, white carnation is one of the most demanded in comparison with the rest of coloured carnations that can be found for commercial purposes (Ebrahimzadeh et al., 2008). This plant species is very sensitive to exogenous ethylene but the response to ethylene exposure is also cultivar dependent (Serrano et al., 1991, 1999). Flower senescence in most carnation cultivars is characterized by autocatalytic ethylene production and subsequent petal inrolling (Serrano et al., 1991, 2001; Satoh et al., 2005). However, some carnation cultivars have low or even absence of ethylene production (Wu et al., 1991; Serrano et al., 1991; Ebrahimzadeh et al., 2011), thus influencing their vase life (Nukui et al., 2004). Vase life is the most important quality criteria that influence the consumer demand of cut carnations although appearance, colour and uniformity of the corolla are also remarkable factors (Reid and Jiang, 2012; Scariot et al., 2014). Cut flowers are metabolically active after harvest, carrying out all their vital processes by using available substrates in their tissues (Yakimova et al., 1997). These energy requirements are partially provided by starch hydrolysis but some techniques as the application of ethylene inhibitors can be a useful tool to delay flower metabolism specially in carnations with a climacteric pattern (Serrano et al., 2001; Ebrahimzadeh et al., 2008). Increasing vase life and delaying flower senescence can be achieved by maintaining the normal rate of water absorption, preventing carbohydrate depletion, and reducing the oxidative stress, which are specially stimulated during postharvest senescence period (Halevy and Mayak, 1981; Ebrahimzadeh et al., 2008). Indeed, the maintenance of a strong antioxidant potential to scavenge reactive oxygen species (ROS) is associated with a longer vase life period in cut flower species (Ezhilmathi et al., 2007; Hassan and Ali, 2014a, 2014b; Aalifar et al., 2020; Rashidiani et al., 2020). In this sense, many advances have been made and the number of studies regarding the

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effect of new compounds on the antioxidant potential to eliminate ROS has increased in recent years. Special interest has received some natural compounds as alternatives to common chemicals (Solgi, 2018; Akhtar et al., 2021) since they do not compromise human health and have been reported as environmentally friendly. Some of these natural substances such as essential oils and moringa leaf extract, have been described as potential providers of extra energy demanded during development by cut flower tissues increasing carbon sources availability (Salmi et al., 2018; Hassan and Fetouha, 2019) and thus, delaying senescence process. In this context, melatonin (MT), polyamines or γ-aminobutyric acid (GABA), have been proposed as responsible of GABA shunt pathway activation, providing extra ATP and decreasing accumulation of ROS in vegetal tissues (Aghdam and Fard, 2017; Karimi et al., 2017; Mohammadi et al., 2020) specially under metabolic stress conditions (Aghdam et al., 2016a; Arnao and Herna´ndez-Ruiz, 2019).

MT (N-acetyl-5-methoxytryptamine), is an indole derivative involved in a wide range of cellular and physiological actions in plant processes, such as seed germination, root development, flowering, photosynthesis, leaf senescence and protective effect against biotic or abiotic stress (Arnao and Herna´ndez-Ruiz, 2019; Debnath et al., 2019; Sharma and Zheng, 2019). However, little information is available regarding the effect of MT on cut flowers vase life and senescence. In fact, as far as we know, only one report (Aghdam et al., 2019) studied the effect of MT treatment on increasing anthurium cut flowers resis- tance to chilling injury during cold storage. For this reason, carnation flowers were selected in this report since this specie is considered a model plant for the ornamental industry (Aalifar et al., 2020). Different MT concentrations were applied to cut carnation flowers, to study their effects on different cut flower quality parameters, with particular attention to the effect on delaying petals and sepals senescence.

6. Experimental design

6.1. *Plant material and treatments*

Cut carnation flowers (*Dianthus caryophyllus* cv. Baltico) were harvested from a commercial orchard in Murcia (Spain) at commercial harvesting stage. Then, flowers were transported to the laboratory with stems immersed in tap water on the harvest day. At the laboratory, 66 flowers were selected (discarding any flower with visual defects) for each treatment (MT at 0.01, 0.1 and 1 mM and distilled water as control) at the development stage 3 (Fig. 1). To select the optimal MT doses to test in the entire flower we carried out a previous study and different MT doses were tested on 'Baltico' carnation petals. 20 petals per MT dose (0.005, 0.01, 0.1 and 1 mM) were individually evaluated at room temperature in comparison with 20 petals in distilled water as control. Petal fresh weight (FW) was reduced, specially, for the 0.1 mM MT dose (Fig. S1). Melatonin (Sigma-Aldrich, USA, > 98 % M5250) was previ- ously dissolved for each concentration with 0.5 mL ethanol. Similar ethanol volume was added to the control solution. Stems were cut to 10 cm and placed individually in falcon tubes with 10 mL of the different treatments. Carnations were kept at room temperature of about 20 ˚C, relative humidity 65–70 % and a 12 h photoperiod using white fluorescent light with an intensity of 80.5 μmol m ^{2} s ^{1}. These conditions were maintained for four weeks, and distilled water or MT solutions were added to falcon tubes when necessary. Non-destructive measurements as vase life, FW and vase solution uptake (VSU) were evaluated almost daily in one lot of 30 flowers per treatment. For respiration rate and ethylene production another lot of 6 flowers per treatment was used exclusively to evaluate these parameters. The different solutions in each tube were replaced when necessary. Additionally, 30 flowers per treatment were divided in 5 lots of 6 flowers, to evaluate destructive parameters as membrane stability index (MSI), total polyphenol content (TPC) and total antioxidant activity (TAA) in petals or total chlorophyll content (TCC) in sepals after 5, 10, 15, 20, and 25 d of storage at room temperature. Also, the different parameters were evaluated in an extra lot of 10 carnations at the day of harvest. All parameters were evaluated in fresh samples except TAA and TPC content which were measured in petals separated from 6 flowers for each treatment, mixed, powdered in liquid nitrogen and stored at 80 ˚C for later analysis.

2.1 *Vase life*

Vase life was evaluated daily in each flower individually. This parameter was determined by the number of days in which flowers maintained their decorative properties, until carnations had no ornamental value due to the petal in-rolling or tip browning appearance (Satoh et al., 2005). This period in our study match with the 7th senescence stage (Fig. 1). Results were expressed as the means ± SE of 30 flowers per treatment.

2.2 *Fresh weight and vase solution uptake*

FW of each flower was expressed as percentage with respect to its initial FW which was assumed to be 100 %. Results were the mean ± SE of 30 flowers. To evaluate the VSU the falcon tube without the flower was weighted, as well as the weight of the flower and the solution. Results were expressed as the mean ± SE of 30 flowers per treatment and the following formula was used to calculate the VSU (mL d ^{1} g ^{1} FW) = $(W_{(t-1)}-W_{(t)}) / FW_{t=0}$, where W_t = solution weight (g) at t days (3, 4, 5, etc.), W_{t-1} =water weight (g) on the previous day and $FW_{t=0}$ = FW of the flower (g) on day 0.

2.3 *Respiration rate and ethylene production*

Respiration rate and ethylene production were measured in 6 flowers by placing each flower in 1 L glass jar hermetically sealed with a rubber stopper for 2 h. After that, one mL gas sample was taken from head space and injected into a Shimadzu TM 14A gas chromatograph (Kyoto, Japan) equipped with a thermal conductivity detector under the chromatographic conditions previously described by Medina-Santamarina et al. (2021) to quantify CO_2 concentration. Respiration rate was expressed as nmol of CO_2 released by kg ^{1} s ^{1} and results were the mean ± SE (n = 12).

Ethylene production was determined by injecting another mL gas sample taken from the same atmosphere into a Hewlett-Packard TM model 5890A gas chromatograph (Wilmington, DE), equipped with a flame ionization detector and a 3 m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. Chromatographic conditions were similar to those previously reported (Medina-Santamarina et al., 2021). Ethylene production was expressed

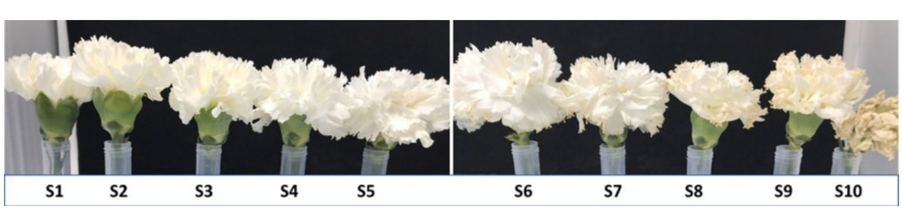

Fig. 1. Ten stages of flower development in carnation cultivar Baltico during vase life (growth: S1-S5, Senescence: S6-S10).

as nmol kg ^{1}s ^{1} and results were the mean ± SE (n = 12).

2.4 Membrane stability index

Four external petals were taken from each one of the 6 flowers sampled from each treatment and sampling date and from each petal one disc was cut with a corkborer (measuring 1 cm in diameter) and placed in testing tubes as indicated by Rashidiani et al. (2020) with small modifications. Thus, 12 testing tubes per treatment and sampling date containing 2 petal discs were used. These tubes were added with 15 mL ultrapure water and 6 tubes were placed in a warm bath (40 ˚C) for 30 min, cooled down to 25 ˚C and then the electrical conductivity (EC) was measured (C1). Then, a second series of 6 testing tubes was placed in a warm bath (100 ˚C) for 20 min and then EC was read after being cooled down rapidly in ice bath until 25 ˚C (C2). MSI was calculated using the following formula: MSI= [1 - (C1/C2)] 100 where C1 = EC after heat treatment at 40 ˚C and C2 = EC after exposure to 100 ˚C. (n = 6)

2.5 Total chlorophyll content

For chlorophyll measurement in sepals, six calix disks, each of 6.25 mm in diameter, were punched from each flower. The disks were placed immediately into 8 mL of 100 % methanol, and pigments were allowed to be extracted in the dark at 30 ˚C for 24 h. Absorbance of the extract was measured using spectrophotometer (1900 UV/ Vis, Shimadzu, Kyoto, Japan) at 652 and 665 nm (Porra et al., 1989). Results were the mean ± SE of six individual flowers per treatment (n = 12).

2.6 Total polyphenol content

To extract phenolic compounds, 0.5 *g* of petal tissue were homogenized with 20 mL of water: methanol (2:8, v/v) containing 2 mM NaF by using a homogenizer (Ultraturrax, T18 basic, IKA, Berlin, Germany) for 60 s. 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 in each extract) by using the Folin-Ciocalteu reagent as previously described for petals and other tissues by Lezoul et al. (2020). The results were expressed as g gallic acid equivalent (GAE) kg^{-1} and are the mean ± SE of measures performed in 6 individual flowers (n = 12).

2.7 Total antioxidant activity

The determination of the antiradical activity by the ABTS test was carried out using the method described by Lezoul et al. (2020). 10 mL of 50 mM phosphate buffer solution and 10 mL ethyl acetate were added to 1 *g* of petal tissue in three replicates and the mixture was homogenized for 1 min and then centrifuged at 10,000 rpm for 20 min at 4 ˚C. Then, the determination of the TAA was evaluated in the hydrophilic fraction and lipophilic fraction. The results were expressed in g trolox equivalent kg ^{1} FW with reference to the trolox calibration curve (n = 6).

2.8 Statistical analysis

The experiment was conducted in a complete randomized design. The analysis of variance (ANOVA) was performed, and data were ana- lysed using SPSS software package v. 20.0 for windows. Means ± SE values were compared by a Least Significant Difference (LSD) test at $P < 0.05$.

3 Results

3.1 Fresh weight and water uptake

FW increased from day 0 to day 5 (Fig. 2) due to petal opening manifested as an apparent corolla growth as flowers evolved from stage S3 to S5 (Fig. 1). From day 5, a slow decrease of FW occurred in control carnations which was accelerated from day 11. Carnations with stems submerged in the most concentrated MT solutions (0.1 and 1 mM) remained with a constant FW, ≈ 107 % of the initial stage, for longer time than the rest of the treatments (Fig. 2A). Nevertheless, it is worth noting that all melatonin treatments delayed significantly ($P < 0.05$) flower weight losses. Also, carnations in MT 0.1 mM solutions showed the highest FW in comparison with the rest of the treatments and especially significant in comparison with control flowers ($P < 0.05$) during the whole experiment. In fact, the FW percentage observed on d 21 of the vase period for control and 0.1 mM MT solution was 66.05 ± 5.47 and 97.27 ± 0.55 % respectively.

VSU was high and without significant changes during the first days of vase life of all carnation flowers and decreased sharply after 10 d in control carnations and after 15, 17 and 18 days in those treated with 0.01, 1 and 0.1 mM MT respectively. Nevertheless, all the concentra- tions maintained higher VSU during storage (Fig. 2B) in comparison with control carnations though a lower VSU was observed in 0.1 mM MT flowers during first period of storage.

3.2 Vase life

Vase life was affected by the different treatments applied. In fact, vase life was significantly ($P < 0.05$) higher when flowers were stored in falcon tubes containing the different melatonin solutions, compared to controls in distilled water (Fig. 3).

MT 0.1 mM was the best dose for carnations in this study with a vase life of 20 d in which stage 7 of senescence was reached, while the rest of the treatments reached this stage several days earlier (10, 16 and 12 days for control, 0.01 and 1 mM MT solutions respectively). Thus, 0.1 mM MT treatments led to a 2-fold increase in the storage time that flowers maintain an aesthetic value with respect to control ones. The melatonin effect on vase life can be clearly observed in the photographs of the evolution of the senescence process (Fig. 4). However, there was no dose-dependence in the range of melatonin concentration assayed, since 0.1 mM led to higher vase life than 0.01 mM but carnation petals of

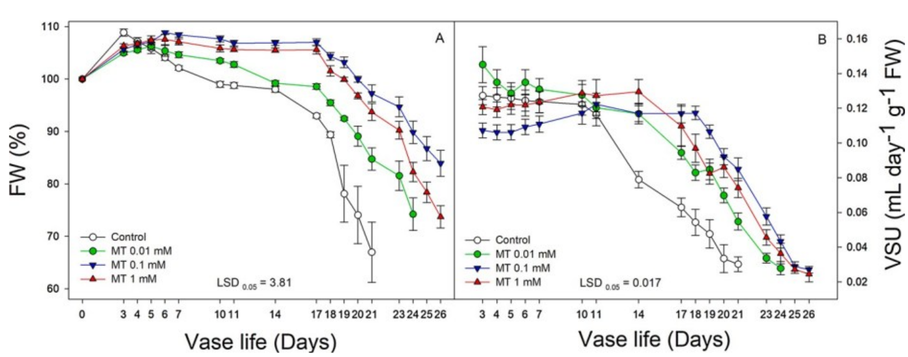

Fig. 2. Carnation fresh weight (FW) evolution (% initial FW) (A) and vase solution uptake (VSU) (B) of control and melatonin (MT, 0.01, 0.1 and 1 mM) in cut carnation flowers cv. Baltico during vase life period. Values are the means ± standard error (SE) (n = 30). LSD at $P < 0.05$ was used for means comparison.

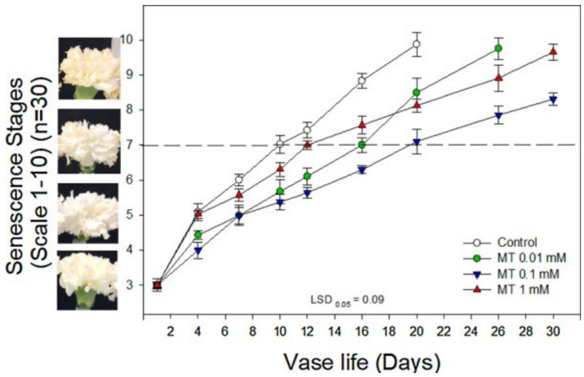

Fig. 3. Effects of melatonin treatments (MT, 0.01, 0.1 and 1 mM) and distilled water (control) on the evolution of the senescence process of carnation flowers during vase life period at room temperature. Values are the means ± standard error (SE) (n = 30). LSD at $P < 0.05$ was used for means comparison.

flowers that received the highest MT dose (1 mM) showed tip burn.

3.3 *Ethylene production, respiration rate and membrane stability index*

Ethylene production was very low in all carnation flowers during the whole vase life period, with values ranging from 3×10^{4} to 1×10^{3} nmol kg^{1} s^{1} and no significant differences were observed attributed to melatonin treatments (data not shown). Respiration rate in carnation flowers was similar at the beginning of the storage for all carnation flowers showing an increase until day 4 without significant differences among treatments (Fig. 5A).

However, from day 6 until the end of the experiment, lower respiration values were obtained when MT solutions were applied. The highest respiration rate was measured on control carnations while the lowest values were obtained on d 25 of the vase life period in 0.1 mM MT treatment.

At the beginning of the experiment, the MSI was 90 % and values decreased during vase life period for all evaluated treatments (Fig. 5B). However, compared with control flowers, significantly ($P < 0.05$) higher MSI values were found in the flowers treated with MT from day 5 until last sampling date. For instance, a MSI value of ≈ 53 % was reached on control flowers at day 15, while similar values were reached after 20 d in 0.01 and 1 mM MT treated flowers and after 25 d in those treated with 0.1 mM MT.

3.4 *Bioactive compounds and antioxidant activity*

TCC values in carnation sepals showed that the effect of different MT concentrations was significant ($P < 0.05$) throughout vase life of carnations (Fig. 6A).

TCC decreased in control carnations from day 0 to the end of vase life, while this decrease started at day 5 in 0.01 mM MT treated flowers and after 10 and 20 d in 1 and 0.1 mM MT treated flowers respectively. Thus, although all MT treatments delayed chlorophyll degradation in the carnation sepals as compared with controls, the highest effect was found for 0.1 mM MT concentration. The highest chlorophyll content was observed when 0.1 mM MT dose was applied after 15 d of vase life while control carnation sepals reached the lowest TCC as compared with MT doses at the same sampling date (Fig. 6 A).

Results showed a decreasing pattern for TPC along the experiment in all petal samples evaluated. However, this decrease evolved sharply in control flowers after 10 d postharvest storage and was significantly ($P < 0.05$) delayed when MT treatments were employed. The highest delay was observed for 0.1 mM MT treatments (Fig. 6 B).

TAA was evaluated in the hydrophilic (H-TAA) and lipophilic (L-TAA) fractions of petal extracts and, in general, both showed a decreasing pattern during vase life (Fig. 6 C and D). However, H-TAA and L-TAA values were significantly higher ($P < 0.05$) when different MT solutions were employed in comparison with control samples. There was an increase in H-TAA until the 10 d for control and 0.01 mM MT treatment which was delayed when higher MT doses were applied (Fig. 6C). After 10 days H-TAA decreased for control and 0.01 mM MT treatment while the higher doses employed maintained a higher H-TAA level for a longer period. In this sense, when 0.1 mM MT was used, carnation petals showed the highest H-TAA level during the whole vase life period. A similar pattern was observed when L-TAA was evaluated (Fig. 6D). L-TAA values were higher in carnation petals when different MT solutions were applied in comparison with control flowers, though 0.1 mM MT dose just maintained the highest L-TAA values at the end of the vase life period evaluated in comparison with other MT concentrations.

3.5 *Discussion*

Several studies reflected that flower senescence could be affected by the endogenous MT content. Despite variations between flower species, it seems that MT levels decreased along the development, specially at latter senescence stages (Murch et al., 2009; Zhao et al., 2017). In the present study we demonstrated that exogenous MT treatments exerted an anti-senescent effect in carnations increasing vase life in MT treated cut flowers and the highest effect was found for 0.1 mM dose. This finding was related with the FW evolution since MT treatments, maintained water relation during postharvest vase life for a longer period. An adequate balance between organs dehydration and the water uptake index is critical to maintain cell turgor in cut flowers (Rot and Friedman, 2010; Reid and Jiang, 2012). 'Baltico' carnations treated with 0.1 mM MT showed a higher FW during vase life period but a delayed VSU as compared with control flowers and other MT doses employed. Senescence evolution in cut flowers is affected through the balance transpiration/water uptake. For this reason, FW decreases when transpiration exceeds the VSU (Rot and Friedman, 2010; Ebrahimzadeh et al., 2011). However, regarding water balance in cut carnations, transpiration rate could be more critical for FW maintenance than VSU during vase life (Solgi, 2018; Hassan and Ali, 2014a). According to this, a lower flower transpiration in petals and other tissues could be affecting the VSU probably through a maintenance of the stomatal properties as has been recently reported (Mohamed et al., 2020).

Carnations are characterized by autocatalytic ethylene production which accelerates flower senescence phenomena also affecting other plant organs (Serrano et al., 1999; Ebrahimzadeh et al., 2008). However, 'Baltico' carnation flowers exhibited longer vase life with a low ethylene production pattern at basal level without a pronounced peak of ethylene synthesis and no associated increases of respiration rate as reported by 'Sandra' 'Killer' and 'Pilar' cultivars (Wu et al., 1991; Serrano et al., 1991; Ebrahimzadeh et al., 2011). However, respiration rate was higher in control than in MT treated flowers, also coincident with advanced senescent stages earlier reached in control carnations. The beginning of the senescence process requires the contribution of metabolic energy (Ebrahimzadeh et al., 2008; Reid and Jiang, 2012). MT postharvest treatments enhanced GABA shunt activity increasing ATP supply in strawberry (Aghdam and Fard, 2017) and electron transport in mitochondria increasing the energy status (Aghdam et al., 2016a, b) in cut anthurium flowers. These associated biochemical processes could explain the lower respiration rate found in MT treated carnation flowers in comparison with control ones, specially, when using 0.1 mM MT dose. According to this, we hypothesize that the additional energy provided by the exogenous MT treatment could be maintaining the energy status in 'Baltico' carnation cell tissues decreasing energetic requirements and respiration increasing vase life. In fact, GABA shunt pathway is involved in maintaining general quality and vase life of cut flowers and several studies have demonstrated that exogenous treatments with solutions capable to stimulate GABA shunt activity increase gerbera vase life (Mohammadi et al., 2020) and confers resistance against abiotic and

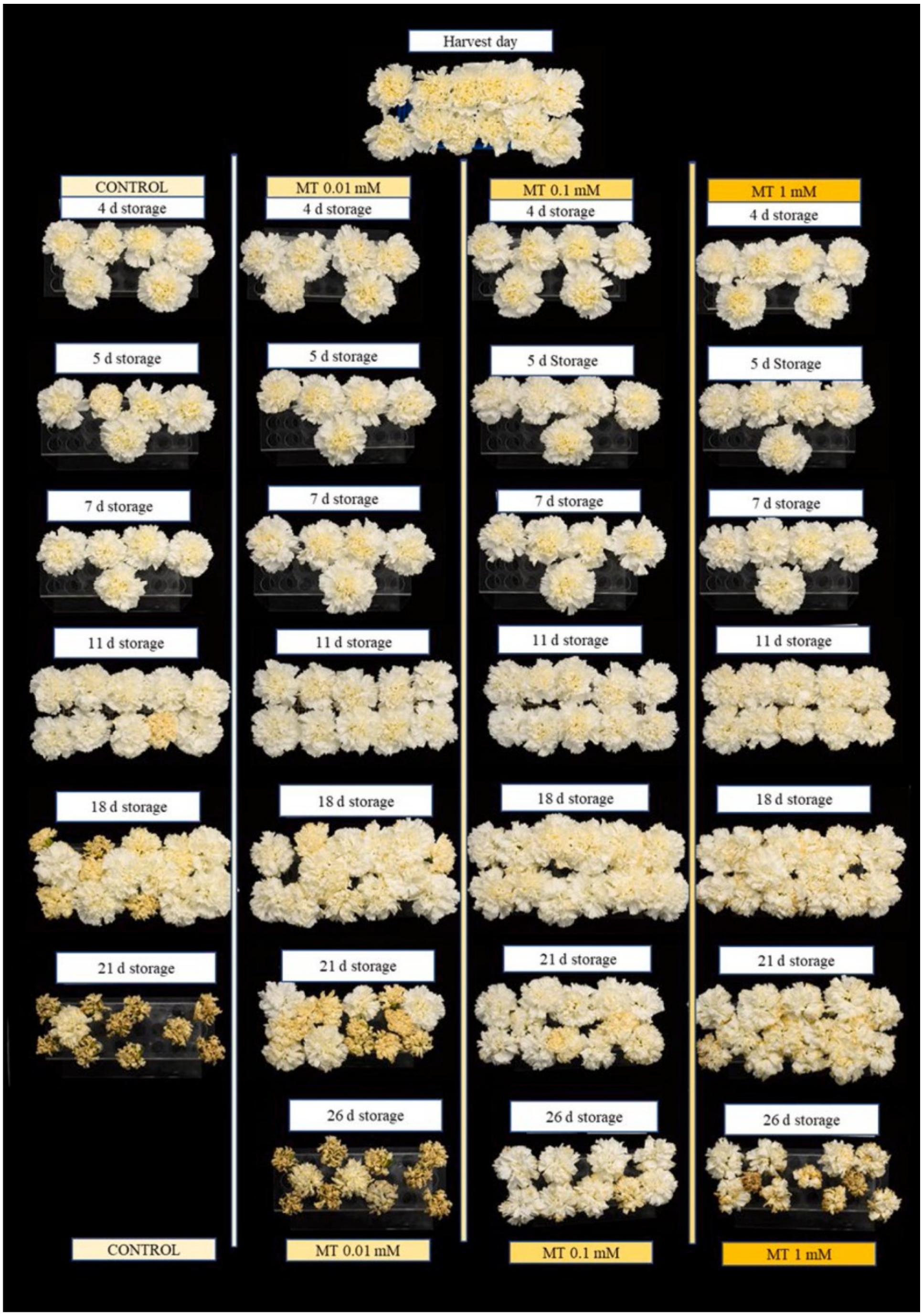

Fig. 4. Carnation cut flowers cv. Baltico appearance during vase life period as affected by different melatonin solutions (MT, 0.01, 0.1 and 1 mM) with respect to control flowers in distilled water.

biotic stress to anthurium and tuberose cut flowers (Aghdam et al., 2015, 2016a, b; Babarabie et al., 2019) during storage. There are no previous studies regarding vase life in melatonin treated cut flowers though the MT effect on chilling injury in cut anthurium flowers have been demonstrated by Aghdam et al. (2019) confirming our results despite of being a very different ornamental plant species. This positive effect on delaying chilling injury was mainly related to MSI. The present results show that MSI exhibited a positive relationship with flower vase life and FW maintenance, as has been described in previous studies in carnation (Hassan et al., 2020; Rashidiani et al., 2020; Ranjbar and Ahmadi, 2015) and other cut flower species (Hemati et al., 2019; Kazemzadeh-Beneh et al., 2018; Perinban et al., 2015). Based in our results, MT treatments resulted in higher MSI than control flowers during the whole vase life period. These observations could be associated with the maintenance of the membrane unsaturated/saturated fatty acids ratio which is affected by ROS in petals (Wang et al., 2020) and other plant tissues (Aghdam et al., 2016a, b). In this sense, Aghdam et al. (2019) in cut anthurium flowers described the role of MT maintaining redox homeostasis, increasing the antioxidant capacity and thus, modulating reparation of oxidatively damaged proteins. This reported effect would be responsible for maintaining higher MSI in MT treated flowers in comparison with control carnations. Also, for leaves (Ali et al., 2020; Mohamed et al., 2020) and fruit tissues in higher plants, antioxidant system activity was stimulated after preharvest

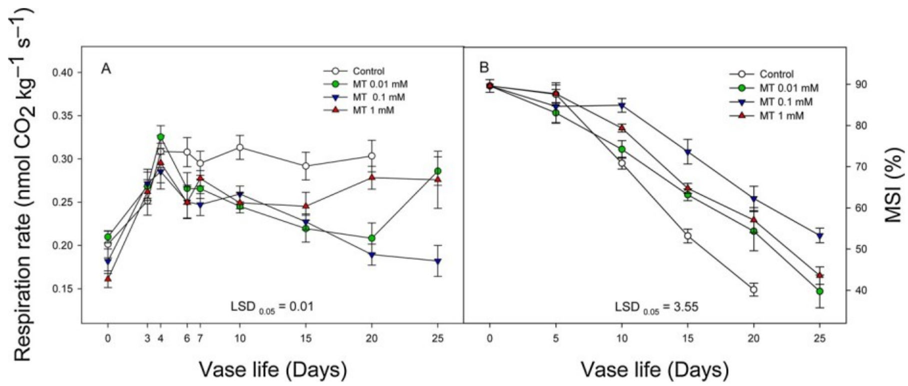

Fig. 5. Respiration rate (n = 12) (A) and membrane stability index (MSI) (n = 6) (B) of control and melatonin (MT, 0.01, 0.1 and 1 mM) in cut carnation flowers cv. Baltico during vase life period. Values are the means ± standard error (SE) (n = 12). LSD at $P < 0.05$ was used for means comparison.

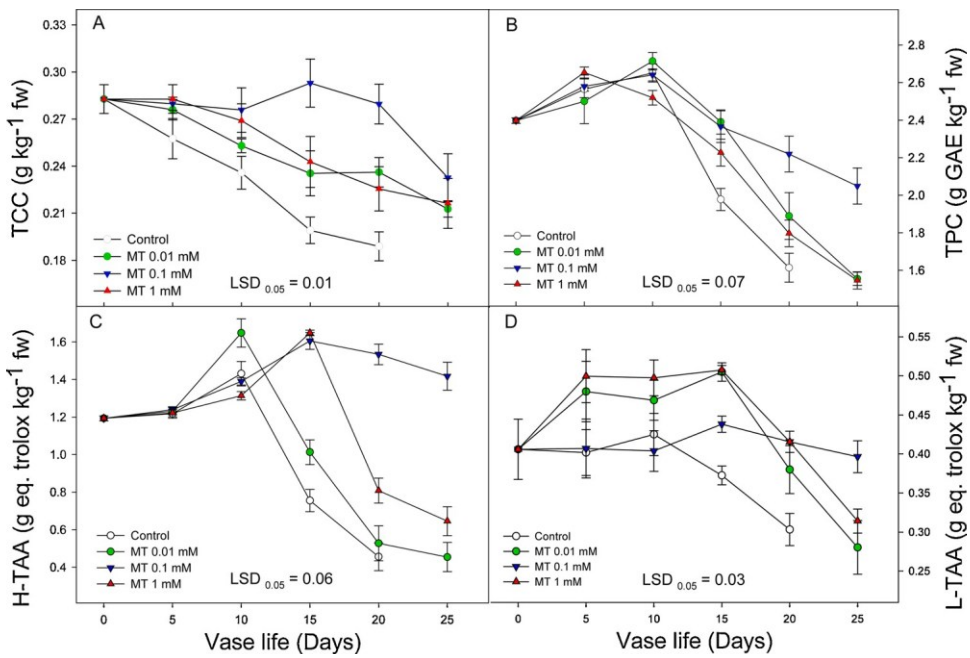

Fig. 6. Total chlorophyll content (TCC) in sepals (n = 12) (A), total polyphenol content (TPC) (n = 12) (B) and total antioxidant activity in the hydrophilic (H-TAA) (n = 6) (C) and lipophilic (L-TAA) (n = 6) (D) soluble phase in petals of carnation cut flowers cv. Baltico treated with melatonin solutions (0.01, 0.1 and 1 mM) and distilled water (control) during vase life period. Values are means ± standard error (SE) and LSD at $P < 0.05$ was used for means comparison.

(Medina-Santamarina et al., 2021) or postharvest melatonin applications (Zhang et al., 2018; Liu et al., 2018; Sharif et al., 2018) leading to maintenance of MSI for longer period.

In the current study, the TCC in sepals was maintained during storage in all MT treated flowers but specially, when 0.1 mM MT solution was employed. Chloroplasts are easy targets of ROS-linked damage during various stresses and natural senescence since ROS detoxification systems decrease during postharvest in leaves and other green parts of the plant (Khanna-Chopra, 2012). In this sense, and based on the results, the MT effect on protecting chlorophyll content could be related to an improved water relation through a preserved membrane stability, supported by a higher antioxidant activity system. This is consistent with the finding reported in gardenia MT treated leaves by Zhao et al. (2017) who observed a higher chlorophyll content in comparison with control, with an increased ROS scavenging capacity and membrane integrity. On the other hand, in cucumber seedling similar results were found by Zhao et al. (2016) who also observed an adjusted photosynthetic electron flux capable to suppress the production of ROS in MT treated leaves.

Phenolic compounds protect lipid membrane oxidation avoiding initiation or propagation of oxidizing chain reactions preventing the damage of ROS (Mohammadi et al., 2020). Different flavonoids as kaempferol or isovitexin have been reported in different white carnation cultivars (Iwashina et al., 2010). In this sense, flavonoids and other phenolic compounds can be found in white rose petals including gallic acid, (+)-catechinic acid, caffeic acid, hesperidin and cinnamic acids between others (Yon et al., 2018; Yeon and Kim, 2020). MT treatments delayed carnation polyphenol degradation and 0.1 mM MT treatment maintained TPC content for a longer period. Similarly, higher phenolic concentrations were observed in different cut flower species, such as anthurium, gladiolus and gerbera, after GABA, polyamines or GA3 treatments as well as an extension in their vase life (Aghdam et al., 2015; Sajjad et al., 2015; do Nascimento Simo˜es et al., 2018; Mohammadi et al., 2020). The higher TPC in MT treated flowers could be attributed to a reduction in the PPO activity and to an increase in antioxidant activity as has been described in cut anthurium flowers by Aghdam et al. (2019). Regarding this, the present study showed a higher TAA in both hydrophilic (H) and lipophilic (L) fractions in petals of MT-treated carnations as compared to control ones. Higher antioxidant capacity was observed and for a longer period when 0.1 mM MT was used. Citric acid, vitamins C, B1 and phenolic compounds are major contributors to H-TAA and the most abundant in plants while carotenoid, vitamin E and terpene compounds are major lipophilic radical-scavenging antioxidants (Niki, 2014; Lezoul et al., 2020). The critical role played by oxidative stress in cut flower vase life, and the stimulation of the antioxidant system activity using different anti-senescent compounds has been studied by several authors (Ezhilmathi et al., 2007; Rashidiani et al., 2020; Mohammadi et al., 2020; Ranjbar and Ahmadi, 2015; Hassan and Ali, 2014a, 2014b). Melatonin has radical scavenge activity by itself and stimulates the antioxidant enzyme system in cut flowers and other tissues also reducing oxidative enzymes activity (Arnao and Hern´andez--Ruiz, 2019; Aghdam et al., 2019). As far as we know this is the first report in which MT impact on cut flower vase life is described. The longer vase life obtained when MT solutions were applied could be attributed to a lower flower metabolism, improved water relation in flower tissues and maintenance of membrane stability. In addition, the higher antioxidant activity and phenolic content stimulated by MT treatments could modulate oxidative damage in carnation tissues increasing the vase life period. Considering the cost of MT as well as the daily VSU, this postharvest treatment could be cost effective increasing vase life up to 10 days more when using the intermediate concentration (0.1 mM MT) providing benefits for cut-flowers retailers. For this reason, MT as a postharvest treatment could be a useful tool to delay carnation senescence.

Author statement

Nour ElHouda Lezoul: Investigation, Formal analysis, Data cura- tion, Visualization, Writing-Original draft preparation. **María Serrano:** Conceptualization, Methodology, Formal analysis, Writing-Reviewing and Editing. **María Celeste Ruiz-Aracil:** Investigation, Formal analysis, Data curation. **Mohamed Belkadi:** Formal analysis, Validation, Visualization **Salvador Castillo:** Investigation, Formal analysis, Software. **Daniel Valero:** Investigation, Formal analysis, Data curation. **Fabia´n Guille´n:** Conceptualization, Investigation, Methodology, Data curation, Writing-Reviewing and Editing, Supervision.

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Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2021.111 759.

References

Aalifar, M., Aliniaeifard, S., Arab, M., Mehrjerdi, M.Z., Serek, M., 2020. Blue light postpones senescence of carnation flowers through regulation of ethylene and abscisic acid pathway-related genes. Plant Physiol. Biochem. 151, 103–112. https://doi.org/10.1016/j.plaphy.2020.03.018.

Aghdam, M.S., Fard, J.R., 2017. Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (Fragaria × anannasa cv. Selva) by enhancing GABA shunt activity. Food Chem. 221, 1650–1657. https://doi.org/10.1016/j.foodchem.2016.10.123.

Aghdam, M.S., Naderi, R., Sarcheshmeh, M.A.A., Babalar, M., 2015. Amelioration of postharvest chilling injury in anthurium cut flowers by γ-aminobutyric acid (GABA) treatments. Postharvest Biol. Technol. 110, 70–76. https://doi.org/10.1016/j.postharvbio.2015.06.020.

Aghdam, M.S., Jannatizadeh, A., Sheikh-Assadi, M., Malekzadeh, P., 2016a. Alleviation of postharvest chilling injury in anthurium cut flowers by salicylic acid treatment. Sci. Hortic. 202, 70–76. https://doi.org/10.1016/j.scienta.2016.02.025.

Aghdam, M.S., Naderi, R., Malekzadeh, P., Jannatizadeh, A., 2016b. Contribution of GABA shunt to chilling tolerance in anthurium cut flowers in response to postharvest salicylic acid treatment. Sci. Hortic. 205, 90–96. https://doi.org/10.1016/j.scienta.2016.04.020.

Aghdam, M.S., Jannatizadeh, A., Nojadeh, M.S., Ebrahimzadeh, A., 2019. Exogenous melatonin ameliorates chilling injury in cut anthurium flowers during low temperature storage. Postharvest Biol. Technol. 148, 184–191. https://doi.org/10.1016/j.postharvbio.2018.11.008.

Akhtar, G., Rajwana, I.A., Sajjad, Y., Asif, M., Muhammad, S., Kashif, A., Sami, R., Hafiz, U., Faried, N., Farooq, A., 2021. Samiullah Do natural leaf extracts involve regulation at physiological and biochemical levels to extend vase life of gladiolus cut flowers? Sci. Hortic. 282, 110042 https://doi.org/10.1016/j.scienta.2021.110042.

Ali, M., Kamran, M., Abbasi, G.H., Saleem, M.H., Ahmad, S., Parveen, A., Malik, Z., Afzal, S., Ahmar, S., Dawar, K.M., 2020. Melatonin-induced salinity tolerance by ameliorating osmotic and oxidative stress in the seedlings of two tomato (Solanum lycopersicum L.) cultivars. J. Plant Growth Regul. 1–13. https://doi.org/10.1007/s00344-020-10273-3.

Arnao, M.B., Herna´ndez-Ruiz, J., 2019. Melatonin: a new plant hormone and/or a plant master regulator? Trends Plant Sci. 24 (1) https://doi.org/10.1016/j.tplants.2018.10.010, 38-4.

Babarabie, M., Zarei, H., Eskandari, A., 2019. The impact of pre-harvest treatment with gamma-aminobutyric acid (GABA) and salicylic acid on vase life and post-harvest traits of tuberose cut flowers. Acta Sci. Pol.-Hort. 18 (4), 83–92. https://doi.org/10.24326/asphc.2019.4.8.

Debnath, B., Islam, W., Li, M., Sun, Y., Lu, X., Mitra, S., Hussain, M., Liu, S., Qiu, D., 2019. Melatonin mediates enhancement of stress tolerance in plants. Inter. J. Mole. Sci. 20 (5), 1040. https://doi.org/10.3390/ijms20051040.

do Nascimento Simo˜es, A., Diniz, N.B., da Silva Vieira, M.R., Ferreira-Silva, S.L., da Silva, M.B., Minatel, I.O., Lima, G.P.P., 2018. Impact of GA3 and spermine on postharvestquality of anthurium cut flowers (Anthurium andraeanum cv. Arizona). Sci. Hortic. 241, 178–186. https://doi.org/10.1016/j.scienta.2018.06.095.

Ebrahimzadeh, A., Jime´nez, S., Da Silva, J.T., Satoh, S., Lao, M.T., 2008. Post-harvest physiology of cut carnation flowers. Fresh Produce 2, 56–71.

Ebrahimzadeh, A., Jimenez-Becker, S., Manzano-Medina, S., Jamilena-Quesada, M., Lao-Arenas, M.T., 2011. Evaluation of ethylene production by ten Mediterranean carnation cultivars and their response to ethylene exposure. Span. J. Agric. Res. 9 (2), 524–530. https://doi.org/10.5424/sjar/20110902-124-10.

Ezhilmathi, K., Singh, V.P., Arora, A., Sairam, R.K., 2007. Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of Gladiolus cut flowers. Plant Growth Regul. 51 (2), 99–108. https://doi.org/10.1007/s10725-006-9142-2.

Halevy, A., Mayak, S., 1981. Senescence and postharvest physiology of cut flowers—part 2. Hort. Rev. 3, 59–143. https://doi.org/10.1002/9781118060766.ch3.

Hassan, F.A.S., Ali, E.F., 2014a. Protective effects of 1-methylcyclopropene and salicylic acid on senescence regulation of gladiolus cut spikes. Sci. Hortic. 179, 146–152. https://doi.org/10.1016/j.scienta.2014.09.025.

Hassan, F.A.S., Ali, E.F., 2014b. Longevity and postharvest quality of Rosa hybrida L. cv. "Happy Hour" cut flowers as affected by Silver thiosulphate (STS) treatment. Sci. Agric. 1, 85–91. https://doi.org/10.15192/PSCP.SA.2014.1.3.8591.

Hassan, F.A.S., Fetouha, M.I., 2019. Does moringa leaf extract have preservative effect improving the longevity and postharvest quality of gladiolus cut spikes? Sci. Hortic. 250, 287–293. https://doi.org/10.1016/j.scienta.2019.02.059.

Hassan, F.A.S., Ali, E.F., Mazrou, R., 2020. Involvement of ethylene synthetic inhibitors in regulating the senescence of cut carnations through membrane integrity maintenance. J. Agric. Res. 28 (1), 39–48. https://doi.org/10.2478/johr-2020-0010.

Hemati, E., Salehi Salmi, M.S., Daneshvar, M.H., Heidari, M., 2019. The roles of sodium nitroprusside, salicylic acid, and methyl jasmonate as hold solutions on vase life of Gerbera jamesonii 'Sun Spot'. Adv. Hortic. Sci. 33 (2), 187–195. https://doi.org/10.13128/ahs-24261.

Iwashina, T., Yamaguchi, M.A., Nakayama, M., Onozaki, T., Yoshida, H., Kawanobu, S., Ono, H., Okamura, M., 2010. Kaempferol glycosides in the flowers of carnation and their contribution to the creamy white flower color. Nat. Prod. Commun. 5 (12), 1903–1906. https://doi.org/10.1177/1934578X1000501213.

Karimi, M., Akbari, F., Heidarzade, A., 2017. Protective effects of polyamines on regulation of senescence in spray carnation cut flowers (Dianthus caryophyllus'Spotlight'). Acta Agric. Slov. 109 (3), 509–515. https://doi.org/10.14720/aas.2017.109.3.03.

Kazemzadeh-Beneh, H., Samsampour, D., Zarbakhsh, S., 2018. Biochemical, physiological changes and antioxidant responses of cut gladiolus flower 'white prosperity' induced by nitric oxide. Adv. Hortic. Sci. 32 (3), 421–431. https://doi.org/10.13128/ahs-23361.

Khanna-Chopra, R., 2012. Leaf senescence and abiotic stresses share reactive oxygen species-mediated chloroplast degradation. Protoplasma 249 (Jul (3)), 469–481. https://doi.org/10.1007/s00709-011-0308-z.

Lezoul, N.E.H., Belkadi, M., Habibi, F., Guill´en, F., 2020. Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. Molecules 25 (20), 4672. https://doi.org/10.3390/molecules25204672.

Liu, C., Zheng, H., Sheng, K., Liu, W., Zheng, L., 2018. Effects of melatonin treatment on the postharvest quality of strawberry fruit. Postharvest Biol. Technol. 139, 47–55. https://doi.org/10.1016/j.postharvbio.2018.01.016.

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

Mohamed, I.A.A., Shalby, N., M.A. El-Badri, A., Saleem, M.H., Khan, M.N., Nawaz, A., Qin, M., Agami, R.A., Kuai, J., Wang, B., Zhou, G., 2020. Stomata and xylem vessels traits improved by melatonin application contribute to enhancing salt tolerance and fatty acid composition of Brassica napus L. Plants. Agronomy 10, 1186. https://doi. org/10.3390/agronomy10081186.

Mohammadi, M., Aelaei, M., Saidi, M., 2020. Pre-harvest and pulse treatments of spermine, γ-and β-aminobutyric acid increased antioxidant activities and extended the vase life of gerbera cut flowers 'Stanza'. Ornam. Hortic. 26 (2), 306–316. https:// doi.org/10.1590/2447-536X.v26i2.2120.

Murch, S.J., Alan, A.R., Cao, J., Saxena, P.K., 2009. Melatonin and serotonin in flowers and fruits of Datura metel L. J. Pineal Res. 47 (3), 277–283. https://doi.org/ 10.1111/j.1600-079X.2009.00711.x.

Niki, E., 2014. Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. Free Radic. Biol. Med. 66, 3–12. https://doi.org/10.1016/j. freeradbiomed.2013.03.022.

Nukui, H., Kudo, S., Yamashita, A., Satoh, S., 2004. Repressed ethylene production in the gynoecium of long-lasting flowers of the carnation 'White Candle': role of the gynoecium in carnation flower senescence. J. Exp. Bot. 55 (397), 641–650. https:// doi.org/10.1093/jxb/erh081.

Perinban, S., Majumder, J., Rai, P., Singh, B., 2015. Effect of plant bioregulators on the vase life of snapdragon (Antirrhinum majus) cut flowers. Indian J. Agric. Sci. 85 (12), 1565–1570 http://epubs.icar.org.in/…/54303.

Porra, R.J., Thompson, W.A., Kriedemann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. BBA Bioenerg. 975 (3), 384–394. https://doi.org/10.1016/S0005-2728(89)80347-0.

Ranjbar, A., Ahmadi, N., 2015. Effects of 1-MCP and ethylene on antioxidant enzyme activity and postharvest physio-biochemical characteristics of cut carnation flower cv. fortune. Adv. Hortic. Sci. 29 (4), 192–198. https://doi.org/10.13128/ahs-22723.

Rashidiani, N., Nazari, F., Javadi, T., Samadi, S., 2020. Comparative postharvest responses carnation and chrysanthemum to synthesized silver nanoparticles (Agnps). Adv. Hortic. Sci. 34 (2), 133–145. https://doi.org/10.13128/ahsc-7491.

Reid, M.S., Jiang, C.Z., 2012. Postharvest biology and technology of cut flowers and potted plants. Hortic. Rev. 40 (1), 1–54. https://doi.org/10.1002/9781118351871. ch1.

Rot, I., Friedman, H., 2010. Desiccation-induced reduction in water uptake of gypsophila florets and its amelioration. Postharvest Biol. Technol. 57 (3), 189–195. https://doi. org/10.1016/j.postharvbio.2010.03.005.

Sajjad, Y., Jaskani, M.J., Qasim, M., Akhtar, G., Mehmood, A., 2015. Foliar application of growth bioregulators influences floral traits, cormassociated traits and chemical constituents in gladiolus grandiflorus L. Korean J. Hortic. Sci. Technol. 33 (6), 812–819. https://doi.org/10.7235/hort.2015.15052.

Salmi, M.S., Hoseini, M.F., Heidari, M., Daneshvar, M.H., 2018. Extending vase life of cut rose (Rosa hybrida L.) cv. Bacara by essential oils. Adv. Hortic. Sci. 32, 61–69. https://doi.org/10.13128/ahs-21860.

Satoh, S., Nukui, H., Kudo, S., Inokuma, T., 2005. Towards understanding the onset of petal senescence: analysis of ethylene production in the long-lasting carnation cv. white candle. Acta Hortic. 669, 175–182. https://doi.org/10.17660/ ActaHortic.2005.669.22.

Scariot, V., Paradiso, R., Rogers, H., De Pascale, S., 2014. Ethylene control in cut flowers: classical and innovative approaches. Postharvest Biol. Technol. 97, 83–92. https:// doi.org/10.1016/j.postharvbio.2014.06.010.

Serrano, M., Romojaro, F., Casas, J.L., Acosta, M., 1991. Ethylene and polyamine metabolism in climacteric and nonclimacteric carnation flowers. Sci. Hortic. 26 (7), 894–896. https://doi.org/10.21273/HORTSCI.26.7.894.

Serrano, M., Martínez-Madrid, M.C., Romojaro, F., 1999. Ethylene biosynthesis and phenamine and ABA levels in cut carnations treated with aminotriazole. J. Am. Soc. Hortic. Sci. 124 (1), 81–85. https://doi.org/10.21273/JASHS.124.1.81.

Serrano, M., Amoro´s, A., Pretel, M.T., Martínez-Madrid, M.C., Romojaro, F., 2001. Preservative solutions containing boric acid delay senescence of carnation flowers. Postharvest Biol. Technol. 23 (2), 133–142. https://doi.org/10.1016/S0925-5214 (01)00108-9.

Sharif, R., Xie, C., Zhang, H., Arnao, M.B., Ali, M., Ali, Q., Muhammad, I., Shalmani, A., Nawaz, M.A., Chen, P., Li, Y., 2018. Melatonin and its effects on plant systems. Molecules 23 (9), 2352. https://doi.org/10.3390/molecules23092352.

Sharma, A., Zheng, B., 2019. Melatonin mediated regulation of drought stress: physiological and molecular aspects. Plants 8 (7), 190. https://doi.org/10.3390/ plants8070190.

Solgi, M., 2018. The application of new environmentally friendly compounds on postharvest characteristics of cut carnation (Dianthus caryophyllus L.). Rev. Bras. Bot. 41 (3), 515–522. https://doi.org/10.1007/s40415-018-0464-x.

Wang, S., Xue, J., Zhang, S., Zheng, S., Xue, Y., Xu, D., Zhang, X., 2020. Composition of peony petal fatty acids and flavonoids and their effect on Caenorhabditis elegans lifespan. Plant Physiol. Biochem. 155, 1–12. https://doi.org/10.1016/j. plaphy.2020.06.029.

Wu, M.J., Zacarias, L., Reid, M.S., 1991. Variation in the senescence of carnation (Dianthus caryophyllus L.) cultivars. II. Comparison of sensitivity to exogenous ethylene and of ethylene binding. Sci. Hortic. 48 (1-2), 109–116. https://doi.org/ 10.1016/0304-4238(91)90157-T.

Yakimova, E., Atanassova, B., Kapchina-Toteva, V., 1997. Longevity and some metabolic events in postharvest spray-carnation (D. Caryophyllus F. Sapry, Hort) Flowers. Bulg. J. Plant. Physiol. 23, 57–65.

Yeon, J.Y., Kim, W.S., 2020. Floral pigment-scent associations in eight cut rose cultivars with various petal color. Hortic. Environ. Biotechnol. 61 (4), 633–641. https://doi. org/10.1007/s13580-020-00249-3.

Yon, J.M., Kim, Y.B., Park, D., 2018. The ethanol fraction of white rose petal extract abrogates excitotoxicity-induced neuronal damage in vivo and in vitro through inhibition of oxidative stress and proinflammation. Nutrients 10 (10), 1375. https:// doi.org/10.3390/nu10101375.

Zhang, Y., Huber, D.J., Hu, M., Jiang, G., Gao, Z., Xu, X., Jiang, Y., Zhang, Z., 2018. Delay of postharvest browning in Litchi fruit by melatonin via the enhancing of antioxidative processes and oxidation repair. J. Agric. Food Chem. 66 (28), 7475–7484. https://doi.org/10.1021/acs.jafc.8b01922.

Zhao, H., Ye, L., Wang, Y., Zhou, X., Yang, J., Wang, J., Cao, K., Zou, Z., 2016. Melatonin increases the chilling tolerance of chloroplast in cucumber seedlings by regulating photosynthetic electron flux and the ascorbate-glutathione cycle. Front. Plant Sci. 7, 1814. https://doi.org/10.3389/fpls.2016.01814.

Zhao, D., Wang, R., Meng, J., Zhiyuan, Li, Wu, Y., Tao, J., 2017. Ameliorative effects of melatonin on dark-induced leaf senescence in gardenia (Gardenia jasminoides Ellis): leaf morphology, anatomy, physiology and transcriptome. Sci. Rep. 7 (1), 10423. https://doi.org/10.1038/s41598-017-10799-9

4.5. Publicación V (Transcripción literal)

V

Comparative effect of melatonin and 1-methylcyclopropene postharvest applications for extending 'Hayward' kiwifruit storage life

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Article

Comparative Effect of Melatonin and 1-Methylcyclopropene Postharvest Applications for Extending 'Hayward' Kiwifruit Storage Life

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Abstract: Kiwifruit, like many other fruits, is susceptible to dehydration, leading to texture changes and a loss of flavour during storage. Exposing kiwifruit to suboptimal temperatures can control these changes but can cause internal browning. Postharvest treatments with substances such as 1-methylcyclopropene (1-MCP) are some of the most successful commercial technologies in the conservation of fruits and vegetables. In recent years, there has been a growing interest among researchers in alternative technologies based in postharvest treatments with plant growth regulators. In this sense, melatonin (MT) has been shown to improve fruit quality, extending shelf life. The aim of this study was to compare these two different technologies applied at postharvest to evaluate the impact on kiwifruit quality. Optimal 1-MCP fumigations and MT solutions were assayed on 'Hayward' kiwifruit under similar conditions. Quality parameters were evaluated at 14-day intervals during 84 days of cold storage plus 5 days at 20 °C. The results showed that both treatments were similarly effective in maintaining quality parameters such as weight loss, respiration, firmness, and acidity. Although 1-MCP treatments delayed the evolution of kiwifruit colour and chlorophyll degradation as compared to MT, MT treatments controlled chilling injury better than 1-MCP. This effect was not related to a greater cell membrane integrity since fruit batches treated with 1-MCP were the ones that showed the lowest electrolyte leakage level. In conclusion, both treatments maintained fruit quality and delayed ripening in a similar way. In this sense, the results suggest that MT immersion treatments could act as efficient delaying senescence as fumigations with 1-MCP maintaining kiwifruit quality during refrigerated storage.

Keywords: *Actinidia deliciosa*; melatonin; 1-MCP; quality; ripening; storage

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1. Introduction

Kiwifruit is a subtropical crop and the species *Actinidia deliciosa* is the most widely cultivated worldwide, commonly known as green kiwifruit. Kiwifruit contains a large amount of nutrients that provide digestive, immune, and metabolic health benefits to the consumer. It is also an important source of vitamin C and E, dietary fibre, folic acid, potassium, and bioactive compounds such as antioxidants, phytochemicals, and enzymes, which provide functional and metabolic benefits [1–3].

The fruit must be carefully harvested since even small damages would affect the production of endogenous ethylene, thus increasing fruit softening. The decrease in texture (hardness or firmness) and the increase in total soluble solids (TSSs) are considered the main indicators of kiwifruit's ripening stage since, in the case of kiwifruit, the evolution of the ripening process cannot be observed externally since the exocarp does not change colour [4,5]. On the other hand, after harvest, the fruit is susceptible to diseases caused mainly by fungi such as *Botrytis cinerea*, *Penicillium expansum*, *Alternaria alternata*, *Botryos-*

phaeria dothidea, and *Diaporthe* spp., which cause a shorter shelf life and a decrease in its commercial yield, incurring postharvest losses [6].

Kiwifruit is a climacteric fruit; therefore, the ripening process is affected by the rate of ethylene production. Kiwifruit are harvested at physiological maturity but in an immature state with insufficient endogenous ethylene content to induce kiwifruit ripening. When stored below 10 °C, ethylene production in kiwifruit is almost nil and it is not strictly necessary to use exogenous ethylene treatment to induce ripening, since it has been shown that ripening and softening during cold storage also occur in the absence of detectable ethylene (exogenous or endogenous [7]).

Currently, there are several postharvest technologies with positive effects which maintain the quality of the fruit once it has been harvested. Physical methods such as temperature management and variations in the atmosphere concentration are used to both accelerate fruit ripening to reach commercial maturity and reduce the fruit respiration rate, therefore delaying ripening and extending its shelf life [8–10]. In this sense, the effect of ionizing radiation has been applied successfully, reducing spoilage caused by fungal pathogens [11]. Exogenous treatments have also been studied to increase kiwifruit's shelf life, such as edible coatings [12], methyl jasmonate [13], or γ-aminobutyric acid (GABA) solutions to reduce the chilling injury (CI) associated with the postharvest cold storage of kiwifruit [14]. On the other hand, ozone postharvest treatments were successful in maintaining fruit quality and reducing fungal growth [15,16].

Another widely used method to extend kiwifruit's shelf life is the use of commercial ethylene inhibitors such as 1-methylcyclopropene (1-MCP) [17–20]. 1-MCP binds to ethylene receptors and inhibits the ethylene action, reducing the fruit respiration rate, thus delaying fruit ripening and softening processes, and thereby preventing quality loss [21–24]. The preharvest application of 1-MCP delays fruit ripening, reducing the negative impact of late harvest and its subsequent effects during storage [25]. Postharvest treatment with 1-MCP delays the increase in membrane permeability and lipid peroxidation, preserv- ing the unsaturated fatty acid content, which is related to the maintenance of membrane integrity [26]. It has also been shown that 1-MCP maintains the content of bioactive compounds and total antioxidant capacity [27] as well as ascorbic acid and phenol content [28]. 1-MCP is also able to effectively eliminate off-flavours by suppressing ethanol metabolism in kiwifruit [29], but also, different studies described a decrease in the fruit aroma properties [30] including kiwifruit [31].

Melatonin (MT) is a multifunctional signalling molecule present in plants that plays a biostimulatory, growth-regulatory, and antioxidant role through the direct scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) under abiotic and biotic stress conditions [32]. MT-treated fruit exposed to suboptimal storage conditions maintained an optimal intracellular energy status, reducing physiological disorders during postharvest storage [33]. Exogenous MT treatment delays softening and preserves kiwifruit quality during low-temperature storage by suppressing cell-wall-degrading enzyme activity, reducing lipid peroxidation, and increasing the accumulation of antioxidant compounds [34–39]. In this sense, MT contributes to the enhancement of defence responses and, interestingly, reduces the alcoholic off-flavour produced by ethanol fermentation during kiwifruit's postharvest stage [40,41].

Both MT and 1-MCP have been studied individually in kiwifruit but the effectiveness of these treatments in increasing or maintaining quality traits in kiwifruit has not been compared. For this reason, the aim of this research was to elucidate the potential of both technologies under similar conditions to maintain kiwifruit quality during cold storage.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Kiwifruit (*Actinidia deliciosa* cv. Hayward) were manually harvested from a commercial plot in Carlet (Valencia) on 10 November 2021 when the fruit reached harvest maturity (6–7 °Brix) based on the literature guidelines [38,39]. A total of 315 kiwifruit without pests, diseases, or mechanical damage were graded and selected for uniform size. After selection, the kiwifruit were grouped into 3 replicates of 5 fruits for each treatment and sampling day. For the control treatment, distilled water immersions were applied for 10 min. For MT treatment, immersions in 0.1 mM solutions were performed for 10 min following optimal conditions in previous studies [36,40]. 1-MCP treatments were applied by mixing commercial tablets releasing 0.5 μL L^{-1} [21,42] using a commercial activator solution provided by SmartFresh (Agro-Fresh Inc., Philadelphia, PA, USA). All the dips of the different MT and control batches also contained Tween 20 (0.05%). Once the fruits were treated, they were left to dry at 20 °C for 1 h and then placed in individual 130 L plastic containers per treatment, providing the same conditions for the different batches. The containers were then closed and hermetically sealed and, thus, the released 1-MCP was left to act for 24 h at 20 °C while the control batches were exposed in these containers to normal air at the same temperature conditions. All these fruits were then stored for 84 days at 2 °C and 90% RH plus an additional period of 5 days at 20 °C for subsequent shelf life determinations.

2.2. Postharvest Quality Parameters

Three replicates of 5 fruits from each treatment lot were randomly selected at 14-day intervals during cold storage plus 5 days at 20 °C. The weight loss of individual kiwifruit was calculated as a percentage of the weight loss obtained with respect to day 0 and expressed as a % using a KERN 440-35N digital balance (Balingen, Germany).

CO_2 and ethylene production were determined in triplicate by placing 5 fruits from each replicate in a 3.4 L plastic container hermetically sealed with a rubber stopper for 60 min using the static method. After that, 1 mL of gas sample was taken in duplicate from the headspace and carbon dioxide was quantified using a Shimadzu 14B (Shimadzu Europa GmbH, Duisburg, Germany), and ethylene production was evaluated with a Shimadzu GC 2010 gas chromatograph according to a previously described method [43]. The respiration rate ethylene production was expressed as mg of CO_2 kg^{-1} h^{-1} and nL g^{-1} h^{-1}, respectively.

Firmness was determined individually using a TX-XT2i texture analyser (Stable Microsystems, Godalming, UK) equipped with a flat probe. The rate of descent of the disc was 20 mm min^{-1} until a deformation of 5% was reached. Two equidistant readings were taken in the equatorial region of each fruit. Fruit firmness was expressed as the ratio of applied force to distance travelled (N mm^{-1}).

The colour was measured with a Minolta colorimeter (CRC400, Minolta Camera Co.; Kanto, Tokyo, Japan); three colour measurements were made for each peeled fruit at three points equidistant from the equatorial zone and expressed as CIE *Hue** (arctg b*/a*) according to CIELab coordinates.

The determination of the kiwifruit susceptibility to CI was performed with a visual assessment of the kiwifruit flesh after peeling, similarly to a previously described method [44]. Visual assessment was performed individually on each fruit using a 5-point scale (0–4 scale) according to the surface area of surface pitting and dark watery spots: 0 (no symptoms), 1 (<25%), 2 (25–50%), 3 (51–75%), and 4 (>75%). The CI index was expressed as CI index = Σ [(scale × N)/total fruit number]. N is the number of fruits on the corresponding scale.

Electrolyte leakage (EL) was evaluated in the flesh tissue, following the method described by McCollum and McDonald [45] with some modifications. First, slices of the three replicates per treatment were cut to 2 mm thick in the equatorial zone of the kiwifruit. Fifteen discs were extracted for each replicate using a 0.5 cm diameter cork borer. After 3 rinses of 3 min for each replicate with deionized water, they were subjected to constant shaking with 50 mL of deionized water at room temperature. After 30 min, the initial electrical conductivity (C1) was measured using a Crison conductivity meter. The samples were frozen and then brought to 121 °C for 15 min. Total conductivity (C2) was evaluated with samples at room temperature (20 °C). EL was calculated as (C1/C2) × 100.

The total chlorophyll content (TCC) was evaluated by extracting a homogeneous mixture of 5 kiwifruit halves from each of the 5 fruits of each replicate following the method described by Vu et al. [46]. Pigment extraction was achieved through homogenization in methanol for 2 min; then, the samples were centrifuged, and the supernatant was evaluated using a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan) at 652 and 665 nm. The data, expressed as mg 100 g^{-1} of kiwifruit flesh, are the mean ± SE of 3 determinations.

The TSS and titratable acidity (TA) were determined in duplicate in the filtered juice extracted from the mixture of the remaining 5 kiwifruit halves for each replicate per batch. The TSSs in kiwifruit juice were measured using an Atago PR-101 digital refractometer (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and the TA was determined in each sample using automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland). The TSSs and TA were expressed as a%.

2.3. Statistical Analysis

A completely randomized design was used in this study. All data in this document are expressed as mean ± standard error (SE). Data were subjected to an analysis of variance (ANOVA). Mean comparisons were performed using a multiple range test (Tukey's HSD test) to find significant differences ($p < 0.05$). Different lowercase letters indicate a significant difference between treatments on the same sampling date. All analyses were performed with the SPSS software package, version 22 (IBM Corp.; Armonk, NY, USA).

3. Results and Discussions

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

Water loss in kiwifruit is related to a degradation of the outer layers, hairiness, and cell death of the skin tissue [47]. As shown in Figure 1A, the weight loss of the postharvest kiwifruit for each treatment steadily increased gradually during the storage period at 2 °C + 5 days at 20 °C. We observed that treatments containing MT (6.59 ± 0.22) or 1-MCP (6.73 ± 0.19) were effective in delaying weight loss significantly ($p < 0.05$) as compared to the control fruit (7.69 ± 0.28) after 28 days of storage at 2 °C + 5 days at 20 °C. On the other hand, it was proven that MT was equally effective as 1-MCP since there were no significant differences ($p > 0.05$) between these treatments. Although both 1-MCP and MT delayed weight loss, this effect was reduced at the end of storage, without significant differences ($p > 0.05$) as compared to the control batch (Figure 1A). In this cultivar, weight losses higher than 8–10% are related to kiwifruit shrivelling and, in this sense, this parameter was delayed for 2 weeks in melatonin- and 1-MCP-treated fruit as compared to the control fruit [48]. Postharvest weight losses are caused by transpiration and fruit respiration processes, so the observed differences could be related to a higher tissue integrity and a reduced respiration in kiwifruit treated with 1-MCP or MT [49]. The 1-MCP treatments delayed the weight loss of different vegetal products as avocado, tomato, and peach [50–52], and MT as a postharvest treatment had a positive effect, delaying this parameter in peach, strawberry, and apple [53–55]. Similar results have been observed for kiwifruit treated with 1-MCP [18,22,28] or MT [35].

Firmness is a key parameter that reflects fruit quality and influences consumer acceptability [56]. Fruit softening has been linked to fruit dehydration, which consequently results in water loss and reduced turgor pressure [47,57]. As expected, due to fruit ripening and senescence, kiwifruit firmness decreased throughout the cold storage, regardless of the treatment applied (Figure 1B). However, the highest fruit firmness levels were obtained for MT and 1-MCP-treated fruit, maintaining values of 22.72 ± 1.06 and 23.05 ± 1.15 N mm^{-1}, respectively, at day 14 while a measurement of 19.63 ± 1.18 N mm^{-1} was obtained for the control batch. The 1-MCP and MT treatments were significantly ($p < 0.05$) effective in delaying fruit softening as compared to the control fruit during storage. In this sense, fruit firmness was maintained at higher firmness levels than those that negatively affect the consumer's acceptance during cold storage [58]. The MT and 1-MCP treatments did not show significant differences ($p > 0.05$) between lots in the previously evaluated parameters (weight loss and fruit firmness). In this sense, it is interesting to highlight that MT, being a natural-origin substance, displayed similar results to those observed for 1-MCP-treated fruit for these important fruit quality traits. Therefore, the positive effect of both treatments on reducing weight loss (Figure 1A) would be one of the causes of the firmness maintenance as observed in Figure 1B. Similar studies have previously demonstrated that 1-MCP postharvest applications on different fruit such as kiwifruit [20,21], pears [59], and blueberry fruit [60] delayed weight losses and maintained fruit firmness through a delayed respiration rate and a reduced transpiration process. Although, in this study, ethylene levels were in general detected at lowlevels as basal ethylene production, 1-MCP blocked the perception of these low ethylene levels [7], reducing kiwifruit respiration which also affected transpiration processes [61]. Additionally, improved tissue integrity related to the reduced activity of cell-wall-degrading enzymes was observed in other studies [18,23]. In this sense, 1-MCP applications [26,62,63] and MT postharvest treatments [34,35,40] on different fruit species also reduced ROS species and degrading-enzyme activity, thus maintaining cell membrane fluidity.

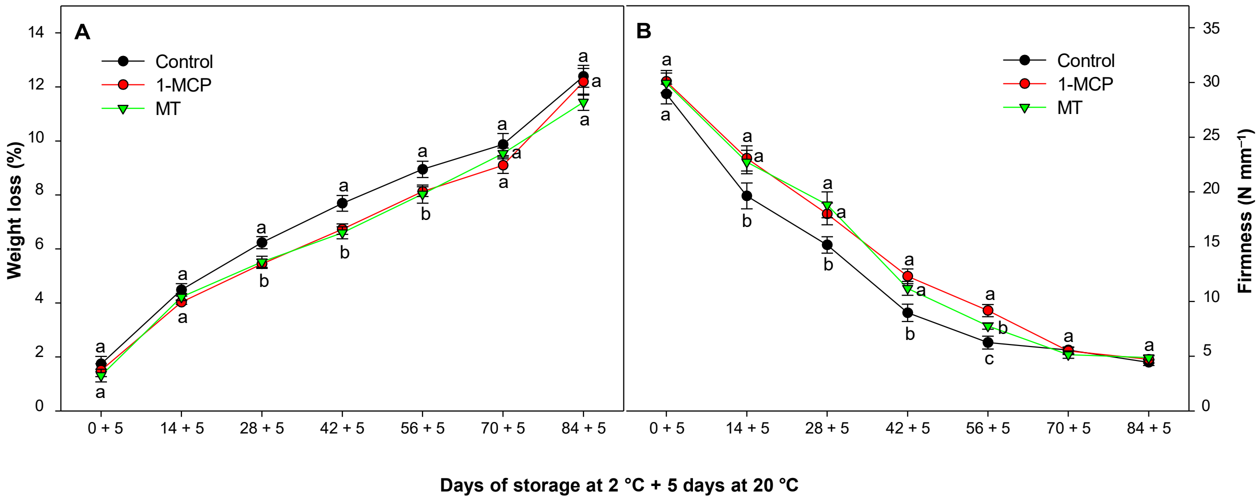

Figure 1. Evolution of weight losses (%) (**A**), and fruit firmness (N mm^{-1}) (**B**) of 'Hayward' kiwifruit treated with distilled water (Control), 1-MCP at 0.5 μL L^{-1} or melatonin at 0.1 mM (MT) during cold storage plus 5 days at 20 °C. Data are the mean ± SE (n = 3). Different lowercase letters show significant differences (p < 0.05) among treatments for each sampling date.

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

A higher respiration rate leads to a shorter fruit shelf life, mainly due to higher metabolic activity in the vegetal product. Once kiwifruits are harvested, an increase in the rate of respiration that accelerates fruit ripening and softening can be observed [23]. When studying the evolution of this parameter during storage, a respiration increase was accompanied by a maximum peak as fruit ripening progressed, showing a typical climacteric pattern (Figure 2A). The respiration evolution was significantly controlled (p < 0.05) in all fruit lots treated with MT and 1-MCP, displaying lower respiration production during storage as compared to the control lot. However, there were also significant differences (p < 0.05) between the MT- and 1-MCP-treated batches, since, at the beginning of the respiration curve, the MT treatment had the lowest respiration levels, while 1-MCP reached the lowest respiration levels at the end of the storage period.

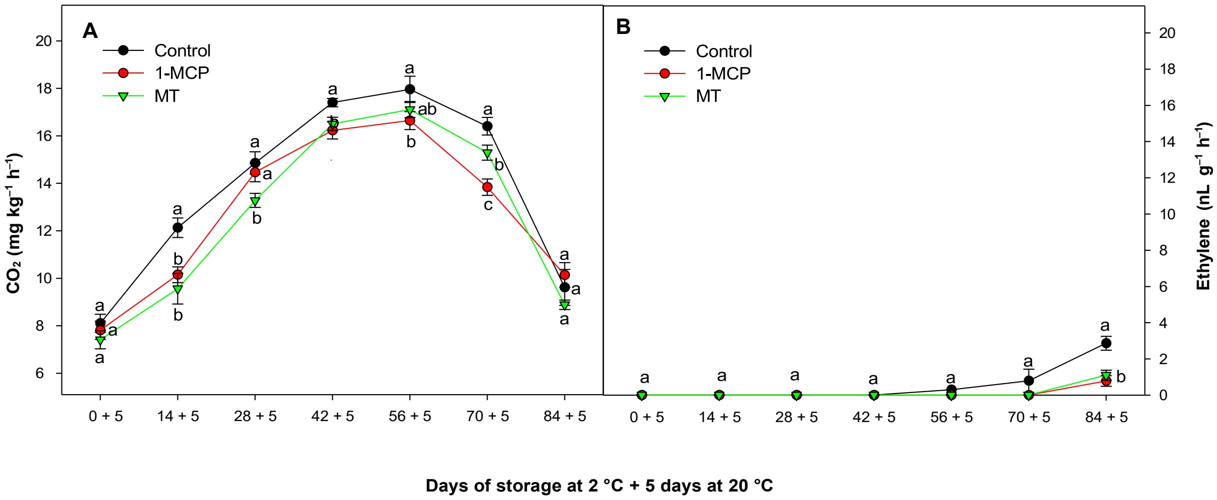

Figure 2. Evolution of respiration rate (mg CO_2 kg^{-1} h^{-1}) (**A**) and ethylene production (nL g^{-1} h^{-1}) (**B**) of 'Hayward' kiwifruit treated with distilled water (Control), 1-MCP at 0.5 µL L^{-1}, or melatonin at 0.1 mM (MT) during cold storage plus 5 days at 20 °C. Data are the mean ± SE (*n* = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

The lower respiration observed for the 1-MCP-treated kiwifruit could indicate that this compound is delaying senescence through reduced fruit metabolism by competitively binding to the ethylene receptor. A significant reduction in CO_2 production has been reported for kiwifruit treated with 1-MCP in the presence or absence of exogenous ethylene [18,21,28,64]. In this study, ethylene production was very low, being detected just at basal levels, and did not show significant differences ($p > 0.05$) between treatments (Figure 2B). In this sense, a significantly higher ethylene level was detected at the end of the storage period only for the control fruit as compared to the rest of the treatments (1-MCP and MT), which did not display significant differences ($p > 0.05$) between them. A reduced respiration pattern has also been observed in MT-treated kiwifruit [34,41]. In addition, several studies have demonstrated that MT applications in strawberry and mango increase the GABA shunt pathway, leading to increases in this metabolite, which is an immediate substrate for respiration, increasing the energy status of plant cells and leading to an improved metabolic balance [65,66].

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

Total soluble solids (TSSs) are one of the main quality parameters for assessing the ripening stage of kiwifruit and this content increases after harvest even when kiwifruits are stored at 0 °C [67]. Figure 3A shows how the TSSs increased gradually throughout storage for all treatments. Overall, the amount of TSSs throughout storage was not significantly affected by the MT and 1-MCP treatments. However, the MT-treated fruit delayed TSS accumulation, displaying significant differences ($p < 0.05$) at the end of storage with respect to both the control and 1-MCP-treated fruit (Figure 3A). The minimum TSSs content at harvest for 'Hayward' kiwifruit has been previously described as 6.2 % and has been used for many years [68]. This level matched with that observed at harvest in the kiwifruit used in this research. However, Goldberg et al. [69] found that the appropriate value for TSSs in order to increase the potential for longer storability in this cultivar should be 7 %. Our results are in consonance with other studies on kiwifruit in which a nonsignificant effect on TSS content was observed in general when 1-MCP was applied ($p > 0.05$) [23,34,36], and even lower values were obtained as compared to control fruit when MT postharvest treatments were assayed [18,26,29]. In this sense, the additive effect exhibited by MT applications in delaying TSS accumulation could indicate a stronger antisenescent effect than that observed in 1-MCP-treated fruit.

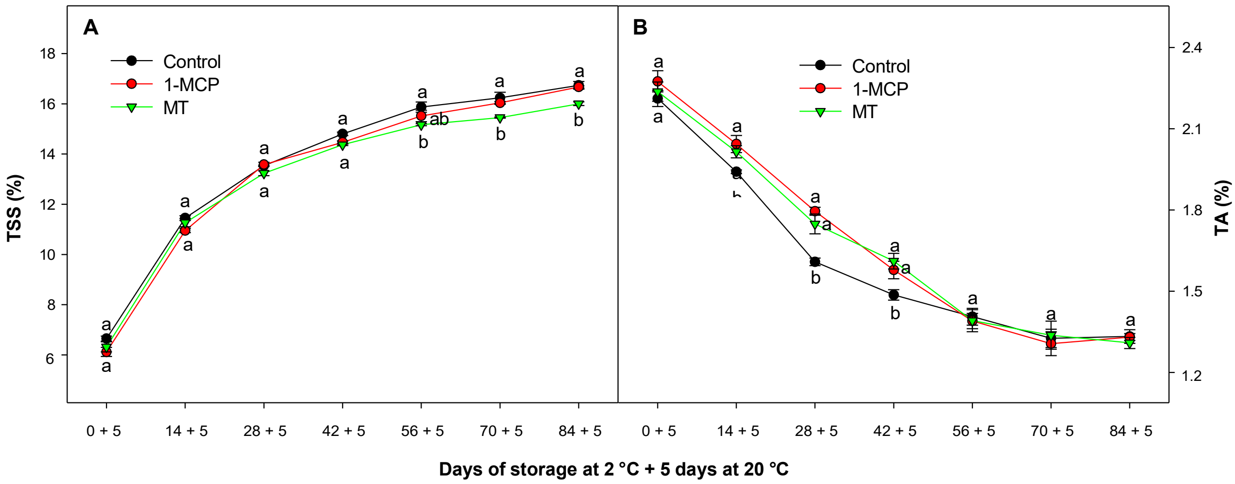

Figure 3. Evolution of total soluble solids (TSSs) (%) (**A**) and titratable acidity (TA) (%) (**B**) of 'Hayward' kiwifruit treated with distilled water (Control), 1-MCP at 0.5 μL L^{-1}, or melatonin at 0.1 mM (MT) during cold storage plus 5 days at 20 °C. Data are the mean ± SE (n = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

With respect to TA, gradual decreases in this parameter were observed as storage progressed (Figure 3B) since organic acids are substrates for the enzymatic reactions of the respiration process [20,70,71]. TA evolution in fruit batches treated separately with 1-MCP or MT displayed a significant ($p < 0.05$) effect, reducing the acidity losses as compared to the control batches. On the other hand, at the end of the storage period, the TA mean values for the control fruit did not differ significantly ($p > 0.05$) from those of the treatments applied after 56 days of cold storage (Figure 3B). The higher TA observed in fruit treated with 1-MCP [18,21,29] or with MT [34] could be related to a lower degradation metabolism of organic acids since 1-MCP reduced ethylene perception, thus delaying kiwifruit ripening. On the other hand, MT could be increasing the energy cell status, in turn being responsible for a higher maintenance of mitochondrial activity, delaying the catabolism of primary substrates such as sugars and organic acids, and, thus, delaying ripening and senescence [34,66].

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

Chlorophyll is the main pigment responsible for the colour of kiwifruit flesh and is considered an important index of kiwifruit maturity and senescence [72]. When we studied the chlorophyll content evolution, a decrease in this parameter throughout storage was observed for all kiwifruit studied (Figure 4A). After 14 days of cold storage + 5 days at 20 °C, the 1-MCP treatment was effective in delaying chlorophyll loss significantly ($p < 0.05$) (6.18 ± 0.07 mg 100 g^{-1}) as compared to the control fruit (5.39 ± 0.09 mg 100 g^{-1}) and the kiwifruit treated with MT (4.95 ± 0.08 mg 100 g^{-1}). Although, at the beginning of the experiment, the 1-MCP-treated fruit displayed a positive effect on delaying chlorophyll loss in kiwifruit, this effect was reduced as the storage progressed until the end of the study. At this point, the mean values of chlorophyll losses were similar between the different fruit batches (Figure 4A). Treatments with 1-MCP have been shown to maintain chlorophyll content by delaying ripening and senescence in both climacteric and nonclimacteric fruit [18,49,73]. In kiwifruit, 1-MCP fumigations reduced the ethylene perception in 'Hayward' kiwifruit even at the basal levels observed in this study (Figure 2B) but also in a previous study designed under similar conditions [7]. On the other hand, photosynthetic pigments such as chlorophylls are the main targets of ROS which increase their content in the plant cells as senescence accelerates [18,74]. Treatments with 1-MCP improved the levels of protective enzymes (APX, POD, SOD, and CAT) against reactive oxygen damage, delaying fruit senescence and maintaining kiwifruit's shelf life [75]. Likewise, it has been documented that exogenous MT delays tissue degradation through an antioxidant effect on free radicals, also protecting chlorophyll-related proteins [32,37]. However, the chlorophyll content of the MT-treated kiwifruit in this experiment (Figure 4A) did not differ from the control batch. In this sense, although chlorophyll content has been linked to fruit ripening and softening, in this case, chlorophyll degradation in the MT-treated fruit was not coincident with a greater weight loss (Figure 1A), higher firmness losses (Figure 1B), or higher ethylene production (Figure 2B) as compared to the control fruit.

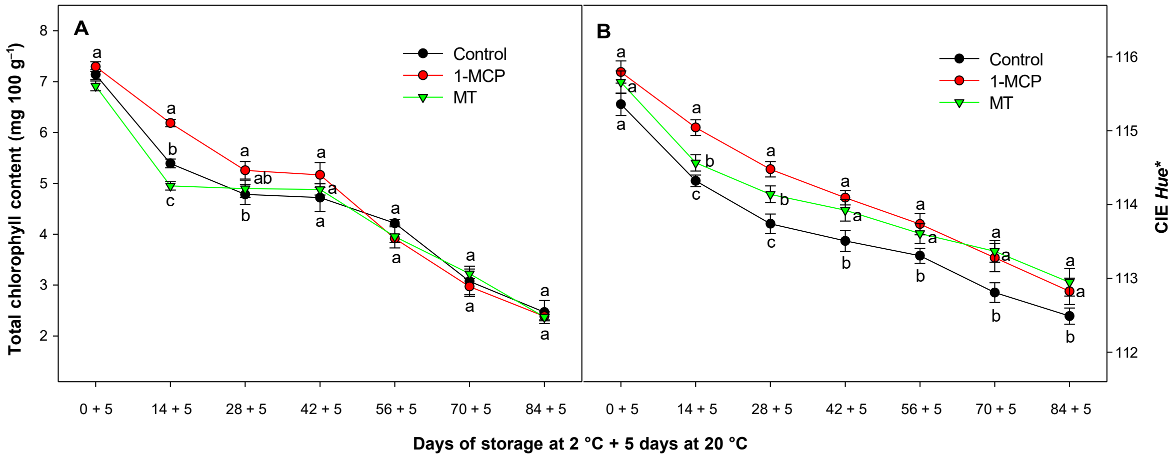

Figure 4. Evolution of total chlorophyll content (mg 100 g^{-1}) (**A**) and CIE *Hue** (**B**) of 'Hayward' kiwifruit treated with distilled water (Control), 1-MCP at 0.5 µL L^{-1}, or melatonin at 0.1 mM (MT) during cold storage plus 5 days at 20 °C. Data are the mean ± SE (n = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

In contrast to other fruits, visible changes in kiwifruit skin colour do not occur during development but do occur in the flesh. Colour fruit changes in kiwifruit are mainly due to the chlorophyll content evolution [69], which decreases during storage, as we evaluated previously (Figure 4A). Colour evaluation was expressed as CIE *Hue** which represents the evolution to darker fruit shades, and for this reason, this parameter is useful for assessing fruit senescence. Figure 4B shows that the CIE *Hue** decreased during storage in kiwifruit. In this sense, the lower the CIE *Hue**, the darker the external pulp shade is. The MT and 1-MCP treatments were effective in delaying colour evolution significantly ($p < 0.05$) as compared to the control fruit batch. However, no significant differences ($p > 0.05$) were found between the MT- and 1-MCP-treated fruit, although at the beginning of the experiment, the 1-MCP treatment controlled the decrease in CIE *Hue** (Figure 4B), probably due to a higher chlorophyll content, as observed in Figure 4A. The chlorophyll content and CIE *Hue** were not in consonance, probably due to the fact that not only chlorophylls are related to CIE *Hue** evolution, but carotenoids also are present in all green tissues of plants, and the evolution of these carotenoids in kiwifruit also affects the CIE *Hue** colour evolution at immature green stages, as they do for yellow flesh cultivars [76]. MT applications on different fruit species such as strawberry and sweet cherry [54,77] also displayed a similar pattern, maintaining different colour parameters as compared with control fruit. In kiwifruit, the carotenoid content shows a decreasing trend during the beginning of the storage, whereas the chlorophyll content remains stable and starts to decrease once ripening is more advanced [78]. The major colour changes in kiwifruit are caused by the enzymatic and nonenzymatic development of brown-pigmented substances and by decolouration due to chlorophyll and carotenoid degradation decreasing the CIE *Hue** values in the flesh, but this degradative evolution is directly related to membrane integrity [79]. For this reason, the effect of these treatments maintaining the chlorophyll content and CIE *Hue** values could be affected by the maintenance of fruit firmness and membrane structure provided by both 1-MCP and MT treatments, as was shown in our results.

3.5. Effect of Exogenous 1-MCP and MT on EL and Chilling Injury

Cold storage induces an increase in ROS in kiwifruit, which ultimately cause oxidative damage to cell membranes, increasing the levels of EL due to the loss of cellular integrity [80]. EL increased throughout cold storage for all treatments. However, the 1-MCP treatment was significantly effective ($p < 0.05$) in delaying the electrolyte outflow as compared to the control fruit. On the other hand, although a delay in the evolution of this parameter was also observed in the MT-treated fruit, the differences displayed as compared to the control fruit were smaller (Figure 5A). Thus, as described in this study, 1-MCP and MT, by maintaining membrane integrity [22,26,34] and probably through an improvement in the oxidative balance [40,63,81], could be the main causes for reducing oxidative stress through maintaining the structure of the cell membranes and, thus, delaying EL evolution in fruit treated with these substances.

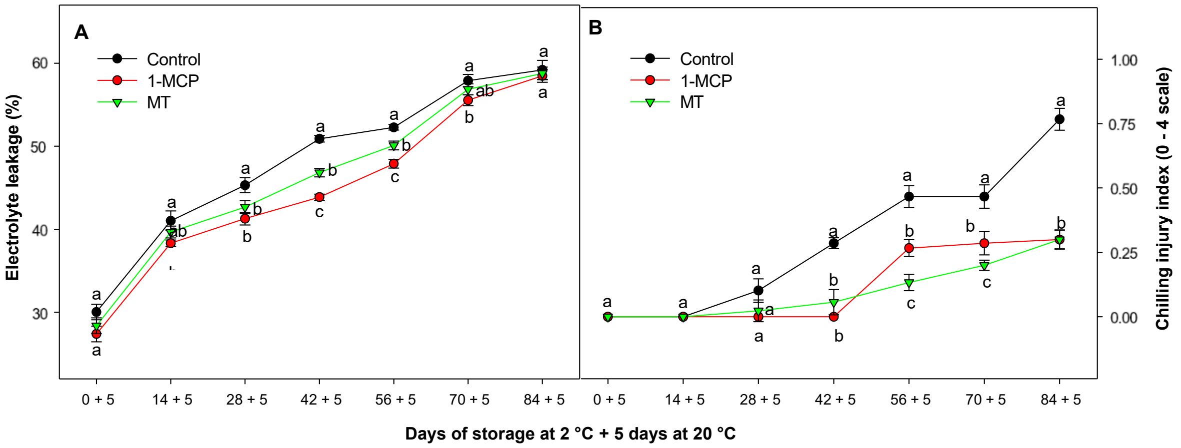

Figure 5. Evolution of electrolyte leakage (%) (**A**) and chilling injury index (0–4 scale) (**B**) of 'Hayward' kiwifruit treated with distilled water (Control), 1-MCP at 0.5 μL L^{-1}, or melatonin at 0.1 mM (MT) during cold storage plus 5 days at 20 °C. Data are the mean ± SE (n = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

Chilling injury is a physiological disorder that manifests itself in fruit after being exposed to temperatures close to the freezing point. Cold tolerance depends on several factors such as the species, cultivar, harvest time, temperature, and time of exposure to cold storage [19,82]. Usually, chilling injury symptoms increase after fruit are moved from cold storage to an ambient temperature. Chilling injury in kiwifruit appeared after 42 days of cold storage plus an additional period of 5 days at 20 °C, increasing until the end of storage. As expected, the impact of CI during storage was, in general, low at 2 °C for kiwifruit, but small watery spots appeared in a small percentage of the tested fruit. A recent study has confirmed that although a 2.5 °C storage temperature prevents chilling injury in kiwifruit [83], lower temperatures such as 2 °C may affect kiwifruit CI, especially when the fruit are stored at this temperature rapidly after harvest [58], as was observed in this experiment. However, MT and 1-MCP increased cold tolerance significantly ($p < 0.05$) as compared to the control fruit during storage (Figure 5B). The MT-treated fruit displayed a lower CI impact as compared to 1-MCP after 56 and 70 days of storage; however, this additional effect on MT-treated fruit was not related to EL evaluations. MT and 1-MCP postharvest treatments may increase cold tolerance by protecting the cell membranes, as has been observed in other studies with these substances in other plant species [58,84–86]. In kiwifruit, both MT treatments [40] and 1-MCP fumigations [64] have been shown to reduce the symptoms of CI caused by suboptimal temperatures. A higher fruit firmness and membrane integrity could be the main factors increasing kiwifruit's cold tolerance, as observed in the MT and 1-MCP-treated fruit in this study.

4. Conclusions

1-Methylcyclopropene and melatonin postharvest treatments delayed kiwifruit ripening, reducing weight losses and fruit respiration and maintaining higher fruit firmness and acidity level in a similar way. 1-Methylcyclopropene fumigations were more effective in maintaining higher values when the colour evolution and total chlorophyll content were evaluated as compared to the rest of the batches in this study. However, these parameters are only visible when the kiwifruit peel was removed. In this sense, kiwifruit chilling injury was lower when melatonin treatments were applied as compared to 1-methylcyclopropene. Therefore, alternative postharvest treatments based on melatonin solutions could be considered as effective as 1-methylcyclopropene fumigations which are applied commercially nowadays. However, further research including the effect of the combination of these treatments at different concentrations and the impact of these technologies on advancing the ripening stages at harvest should be conducted.

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References

1. Satpal, D.; Kaur, J.; Bhadariya, V.; Sharma, K. *Actinidia deliciosa* (Kiwi fruit): A comprehensive review on the nutritional composition, health benefits, traditional utilization, and commercialization. *J. Food Process. Preserv.* **2021**, *45*, e15588. [CrossRef]
2. Wang, S.; Qiu, Y.; Zhu, F. Kiwifruit (*Actinidia* spp.): A review of chemical diversity and biological activities. *Food Chem.* **2021**, *350*, 128469. [CrossRef]
3. Richardson, D.P.; Ansell, J.; Drummond, L.N. The nutritional and health attributes of kiwifruit: A review. *Eur. J. Nutr.* **2018**, *57*, 2659–2676. [CrossRef]
4. Wang, H.; Li, X.; Wang, J.; Vidyarthi, S.K.; Wang, H.; Zhang, X.G.; Gao, L.; Yang, K.W.; Zhang, J.S.; Xiao, H.W. Effects of postharvest ripening on water status and distribution, drying characteristics, volatile profiles, phytochemical contents, antioxidant capacity and microstructure of kiwifruit (*Actinidia deliciosa*). *Food Control* **2022**, *139*, 109062. [CrossRef]
5. Yi, J.; Kebede, B.T.; Grauwet, T.; Loey, A.V.; Hu, X.; Hendrickx, M. A multivariate approach into physicochemical, biochemical and aromatic quality changes of purée based on 'Hayward' kiwifruit during the final phase of ripening. *Postharvest Biol. Technol.* **2016**, *117*, 206–216. [CrossRef]
6. Dai, Y.; Wang, Z.; Leng, J.; Sui, Y.; Jiang, M.; Wisniewski, M.; Liu, J.; Wang, Q. Eco-friendly management of postharvest fungal decays in kiwifruit. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 8307–8318. [CrossRef]
7. Mitalo, O.W.; Tokiwa, S.; Kondo, Y.; Otsuki, T.; Galis, I.; Suezawa, K.; Kataoka, I.; Doan, A.T.; Nakano, R.; Ushijima, K.; et al. Low temperature storage stimulates fruit softening and sugar accumulation without ethylene and aroma volatile production in kiwifruit. *Front. Plant Sci.* **2019**, *10*, 888. [CrossRef] [PubMed]
8. Cha, G.H.; Kumarihami, H.P.C.; Kim, H.L.; Kwack, Y.B.; Kim, J.G. Storage temperature influences fruit ripening and changes in organic acids of kiwifruit treated with exogenous ethylene. *Hortic. Sci. Technol.* **2019**, *37*, 618–629.
9. Chai, J.; Wang, Y.; Liu, Y.; Gu, Z.; Liu, Z. High O_2/N_2 controlled atmosphere accelerates postharvest ripening of 'Hayward' kiwifruit. *Sci. Hortic.* **2022**, *300*, 111073. [CrossRef]
10. Han, Y.; East, A.; Nicholson, S.; Jeffery, P.; Glowacz, M.; Heyes, J. Benefits of modified atmosphere packaging in maintaining 'Hayward' kiwifruit quality at room temperature retail conditions. *N. Z. J. Crop Hortic. Sci.* **2022**, *50*, 242–258. [CrossRef]
11. Kim, K.H.; Yook, H.S. Effect of gamma irradiation on quality of kiwifruit (*Actinidia deliciosa* var. deliciosa cv. Hayward). *Radiat. Phys. Chem.* **2009**, *78*, 414–421. [CrossRef]

12. Kumarihami, H.P.C.; Kim, Y.H.; Kwack, Y.B.; Kim, J.; Kim, J.G. Application of chitosan as edible coating to enhance storability and fruit quality of Kiwifruit: A Review. *Sci. Hortic.* **2022**, *292*, 110647. [CrossRef]
13. Öztürk, B.; Yücedag˘ , F. Effects of methyl jasmonate on quality properties and phytochemical compounds of kiwifruit (*Actinidia deliciosa* cv. Hayward) during cold storage and shelf life. *Turk. J. Agric. For.* **2021**, *45*, 154–164. [CrossRef]
14. Liu, Q.; Li, X.; Jin, S.; Dong, W.; Zhang, Y.; Chen, W.; Shi, L.; Cao, S.; Yang, Z. γ-Aminobutyric acid treatment induced chilling tolerance in postharvest kiwifruit (*Actinidia chinensis* cv. Hongyang) via regulating ascorbic acid metabolism. *Food Chem.* **2023**, *404*, 134661. [CrossRef]
15. Minas, I.S.; Karaoglanidis, G.S.; Manganaris, G.A.; Vasilakakis, M. Effect of ozone application during cold storage of kiwifruit on the Development of stem-end rot caused by *Botrytis cinerea*. *Postharvest Biol. Technol.* **2010**, *58*, 203–210. [CrossRef]
16. Luo, A.; Bai, J.; Li, R.; Fang, Y.; Li, L.; Wang, D.; Zhang, L.; Liang, J.; Huang, T.; Kou, L. Effects of ozone treatment on the quality of kiwifruit during postharvest storage affected by *Botrytis cinerea* and *Penicillium expansum*. *J. Phytopathol.* **2019**, *167*, 470–478. [CrossRef]
17. Cantin, C.M.; Holcroft, D.; Crisosto, C.H. Postharvest application of 1-methylcyclopropene (1-MCP) extends shelf life of kiwifruit. *Acta Hortic.* **2011**, *913*, 621–626. [CrossRef]
18. Xu, F.; Liu, S.; Liu, Y.; Xu, J.; Liu, T.; Dong, S. Effectiveness of lysozyme coatings and 1-MCP treatments on storage and preservation of kiwifruit. *Food Chem.* **2019**, *288*, 201–207. [CrossRef]
19. Krupa, T.; Tomala, K.; Zaras´-Januszkiewicz, E. Evaluation of Storage Quality of 'Hardy' Kiwifruit (*Actinidia arguta*): Effect of 1-MCP and Maturity Stage. *Agriculture* **2022**, *12*, 2062. [CrossRef]
20. Cornacchia, R.; Amodio, M.L.; Rinaldi, R.; Colelli, G. Effect of 1-methylcyclopropene and controlled atmosphere on storage of kiwifruits. *Fresh Prod.* **2008**, *2*, 22–25.
21. Koukounaras, A.; Sfakiotakis, E. Effect of 1-MCP prestorage treatment on ethylene and CO_2 production and quality of 'Hayward' kiwifruit during shelf-life after short, medium and long term cold storage. *Postharvest Biol. Technol.* **2007**, *46*, 174–180. [CrossRef]
22. Watkins, C.B. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnol. Adv.* **2006**, *24*, 389–409. [CrossRef]
23. Boquete, E.J.; Trinchero, G.D.; Fraschina, A.A.; Vilella, F.; Sozzi, G.O. Ripening of 'Hayward' kiwifruit treated with 1-methylcyclopropene after cold storage. *Postharvest Biol. Technol.* **2004**, *32*, 57–65. [CrossRef]
24. Salazar, J.; Jorquera, C.; Campos-Vargas, R.; Jorgensen, C.; Zapata, P.; Infante, R. Effect of the application timing of 1-MCP on postharvest traits and sensory quality of a yellow-fleshed kiwifruit. *Sci. Hortic.* **2019**, *244*, 82–87. [CrossRef]
25. Algul, B.E.; Al Shoffe, Y.; Park, D.; Miller, W.B.; Watkins, C.B. Preharvest 1-methylcyclopropene treatment enhances 'stress-associated watercore' dissipation in 'Jonagold' apples. *Postharvest Biol. Technol.* **2021**, *181*, 111689. [CrossRef]
26. Huang, H.; Guo, L.; Wang, L.; Wang, H.; Ma, S.; Jiang, Y.; Qu, H. 1-Methylcyclopropene (1-MCP) slows ripening of kiwifruit and affects energy status, membrane fatty acid contents and cell membrane integrity. *Postharvest Biol. Technol.* **2019**, *156*, 110941. [CrossRef]
27. Park, Y.S.; Im, M.H.; Gorinstein, S. Shelf-life extension and antioxidant activity of 'Hayward' kiwifruit as a result of prestorage conditioning and 1-methylcyclopropene treatment. *J. Food Sci. Technol.* **2015**, *52*, 2711–2720. [CrossRef]
28. Lim, S.; Han, S.H.; Kim, J.; Lee, H.J.; Lee, J.G.; Lee, E.J. Inhibition of hardy kiwifruit (*Actinidia aruguta*) ripening by 1-methylcyclopropene during cold storage and anticancer properties of the fruit extract. *Food Chem.* **2016**, *190*, 150–157. [CrossRef]
29. Huan, C.; Zhang, J.; Jia, Y.; Jiang, T.; Shen, S.; Zheng, X. Effect of 1-methylcyclopropene treatment on quality, volatile production and ethanol metabolism in kiwifruit during storage at room temperature. *Sci. Hortic.* **2020**, *265*, 109266. [CrossRef]
30. Blankenship, S.M.; Dole, J.M. 1-Methylcyclopropene: A review. *Postharvest Biol. Technol.* **2003**, *28*, 1–25. [CrossRef]
31. Zhang, A.; Zhang, Q.; Li, J.; Gong, H.; Fan, X.; Yang, Y.; Liu, X.; Yin, X. Transcriptome co-expression network analysis identifies key genes and regulators of ripening kiwifruit ester biosynthesis. *BMC Plant Biol.* **2020**, *20*, 103. [CrossRef] [PubMed]
32. Debnath, B.; Islam, W.; Li, M.; Sun, Y.; Lu, X.; Mitra, S.; Hussain, M.; Liu, S.; Qiu, D. Melatonin mediates enhancement of stress tolerance in plants. *Int. J. Mol. Sci.* **2019**, *20*, 1040. [CrossRef]
33. Madebo, M.P.; Hu, S.; Zheng, Y.; Jin, P. Mechanisms of chilling tolerance in melatonin treated postharvest fruits and vegetables: A review. *J. Future Foods* **2021**, *1*, 156–167. [CrossRef]
34. Cao, S.; Qu, G.; Ma, C.; Ba, L.; Ji, N.; Meng, L.; Lei, J.; Wang, R. Effects of melatonin treatment on the physiological quality and cell wall metabolites in kiwifruit. *Food Sci. Technol.* **2021**, *42*, e85421. [CrossRef]
35. Hu, M.; Li, J.; Rao, J. Effect of melatonin on ripening and senescence of postharvest kiwifruits. *Sh. Kexue/Food Sci.* **2018**, *39*, 226–232.
36. Wang, X.; Liang, D.; Xie, Y.; Lv, X.; Wang, J.; Xia, H. Melatonin application increases accumulation of phenol substances in kiwifruit during storage. *Emir. J. Food Agric.* **2019**, *31*, 361–367.
37. Liang, D.; Shen, Y.; Ni, Z.; Wang, Q.; Lei, Z.; Xu, N.; Deng, Q.; Lin, L.; Wang, J.; Lv, X.; et al. Exogenous melatonin application delays senescence of kiwifruit leaves by regulating the antioxidant capacity and biosynthesis of flavonoids. *Front. Plant Sci.* **2018**, *9*, 426. [CrossRef]
38. Wang, H.; Wang, J.; Mujumdar, A.S.; Jin, X.; Liu, Z.L.; Zhang, Y.; Xiao, H.W. Effects of postharvest ripening on physicochemical properties, microstructure, cell wall polysaccharides contents (pectin, hemicellulose, cellulose) and nanostructure of kiwifruit (*Actinidia deliciosa*). *Food Hydrocoll.* **2021**, *118*, 106808. [CrossRef]
39. Tavarini, S.; Degl'Innocenti, E.; Remorini, D.; Massai, R.; Guidi, L. Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of 'Hayward' kiwifruit. *Food Chem.* **2008**, *107*, 282–288. [CrossRef]

40. Jiao, J.; Jin, M.; Liu, H.; Suo, J.; Yin, X.; Zhu, Q.; Rao, J. Application of melatonin in kiwifruit (*Actinidia chinensis*) alleviated chilling injury during cold storage. *Sci. Hortic.* **2022**, *296*, 110876. [CrossRef]
41. Cheng, J.; Zheng, A.; Li, H.; Huan, C.; Jiang, T.; Shen, S.; Zheng, X. Effects of melatonin treatment on ethanol fermentation and ERF expression in kiwifruit cv. Bruno during postharvest. *Sci. Hortic.* **2022**, *293*, 110696. [CrossRef]
42. Almeida, D.P.; Gomes, M.H. Uncoupling the sensory effects of 1-Methylcyclopropene and ripening stage on 'Hayward' kiwifruit. *HortScience* **2009**, *44*, 1936–1940. [CrossRef]
43. Martínez-Romero, D.; Serrano, M.; Carbonell, A.; Burgos, L.; Riquelme, F.; Valero, D. Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. *J. Food Sci.* **2002**, *67*, 1706–1712. [CrossRef]
44. Mao, L.C.; Wang, G.Z.; Zhu, C.G.; Pang, H.Q. Involvement of phospholipase D and lipoxygenase in response to chilling stress in postharvest cucumber fruits. *Plant Sci.* **2007**, *172*, 400–405. [CrossRef]
45. McCollum, T.G.; McDonald, R.E. Electrolyte leakage, respiration, and ethylene production as indices of chilling injury in grapefruit. *HortScience* **1991**, *26*, 1191–1192. [CrossRef]
46. Vu, H.T.; Scarlett, C.J.; Vuong, Q.V. Changes of phytochemicals and antioxidant capacity of banana peel during the ripening process; with and without ethylene treatment. *Sci. Hortic.* **2019**, *253*, 255–262. [CrossRef]
47. Celano, G.; Minnocci, A.; Sebastiani, L.; D'auria, M.; Xiloyannis, C. Changes in the structure of the skin of kiwifruit in relation to water loss. *J. Hortic. Sci. Biotechnol.* **2009**, *84*, 41–46. [CrossRef]
48. Burdon, J.; Clark, C. Effect of postharvest water loss on 'Hayward' kiwifruit water status. *Postharvest Biol. Technol.* **2001**, *22*, 215–225. [CrossRef]
49. Valero, D.; Serrano, M. *Postharvest Biology and Technology for Preserving Fruit Quality*, 1st ed.; CRC Press: Boca Raton, FL, USA, 2010.
50. Jeong, J.; Huber, D.J.; Sargent, S.A. Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (*Persea americana*) fruit. *Postharvest Biol. Technol.* **2002**, *25*, 241–256. [CrossRef]
51. Guillén, F.; Castillo, S.; Zapata, P.J.; Martínez-Romero, D.; Serrano, M.; Valero, D. Efficacy of 1-MCP treatment in tomato fruit: 1. Duration and concentration of 1-MCP treatment to gain an effective delay of postharvest ripening. *Postharvest Biol. Technol.* **2007**, *43*, 23–27. [CrossRef]
52. Wang, Q.; Wei, Y.; Chen, X.; Xu, W.; Wang, N.; Xu, F.; Wang, H.; Shao, X. Postharvest strategy combining maturity and storage temperature for 1-MCP-treated peach fruit. *J. Food Process. Preserv.* **2020**, *44*, e14388. [CrossRef]
53. Gao, H.; Zhang, Z.K.; Chai, H.K.; Cheng, N.; Yang, Y.; Wang, D.N.; Yang, T.; Cao, W. Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. *Postharvest Biol. Technol.* **2016**, *118*, 103–110. [CrossRef]
54. Liu, C.; Zheng, H.; Sheng, K.; Liu, W.; Zheng, L. Effects of melatonin treatment on the postharvest quality of strawberry fruit. *Postharvest Biol. Technol.* **2018**, *139*, 47–55. [CrossRef]
55. Onik, J.C.; Wai, S.C.; Li, A.; Lin, Q.; Sun, Q.; Wang, Z.; Duan, Y. Melatonin treatment reduces ethylene production and maintains fruit quality in apple during postharvest storage. *Food Chem.* **2021**, *337*, 127753. [CrossRef]
56. Latocha, P.; Krupa, T.; Jankowski, P.; Radzanowska, J. Changes in postharvest physicochemical and sensory characteristics of hardy kiwifruit (*Actinidia arguta* and its hybrid) after cold storage under normal versus controlled atmosphere. *Postharvest Biol. Technol.* **2014**, *88*, 21–33. [CrossRef]
57. Paniagua, A.C.; East, A.R.; Hindmarsh, J.P.; Heyes, J. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. *Postharvest Biol. Technol.* **2013**, *79*, 13–19. [CrossRef]
58. Gwanpua, S.G.; Zhao, M.; Jabbar, A.; Bronlund, J.E.; East, A.R. A model for firmness and low temperature-induced storage breakdown disorder of 'Hayward' kiwifruit in supply chain. *Postharvest Biol. Technol.* **2022**, *185*, 111789. [CrossRef]
59. Mahajan, B.V.C.; Singh, K.; Dhillon, W.S. Effect of 1-methylcyclopropene (1-MCP) on storage life and quality of pear fruits. *J. Food Sci. Technol.* **2010**, *47*, 351–354. [CrossRef]
60. Xu, F.; Liu, Y.; Dong, S.; Wang, S. Effect of 1-methylcyclopropene (1-MCP) on ripening and volatile compounds of blueberry fruit. *J. Food Process. Preserv.* **2020**, *44*, e14840. [CrossRef]
61. Ma, Q.; Zhang, T.; Zhang, P.; Wang, Z.Y. Melatonin attenuates postharvest physiological deterioration of cassava storage roots. *J. Pineal Res.* **2016**, *60*, 424–434. [CrossRef]
62. Sabban-Amin, R.; Feygenberg, O.; Belausov, E.; Pesis, E. Low oxygen and 1-MCP pretreatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith'apples. *Postharvest Biol. Technol.* **2011**, *62*, 295–304. [CrossRef]
63. Zhang, W.; Zhao, H.; Jiang, H.; Xu, Y.; Cao, J.; Jiang, W. Multiple 1-MCP treatment more effectively alleviated postharvest nectarine chilling injury than conventional one-time 1-MCP treatment by regulating ROS and energy metabolism. *Food Chem.* **2020**, *330*, 127256. [CrossRef] [PubMed]
64. Liu, H.; Pei, H.; Jiao, J.; Jin, M.; Li, H.; Zhu, Q.; Ma, Y.; Rao, J. 1-Methylcyclopropene treatment followed with ethylene treatment alleviates postharvest chilling injury of 'Xuxiang' kiwifruit during low-temperature storage. *Food Control* **2021**, *130*, 108340. [CrossRef]
65. Aghdam, M.S.; Fard, J.R. Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (*Fragaria* × *anannasa* cv. Selva) by enhancing GABA shunt activity. *Food Chem.* **2017**, *221*, 1650–1657. [CrossRef] [PubMed]

66. Bhardwaj, R.; Aghdam, M.S.; Arnao, M.B.; Brecht, J.K.; Fawole, O.A.; Pareek, S. Melatonin alleviates chilling injury symptom development in mango fruit by maintaining intracellular energy and cell wall and membrane stability. *Front. Nutr.* **2022**, *9*, 936932. [CrossRef]
67. Matsumoto, S.; Obara, T.; Luh, B.S. Changes in chemical constituents of kiwifruit during post-harvest ripening. *J. Food Sci.* **1983**, *48*, 607–611. [CrossRef]
68. Burdon, J.; Lallu, N.; Pidakala, P.; Barnett, A. Soluble solids accumulation and postharvest performance of 'Hayward' kiwifruit. *Postharvest Biol. Technol.* **2013**, *80*, 1–8. [CrossRef]
69. Goldberg, T.; Agra, H.; Ben-Arie, R. Quality of 'Hayward' kiwifruit in prolonged cold storage as affected by the stage of maturity at Harvest. *Horticulturae* **2021**, *7*, 358. [CrossRef]
70. Yaman, Ö.; Bayoındırlı, L. Effects of an edible coating and cold storage on shelf-life and quality of cherries. *LWT-Food Sci. Technol.* **2002**, *35*, 146–150. [CrossRef]
71. Aghdam, M.S.; Jannatizadeh, A.; Luo, Z.; Paliyath, G. Ensuring sufficient intracellular ATP supplying and friendly extracellular ATP signaling attenuates stresses, delays senescence and maintains quality in horticultural crops during postharvest life. *Trends Food Sci. Technol.* **2018**, *76*, 67–81. [CrossRef]
72. Gao, S.P.; Hu, K.D.; Hu, L.Y.; Li, Y.H.; Han, Y.; Wang, H.L.; Lv, K.; Liu, Y.S.; Zhang, H. Hydrogen sulfide delays postharvest senescence and plays an antioxidative role in fresh-cut kiwifruit. *HortScience* **2013**, *48*, 1385–1392. [CrossRef]
73. Du, Y.; Jin, T.; Zhao, H.; Han, C.; Sun, F.; Chen, Q.; Yue, F.; Luo, Z.; Fu, M. Synergistic inhibitory effect of 1-methylcyclopropene (1-MCP) and chlorine dioxide (ClO_2) treatment on chlorophyll degradation of green pepper fruit during storage. *Postharvest Biol. Technol.* **2021**, *171*, 111363. [CrossRef]
74. Khanna-Chopra, R. Leaf senescence and abiotic stresses share reactive oxygen species-mediated chloroplast degradation. *Protoplasma* **2012**, *249*, 469–481. [CrossRef] [PubMed]
75. Wang, C.H.; Meng, Q.K.; Liu, X.H.; Xia, X.D.; Li, R.; Zhu, X.H.; Li, S.J. Effect of 1-MCP treatment on ascorbic acid content and its quality, physiological of *Actinidia chinensis*. *J. Chin. Inst. Food Sci. Technol.* **2014**, *14*, 134–141.
76. Fuke, Y.; Sasago, K.; Matsuoka, H. Determination of chlorophylls in kiwi fruit and their changes during ripening. *J. Food Sci.* **1985**, *50*, 1220–1223. [CrossRef]
77. Wang, F.; Zhang, X.; Yang, Q.; Zhao, Q. Exogenous melatonin delays postharvest fruit senescence and maintains the quality of sweet cherries. *Food Chem.* **2019**, *301*, 125311. [CrossRef]
78. Liu, Y.; Lv, G.; Chai, J.; Yang, Y.; Ma, F.; Liu, Z. The effect of 1-MCP on the expression of carotenoid, chlorophyll degradation, and ethylene response factors in 'Qihong' kiwifruit. *Foods* **2021**, *10*, 3017. [CrossRef]
79. Shiri, M.A.; Ghasemnezhad, M.; Moghadam, J.F.; Ebrahimi, R. Effect of $CaCl_2$ sprays at different fruit development stages on postharvest keeping quality of "Hayward" kiwifruit. *J. Food Process. Preserv.* **2016**, *40*, 624–635. [CrossRef]
80. Malekzadeh, P.; Khosravi-Nejad, F.; Hatamnia, A.A.; Mehr, R.S. Impact of postharvest exogenous γ-aminobutyric acid treatment on cucumber fruit in response to chilling tolerance. *Physiol. Mol. Biol. Plants* **2017**, *23*, 827–836. [CrossRef]
81. Wang, S.Y.; Shi, X.C.; Wang, R.; Wang, H.L.; Liu, F.; Laborda, P. Melatonin in fruit production and postharvest preservation: A review. *Food Chem.* **2020**, *320*, 126642. [CrossRef] [PubMed]
82. Parkin, K.L.; Marangoni, A.; Jackman, R.L.; Yada, R.Y.; Stanley, D.W. Chilling injury. A review of possible mechanisms. *J. Food Biochem.* **1989**, *13*, 127–153. [CrossRef]
83. Gwanpua, S.G.; Jabbar, A.; Zhao, M.; Heyes, J.A.; East, A.R. Investigating the potential of dual temperature storage as a postharvest management practice to mitigate chilling injury in kiwifruit. *Int. J. Refrig.* **2018**, *86*, 62–72. [CrossRef]
84. Azadshahraki, F.; Jamshidi, B.; Mohebbi, S. Postharvest melatonin treatment reduces chilling injury and enhances antioxidant capacity of tomato fruit during cold storage. *Adv. Hortic. Sci.* **2018**, *32*, 299–310.
85. Gao, H.; Lu, Z.; Yang, Y.; Wang, D.; Yang, T.; Cao, M.; Cao, W. Melatonin treatment reduces chilling injury in peach fruit through its regulation of membrane fatty acid contents and phenolic metabolism. *Food Chem.* **2018**, *245*, 659–666. [CrossRef]
86. Jiao, W.; Xi, Y.; Cao, J.; Fan, X.; Jiang, W. Regulatory effects of $CaCl_2$, sodium isoascorbate, and 1-methylcyclopropene on chilling injury of banana fruit at two ripening stages and the mechanisms involved. *J. Food Process. Preserv.* **2018**, *42*, e13442. [CrossRef]

4.6. Publicación VI (Transcripción literal)

VI

Innovative postharvest management for Hass avocado at the preclimacteric stage: a combined technology with GABA and 1-MCP

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Innovative Postharvest Management for Hass Avocado at the Preclimacteric Stage: A Combined Technology with GABA and 1-MCP

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Abstract: Avocado (*Persea americana* Mill.) is a subtropical climacteric fruit with a limited shelf life due to its high sensitivity to low temperatures. Chilling injury (CI) produced by cold storage displays symptoms in avocado fruit such as irregular ripening, darkening of the mesocarp, hardening of vascular strands, lipid oxidation with "off flavors", and pitting and darkening of the skin, increasing weight loss. Accordingly, we studied the effect of γ-aminobutyric acid (GABA) and 1-methylcyclopropene (1-MCP) alone or in combination as postharvest treatments to maintain quality and to increase cold tolerance. Hass avocados were stored at 5 °C plus 5 days at room temperature. The results showed that the combined treatment improved fruit quality parameters as compared with control fruit and with those treated with only 1-MCP or GABA. The combined treatment delayed synergistically the postharvest ripening process. This delayed pattern was concomitant with a delayed ethylene pattern in GABA + 1-MCP or 1-MCP fruit batches. CI symptoms and electrolyte leakage were minimized in all GABA and 1-MCP fruit batches specifically in the combined treatment. For this reason, the synergistic effect of the combination of treatments may be recommended as an effective alternative strategy to prolong the postharvest quality of avocado during refrigerated storage.

Keywords: *Persea americana* Mill; cold tolerance; shelf life; ripening; γ-aminobutyric acid; 1-methylcyclopropene

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1. Introduction

Avocado (*Persea americana* Mill.) is native to central and southern Mexico and is a fruit grown worldwide and with nutraceutical attributes, specifically the Hass variety [1]. This fruit is known for its nutritional content and human health benefits, which are primarily due to the source of fat-soluble nutrients or phytochemicals such as xanthophyll, carotenoids, phenols and phytosterols [2]. The consumption of Hass avocados has been linked to beneficial effects, including anticarcinogenic and antioxidant properties, as well as a reduced risk of cardiovascular diseases and obesity [2].

The avocado fruit is very susceptible to mechanical damage, particularly bruising and to chilling injury (CI) during cold storage, as well as physiological breakdown and microbial spoilage. For this reason, ensuring the highest fruit quality and preventing spoilage during transportation or commercial distribution is a significant challenge in avocado fruit due to their delicate nature [3]. Avocados have significant postharvest losses up to 43% according to Yahia et al. [4]. This places avocados among the fruits with some of the highest loss percentages. Recent research establishes that postharvest losses for fruits and vegetables worldwide range from 28 to 55%, depending on the plant species [5]. This highlights the urgent need for technological improvements. This is especially important for avocados, not just to help with distribution but also to enhance storability ensuring a longer shelf life and reducing waste after harvesting. In addition, avocado is a climacteric fruit, meaning that once harvested, it can continue to ripen due to its high respiration rate and abundant ethylene production [6]. Mishandling can greatly diminish the shelf life of avocados, leading to water loss, premature ripening, and structural decay. These issues have a direct impact on the economic viability of both producers and retailers. Several factors stimulate the avocado senescence, including the level of fruit ripening, metabolic and respiration rates, ethylene production, accumulation of carbon dioxide, and the heat generated during the climacteric process. These aspects collectively contribute to the degradation of avocado quality over time [7].

The initial postharvest strategies in the fruit supply chain involve selection and categorization processes aimed at minimizing postharvest losses. To ensure avocados can endure prolonged transportation periods, the level of ripeness often serves as a key classification criterion [8]. On the other hand, storage temperature is one of the most usual and effective technologies to delay fruit ripening, managing to reduce the climacteric increase in respiration, in addition to controlling internal CO_2 outflow and external oxygen inflow [9]. However, exposure of avocados to suboptimal temperatures below their critical threshold (10–15 °C) can suppress the ripening process inducing CI and fruit quality losses [10]. Symptoms of avocado CI are skin pitting, blanching, lack of ripening, gray flesh, vascular browning, bad taste, and decay [11] becoming more evident when products are transferred at room temperature.

Other strategies such as employing ethylene inhibitors to delay ripening or using phytohormones to maintain fruit quality have been suggested to alleviate postharvest losses in avocado fruit. Among these strategies is 1-methylcyclopropene (1-MCP), a commercially ethylene inhibitor that acts to delay the ripening process [12]. In this sense, 1-MCP decelerates the respiration rate and weight loss, mitigates physiological discoloration of the mesocarp (gray flesh), and reduces internal CI. Additionally, 1-MCP has shown effectiveness at diminishing fungal infections, enhancing antioxidant balance during fruit storage [13]. It should also be considered that the efficacy of 1-MCP on climacteric fruits is influenced by various factors such as fruit maturity, genotype, compound concentration, application time and method, storage temperature, packaging material, and controlled atmosphere [14].

The synergistic effect of 1-MCP combined with other substances such as melatonin has been studied in zucchini [15] and with methyl jasmonate in pomegranate [16], improving the general 1-MCP effect on the fruit and reducing CI. In avocado, the effect of the combination of 1-MCP with the *Acremonium strictum* Elicitor Subtilisin (AsES) has also been studied, showing higher levels of firmness and reduction in weight loss at the end of storage [17]. Otherwise, different post-harvest treatments based on other elicitors such as γ-aminobutyric acid (GABA) have also been studied. This substance serves as a signaling molecule, playing an important role in plant growth and development, cellular osmoregulation, cellular nitrogen supply, and free radical scavenging under biotic and abiotic stress conditions [18,19]. GABA also has been demonstrated to enhance CI tolerance by stimulating the activities of antioxidant enzymes and maintaining elevated levels of ATP content increasing the energy supply. This mechanism safeguards cell membranes from CI in different species, including peach and orange [20,21]. On the other hand, GABA treatments applied after harvesting have been shown to reduce lipid peroxidation and sustain membrane integrity by stimulating the activity of antioxidant enzymes in different fruits [21,22]. However, as far as we know, there is not any previous study in which the GABA effect on avocado has been studied. For this reason, in this study, we evaluated the effects of post-harvest GABA applications and the impacts of both substances (GABA + 1-MCP) when they were applied as a combined treatment on the shelf life and CI of avocado during cold storage.

2. Materials and Methods

2.1. Plant Material and Postharvest Treatments

Avocados cv. Hass (*Persea americana* Mill.) were harvested at a commercial plot in Granada (Spain) and transported to the laboratory on the same day of harvest. In the laboratory, the fruit was visually selected considering parameters such as homogenous size and color, with an absence of defects in each piece. After selection (360 fruits), avocados were grouped into 3 replicates of 5 fruits for each treatment and sampling day.

For GABA (Sigma-Aldrich, Madrid, Spain), freshly prepared 1 mM solutions were used for immersion treatments performed for 10 min. This treatment was selected among different GABA concentrations (1–10 mM) and immersion times assayed (10 min and 1 h) in previous experiments (Figures S1 and S2). For the 1-MCP (Smartfresh^{TM}, Agrofresh Inc., Philadelphia, PA, USA) and control treatments, distilled water immersions were also applied for 10 min to provide the same conditions. All solutions contained tween-20 (Sigma-Aldrich, Madrid, Spain) 0.05% v/v. In this sense, 25 avocados were soaked in 10 L of control or GABA solution for each replicate (n = 3) and treatment. This ensured thorough coverage and consistent treatment of the fruit. Following treatments, all the fruits were exposed for a drying period at 20 °C (one hour) before being transferred to individual 130 L plastic containers, ensuring consistent conditions across all batches. These containers were then tightly sealed, allowing 1-MCP to take effect over 24 h at 20 °C, while control and GABA 1 mM batches were exposed to ambient air in similarly sealed containers at the same temperature. The application of 1-MCP treatments involved the utilization of commercial tablets, releasing 0.3 μL L^{-1}, alongside a commercial activator solution provided by SmartFresh (Agrofresh®). For 1-MCP, the optimum concentration applied on these fruits was chosen from those tested on this cultivar by Defilippi et al. [23]. Afterward, these fruits were stored for 35 days at 5 °C and 90% relative humidity, followed by an additional 5-day period at 20 °C for subsequent shelf-life assessments. When stored at 5 °C, CI symptoms could be observed [23], and this temperature was used with simulated shipments studies for this avocado cultivar [24]. All reagents used in this study were of analytical purity.

2.2. Postharvest Quality Parameters

The weight loss of individual avocados was expressed as a percentage relative to their initial weight. The results represented the mean ± SE of 15 fruits per replicate (n = 3).

Fruit firmness was individually determined using a TX-XT2i texture analyzer (Stable Microsystems, Godalming, UK) equipped with a flat probe with a diameter of 100 mm. The disc descended at a rate of 20 mm min^{-1} until a 5% deformation was achieved. Two readings were taken equidistant along the equatorial region of each fruit. Firmness was expressed as the ratio of applied force to the distance traveled (N mm^{-1}).

Respiration and ethylene levels were measured in triplicate by enclosing five avocados per treatment within a 4.6 L plastic container with five fruit per replicate sealed hermetically with a rubber stopper, for a duration of 60 min at room temperature. Following this, a 1 mL gas sample in duplicate (n = 3) was injected into a Shimadzu GC-14B gas chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) to determine the CO_2 concentration, while the other two samples were injected into a Shimadzu GC-2010 gas chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) equipped with an FID detector to measure the ethylene concentration. The chromatographic parameters were detailed by Martínez-Romero et al. [25]. The respiration rate and ethylene production were quantified as mg of CO_2 kg^{-1} h^{-1} and nL g^{-1} h^{-1}, respectively.

The total soluble solids content (TSS) was determined in duplicate in the filtered and centrifuged juice extracted from the mixture of 5 avocado halves for each replicate per batch. The extraction was effectuated after mixing 10 g of avocado pulp with 10 mL of deionized water and finely homogenized (Ultraturrax, T18 basic, IKA, Berlin, Germany). TSSs were measured in filtered and centrifuged juice (10,000 × g) at 4 °C for 20 min and using an Atago PR-101 digital refractometer (Atago Co., Ltd. Tokyo, Japan) at 20 °C. TSS results were expressed in g 100 g^{-1}. The total acidity (TA) was determined in duplicate through automatic titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) with 0.1 N NaOH until reaching pH 8.1, with 1 mL of juice in 25 mL distilled water. The results were expressed as g 100 g^{-1} of the predominant organic acid (malic acid equivalents) using the following formula: $(M \times 0.067 \times N \times D)/m) \times 100$, where M is the amount of NaOH used in mL, 0.067 is the acid milliequivalent conversion factor, N is the normality of NaOH, D is the dilution factor used for the extraction, and m is the amount of sample evaluated.

The skin color was assessed using a reflectance Minolta colorimeter (CRC400, Minolta Camera Co., Kanto¯ , Tokyo, Japan) equipped with a D65 illuminant and a CIE 2° standard observer measuring through an 8 mm aperture. Three measurements were taken per avocado, obtaining 15 measures for each replicate (n = 3) at three equidistant points along the equatorial area based on CIELab coordinates (CIE L^*, CIE a^* and CIE b^*). CIE h*ue** (180 + $\tan^{-1}$ (b^*/a^*), if $a^* < 0$), *chroma** ($(a^{*2} + b^{*2})^{1/2}$), and ΔE values ($((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$ were calculated [26], and the results were expressed as the mean ± SE (n = 3).

The chlorophyll content was evaluated by extracting a homogeneous mixture of skin samples from 5 avocado halves for each replicate per batch following the method described by Vu et al. [27]. In this sense and after the pigments were extracted by homogenization in methanol for 2 min, samples were centrifuged, and the supernatant was measured in duplicate at the wavelengths of 665.2 and 652.4 in a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan). The results were expressed as mg of chlorophyll kg^{-1}.

Determination of the susceptibility of avocado to CI was assayed by visual assessment of mesocarp discoloration according to the method described by Hershkovitz et al. [28]. The fruits were sliced lengthwise into two halves to inspect the pulp's appearance, and the internal damage was evaluated using a 10-point scale as follows: 0 for no damage, 1 for minor damage, 5 for moderate damage, and 10 for severe damage.

Electrolyte leakage (EL) in avocado skin was assessed following the method outlined by Lorente et al. [16] with slight modifications. Fifteen discs were extracted from each replicate of five avocado fruits using a 1 cm diameter cork borer. After three rinses lasting 3 min each with deionized water, the discs were subjected to continuous shaking with 50 mL of deionized water at room temperature. After 60 min, the initial electrical conductivity (C1) was measured using a Crison conductivity meter. Samples were subsequently frozen and then heated to 121 °C for 15 min. Total conductivity (C2) was measured with samples at room temperature (20 °C). EL was calculated as (C1/C2) × 100.

2.3. Statistical Analysis

This study was designed with a randomized design. All data presented were expressed as mean ± standard error (SE) (n = 3). Analysis of variance (ANOVA) and mean comparisons were conducted using Tukey's test to identify significant differences ($p < 0.05$). Different lowercase letters denote significant differences between treatments on the same sampling date. All statistical analyses were carried out using the SPSS software package, version 22 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Effect of 1-MCP and GABA Treatments on Weight Loss and Firmness

The reduction in fruit weight was influenced by two main factors. First, fruit weight loss was connected to vapor-phase diffusion, driven by the vapor pressure difference between the interior and exterior cell fruit compartments. Second, this process was associated with avocado metabolism [29] and the water loss occurring through the stomata, stem scars, and cuticle [3,30]. In this sense, the water loss was determined by the composition and thickness of the cuticle, which was different across different cultivars and maturity stages.

Avocado fruit weight loss increased progressively throughout the storage time, showing a linear behavior (Figure 1A). Both 1-MCP and GABA individually delayed weight losses in avocado throughout storage, with significant differences ($p < 0.05$) observed as compared with control fruit. These differences were more pronounced when both treatments (GABA + 1-MCP) were combined after 28 days of cold storage, with 4.38% less weight loss as compared with control fruit (Figure 1A). The delayed weight loss in 1-MCP-treated fruit was linked to a delayed initiation of the respiratory climacteric phase in 1-MCP-treated fruits such as has been observed previously in avocado [28]. In addi- tion to moisture reduction, the metabolism of organic substances like sugars associated to respiration rate played an important role in the decline of fruit weight. Consequently, managing the factors that trigger metabolic processes within the fruit tissue became crucial to controlling weight loss after harvesting [31].

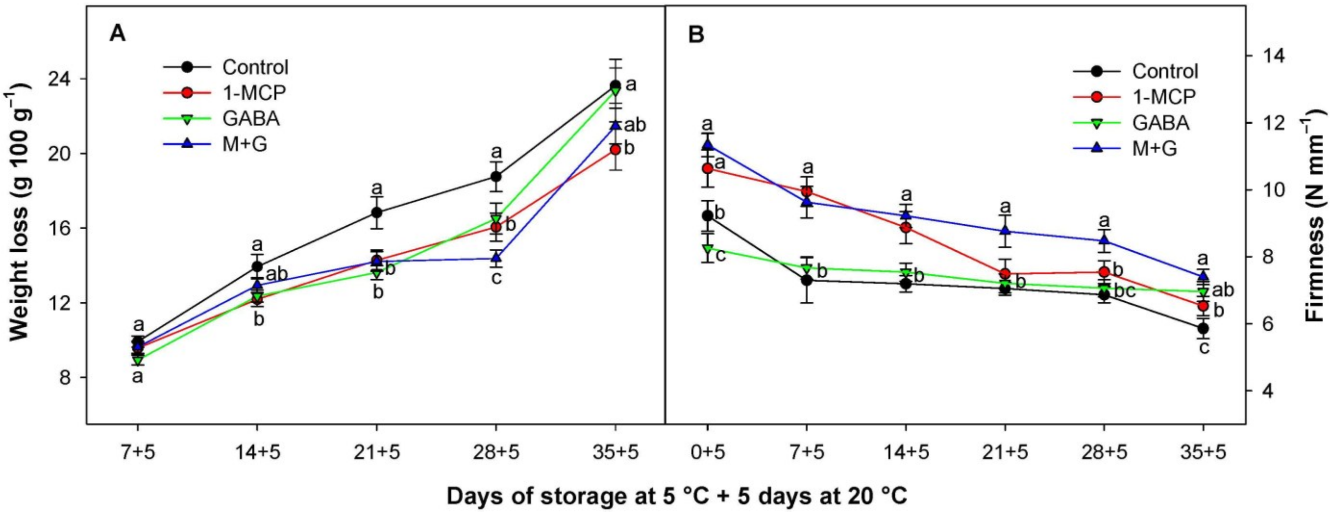

Figure 1. Evolution of weight loss (g 100 g^{-1}) (**A**) and fruit firmness (N mm^{-1}) (**B**) of Hass avocado treated with distilled water (control), 1-MCP at 0.3 µL L^{-1}, GABA at 1 mM, and the combination of 1-MCP and GABA (M+G) during storage at 5 °C plus 5 days at 20 °C. The data are the mean ± SE (n = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

On the other hand, GABA postharvest treatments demonstrated the effect on reducing the degree of membrane lipid peroxidation due to the ability of this compound to improve the antioxidant defense systems, reducing the content of EL and MDA in carambola fruit [32]. In this sense, the higher activity of antioxidant enzymes and the accumulation of bioactive compounds in GABA-treated fruit was related to lower chilling damage, cell membrane permeability, and weight loss in Chinese olive fruit [33].

Texture softening primarily takes place during the fruit ripening process. However, this also makes the fruit more vulnerable to microbial infections and physical damage [31]. In our study, the ripening process of Hass avocados resulted in a gradual reduction in fruit firmness over time, and significant differences in firmness ($p < 0.05$) between the treatments were observed (Figure 1B). Treatments containing 1-MCP maintained significantly higher levels of fruit firmness as compared with the control fruit throughout 14 days of cold storage. Furthermore, when GABA was applied in combination with 1-MCP, fruit firmness was maintained for an additional 7 days. This effect demonstrated synergy between these two substances, as the application of GABA alone did not show any significant difference ($p > 0.05$) as compared with the control batch as it was observed in other fruit species such as peach [22]. While this study did not report any GABA impact on firmness preservation in avocados by itself, previous research indicates that GABA has been linked to maintaining elevated levels of firmness in postharvest mangoes when compared with control samples [34].

Research studies indicate that 1-MCP effectively suppresses the enzymatic activities associated with softening during the ripening process; as a result, the degradation of cell walls is reduced, leading to a delayed softening of fruit [35]. The effect of delayed firmness in avocados treated with 1-MCP has been studied previously [27,36,37], but it has not been studied as a combined treatment with GABA. In this study we observed how the combination of treatments can provide higher values of fruit firmness as compared with control fruit or GABA- and 1-MCP-treated batches when they are applied alone. The GABA pathway is activated, especially against stress responses such as cold storage [38], maintaining cell metabolism against stress conditions in climacteric and non-climacteric fruit [39,40]. For this reason, it seems reasonable to assume that external GABA application could have an improved effect on cell metabolism in MCP-treated tissues, which experience delayed cell wall disassembly compared with control fruit. Consequently, other metabolic parameters, such as respiration and ethylene production, need to be considered to evaluate the impacts of these treatments.

3.2. Effect of 1-MCP and GABA Treatments on Respiration Rate and Ethylene

The climacteric rise of respiration rate was observed on the 14th day of storage at 5 °C plus 5 days at 20 °C in the control and in the treated fruit, resulting in a subsequent decline in both cases. However, 1-MCP significantly ($p < 0.05$) reduced the climacteric rise of the respiration rate as compared with the control batches (Figure 2A). The reduction in the respiratory rate by 1-MCP has been previously studied in avocado [27,41] and in other climacteric fruits such as apricot and kiwi [42,43]. The reduction in the respiration rate can be explained because 1-MCP blocks the ethylene receptors, also decreasing the physiological processes in fruits and vegetables [44]. While GABA treatments did not show significant differences ($p > 0.05$) when applied alone as compared with control fruit, when combined with 1-MCP, they demonstrated the most effective ($p < 0.05$) reduction in the overall respiratory production of the experiment. The combined treatment (GABA + 1-MCP) peaked higher than in fruit treated only with 1-MCP, showing an influenced pattern similar to GABA-treated fruit at that moment. The application of 1-MCP has been demonstrated to increase GABA content in both apples and pears [45,46]. Additionally, exogenous applications of GABA stimulate the GABA pathway, thereby promoting the tricarboxylic acid (TCA) cycle in GABA-treated plants. This stimulation contributes to an increase in the cellular energy status, which demands immediate substrates to obtain energy especially under cold storage [47,48]. For this reason, it is reasonable to hypothesize that with the combined treatment, both technologies together provided additional GABA content stimulated endogenously, with 1-MCP and with GABA exogenous applications reducing the cell stress. Under cold-related stress, maintaining ATP content is crucial in various plant species [49]. This is achieved by regulating the mitochondrial oxidative defense system and preserving the mitochondrial structure. A deficiency in ATP has been shown to increase the content of reactive oxygen species (ROS) [50]. Hence, these findings collectively highlight the intricate interplay between 1-MCP, GABA, and ATP in plants, for enhancing plant resilience under suboptimal temperatures. The respiration rate of climacteric fruits is influenced by ethylene production. In climacteric fruits such as avocado, there is an increase in ethylene production at the onset of ripening [7]. On the other hand, the application of exogenous ethylene increased the respiratory rate, whereas the use of 1-MCP decreased it [51,52].

In this study, ethylene production exhibited typical ethylene climacteric patterns, and the peaks occurred at 7 days of storage in control and GABA treatments and after 14 days of storage in 1-MCP-treated batches alone and in combination with GABA immersions (Figure 2B). The same delay was observed in peak ethylene production by Jeong et al. [53], Hershkovitz et al. [27], and Zhang et al. [36] where the climacteric peak of ethylene occurred 6, 4, and 7 days later, respectively, in the avocado fruit treated with 1-MCP as compared with the control batch. Although 1-MCP delayed the ethylene peak and production, it did not quantitatively reduce the ethylene peak in 1-MCP-treated batches, as reported in previous studies [27,51,53].

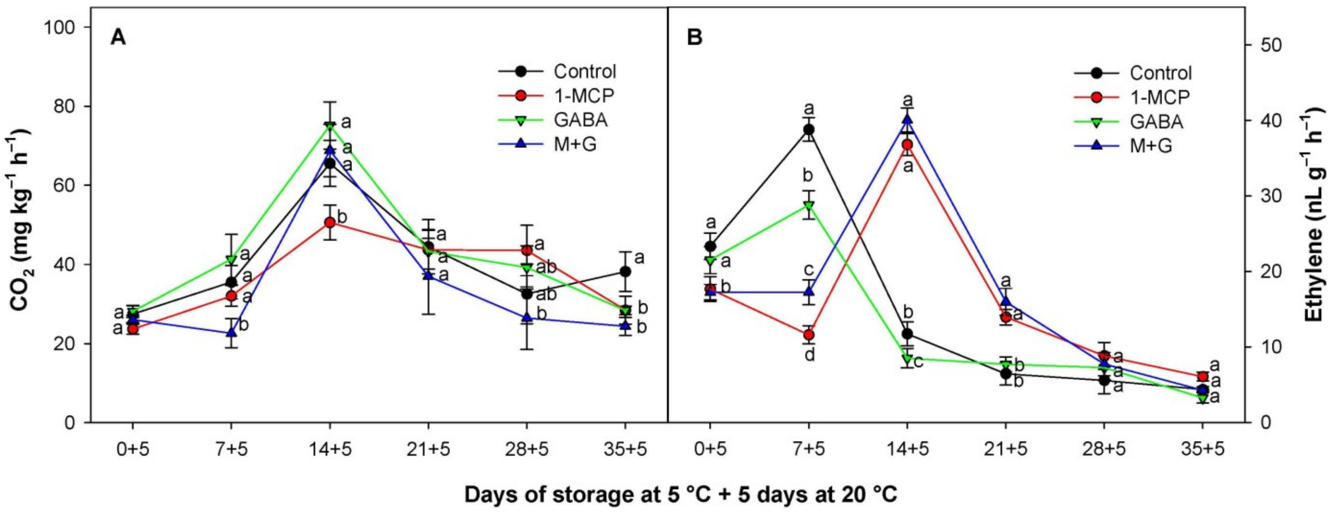

Figure 2. Evolution of respiration rate (mg CO_2 kg^{-1} h^{-1}) (**A**) and ethylene production (nL g^{-1} h^{-1}) (**B**) of Hass avocado treated with distilled water (control), 1-MCP at 0.3 µL L^{-1}, GABA at 1 mM, and the combination of 1-MCP and GABA (M+G) during storage at 5 °C plus 5 days at 20 °C. The data are the mean ± SE (n = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

When GABA treatments were applied alone, they significantly ($p < 0.05$) reduced ethylene production at the beginning of the study. However, after 14 days of storage, there were no significant differences ($p > 0.05$) as compared with control fruit. In other studies, GABA postharvest treatments were also effective in reducing ethylene production in apples [54] and guavas [55]. This effect has been observed in tomatoes treated with GABA, leading to a lower accumulation of key metabolic intermediates such as 1-aminocyclopropane-1-carboxylic acid (ACC), which slightly reduces ethylene production in this fruit. On the other hand, 1-MCP, as a potent inhibitor of ethylene action, reduces ethylene activity, thereby slowing down fruit ripening, affecting also metabolic substrates [44].

3.3. Effect of 1-MCP and GABA Treatments on Total Soluble Solids (TSSs) and Total Acidity (TA)

In the early stages of storage, a gradual increase in TSSs was observed (Figure 3A). As the avocado ripened, this process intensified, and TSSs reached their peak. However, as the avocado continued its ripening process, some sugars may have converted into other compounds or been lost, potentially resulting in a decline in TSSs toward the end of the storage period. Similar values and fluctuations were also previously observed in Hass avocados [56,57], which seemed to occur specifically in late season avocado sucrose content with or without 1-MCP treatments [58]. In this sense, the TSS value of GABA-treated fruits showed a consistent increase with higher values than those observed in both control and 1-MCP-treated fruits from day 0 to day 14, followed by a decline toward the end of the storage period. The 1-MCP-treated fruit alone displayed the lowest TSS level along the storage. The increased TSS levels in GABA-treated batches could be attributed to GABA as an immediate metabolic substrate, potentially resulting in a reduced respiration rate delaying the breakdown of sugars and leading to TSS accumulation, as observed in other fruit species such as peach, apple, and persimmon [20,54,59]. This association was linked to a stimulation of the GABA-shunt pathway covering energy demands reducing stress and metabolism [48]. In this sense, we previously observed in this study a delayed ethylene pattern in GABA-treated avocado batches, as well as in the respiration process in GABA + 1-MCP batches (Figure 2A,B).

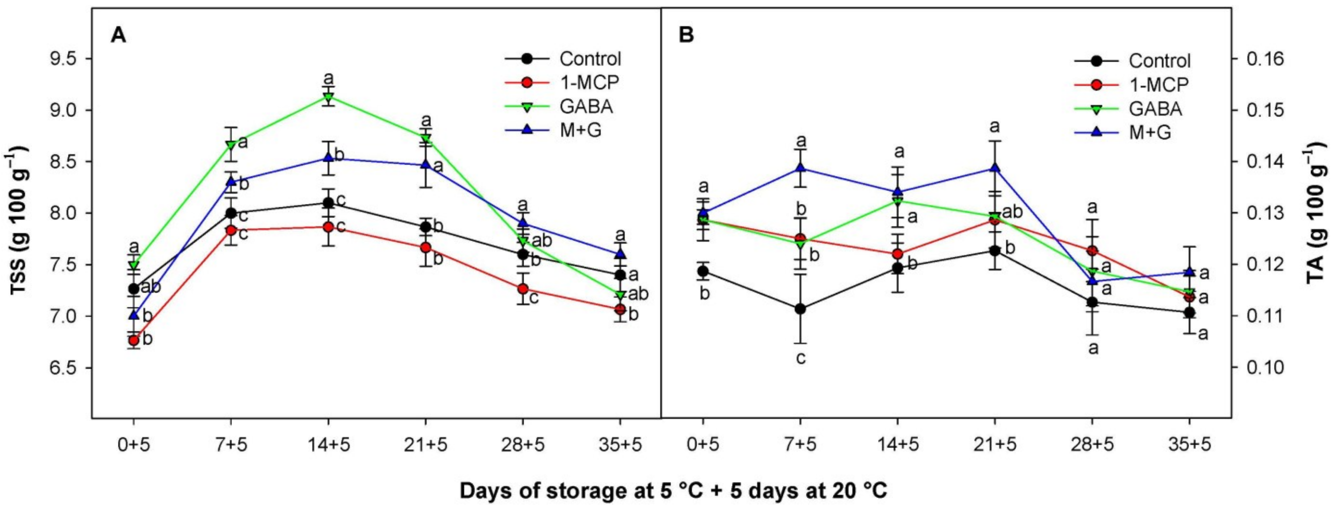

Figure 3. Evolution of total soluble solids (TSSs) (g 100 g^{-1}) (**A**) and total acidity (TA) (g 100 g^{-1}) (**B**) of Hass avocados treated with distilled water (control), 1-MCP at 0.3 μL L^{-1}, GABA at 1 mM, and the combination of 1-MCP and GABA (M+G) during storage at 5 °C plus 5 days at 20 °C. The data are the mean ± SE (n = 3). Different lowercase letters show significant differences (p < 0.05) among treatments for each sampling date.

On the other hand, the TA values of the control fruit slightly decreased along the storage (Figure 3B) in consonance with other authors [57]. These values (0.10–0.14%) were similar than those observed by Salameh et al. [56] in Hass avocados grown in different locations (0.09–0.17%). However, GABA-treated fruit and MCP-treated batches exhibited significantly higher TA values as compared with the control fruit. Furthermore, the GABA + 1-MCP batch consistently displayed the highest TA levels, as compared with the other evaluated batches in general, confirming the delayed metabolism in GABA and 1-MCP treatments alone or combined. In fact, the additional positive effect observed when these substances were combined could be attributed to the synergistic benefits observed when applied individually. In this sense, GABA treatments also delayed TA evolution in other fruit species as persimmon fruit during storage [59] and after 1-MCP treatments [44] including avocado [60], as we have observed in this study, specifically for the GABA + 1-MCP-treated batch.

3.4. Effect of 1-MCP and GABA Treatments on Skin Color and Chlorophyll Content

The change in skin color of Hass avocados during ripening, transitioning from green to purple and ultimately black, occurs due to a reduction in chlorophyll concentration initially, succeeded by a rise in the presence of the anthocyanin known as cyanidin 3-O-glucoside [61]. Regarding exocarp color, the decline in color CIE *L** represented the loss of lightness in avocado during storage [61]. In this study, color CIE *L** maintained lower values during storage for the control fruit as compared with the fruit treated with GABA or 1-MCP both individually and applied as a combination of treatments (Figure 4A). On the other hand, CIE chroma* values were also delayed in all GABA- and 1-MCP-treated batches (Figure 4B). In this sense, 1-MCP treatments alone or combined with GABA displayed higher medium values than control fruit. External color was affected by GABA and 1-MCP in a similar way; batches containing 1-MCP alone or combined with GABA showed significant (p < 0.05) higher values as compared with GABA when it was applied alone, especially after short-term storage. This delayed pattern can be observed also regarding other color parameters evaluated as CIE *hue** and ΔE* (Figure S3).

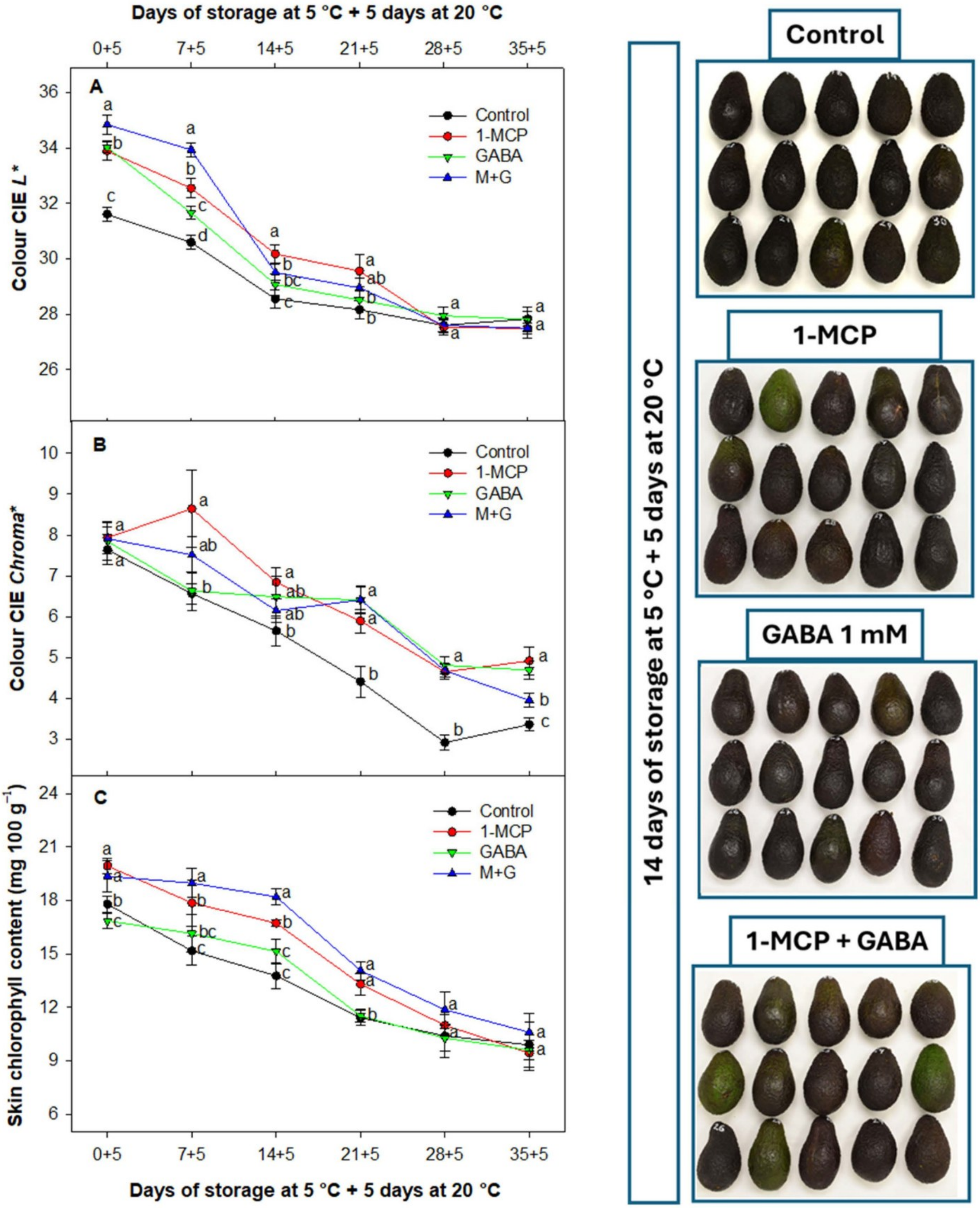

Figure 4. Appearance and evolution of skin external color CIE *L** (**A**), CIE *Chroma** (**B**), and skin chlorophyll content (mg 100 g^{-1}) (**C**) of Hass avocado treated with distilled water (control), 1-MCP at 0.3 μL L^{-1}, GABA at 1 mM, and the combination of 1-MCP and GABA (M+G) during storage at 5 °C plus 5 days at 20 °C. The data are the mean ± SE (n = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

Accordingly, decreases in the total chlorophyll content of avocado skin were observed during storage (Figure 4C). However, this decrease was significantly delayed ($p < 0.05$) in fruit treated with the combination of 1-MCP and GABA as compared with fruit batches treated individually with these compounds and control fruit, mainly after short-term storage. The 1-MCP showed a stronger effect in delaying the reduction in chlorophylls as compared with fruit batches treated only with GABA, although both treatments controlled this parameter with higher medium values in general than those observed for control fruit. Toward the end of the study, no differences were observed between all the different fruit batches.

For many fruits, the reduction in chlorophyll and the evolution of colored pigments are crucial components of the ripening process. The slower progression of CIE L^* evolution has been associated with a reduced rate of fruit weight loss and a delayed ripening process [62]. The 1-MCP inhibits the loss of greenness, or yellowing, in most plant products. Consequently, effective 1-MCP applications delay all these processes but without causing them to become permanently inhibited [44]. The 1-MCP has been observed to delay external color in Hass avocado in different studies through diminished production of ethylene [44,60]. Hershkovitz et al. [27] also elucidated that the delayed color in avocado fruit treated with 1-MCP was related to a delayed ethylene production and a lower chlorophyllase activity.

On the other hand, although the effect of GABA treatments has not been evaluated previously in avocados, several studies have recently confirmed the effectiveness of 40 mM GABA in delaying color evolution and chlorophyll degradation as a preharvest treatment in apples [63] and as a postharvest treatment in pistachios with 10 mM GABA [64]. These concentrations were considerably higher than those applied in this postharvest study (1 mM). The additional effectiveness observed with the combined treatment (1-MCP + GABA) has not been previously studied in any fruit species, but it could be attributed to the synergistic interaction of both technologies producing a combined positive effect.

3.5. Effect of 1-MCP and GABA Treatments on Electrolyte Leakage and Chilling Injury

During ripening, senescence, and in response to stress, a sequence of oxidative metabolic reactions occurs, impacting the integrity of cell membranes. This leads to changes in membrane permeability and results in cellular dysfunctions, including the unregulated movement of electrolytes [65]. On the other hand, one of the frequently observed phenomena associated with CI is an elevation in membrane permeability, characterized by an increase in the release of ions.

EL has been traditionally considered a qualitative indicator of CI in avocados and is a major marker of cell membrane deterioration under cold stress [28]. This parameter in avocado fruit has been shown to be associated with membrane permeability, indicating a strong correlation between EL with fruit softening and ethylene production [66]. For this reason, ethylene blocking substances such as 1-MCP have been previously reported as a successful technology for reducing CI in avocado [27,51].

In this study, we observed a comparable trend between CI symptoms in avocado fruit, manifested as mesocarp discoloration, and increased EL values. Control fruit exhibited higher levels of EL than treated fruit along 35 days of storage since this parameter was reduced by 10.02 and 10.96% for 1-MCP and GABA treatments, respectively, when applied alone (Figure 5A). However, when these two substances were combined, the reduction in CI reached 20.52%. Although the effects of GABA and 1-MCP were similar in reducing EL, the 1-MCP treatment was significantly ($p < 0.05$) more effective in fruit treated with 1-MCP than GABA when CI was evaluated (Figure 5B). In this sense, after 35 days of cold storage, CI was reduced by 42.81% and 20.31% for 1-MCP and GABA treatments, respectively, when applied alone. Additionally, a synergistic effect was observed in this parameter when combining these treatments, reducing CI by 61.56%.

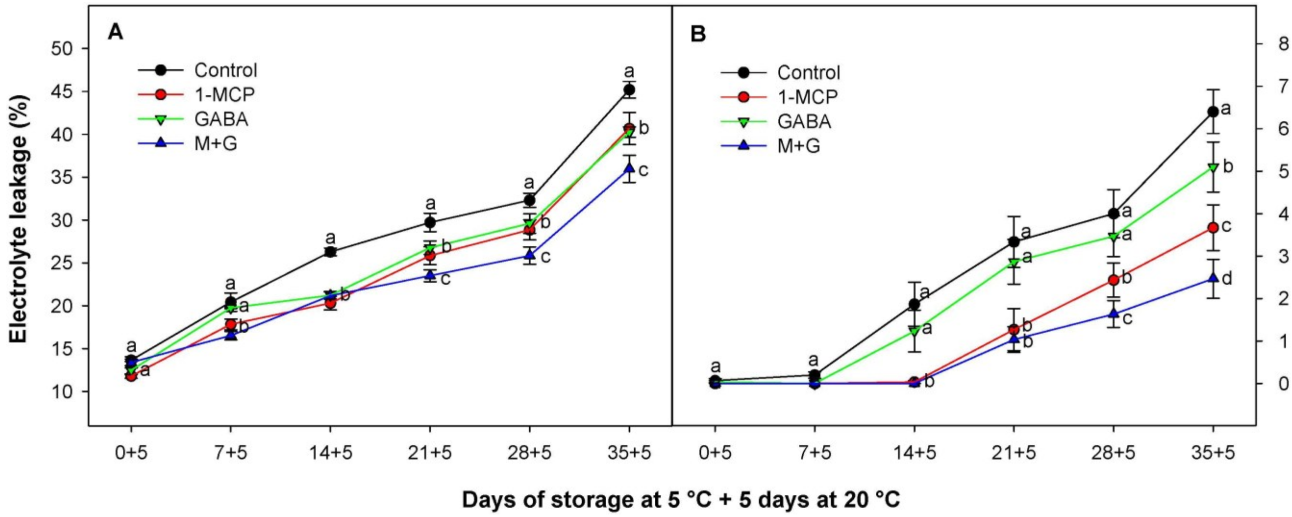

Figure 5. Evolution of electrolyte leakage (%) (**A**) and CI (0–10 scale) (**B**) of Hass avocado treated with distilled water (control), 1-MCP at 0.3 μL L^{-1}, GABA at 1 mM, and the combination of 1-MCP and GABA (M+G) during storage at 5 °C plus 5 days at 20 °C. The data are the mean ± SE (n = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

Loss of membrane integrity facilitates enzyme–substrate interaction, triggering flesh darkening and discolorations because of enzymatic browning [67]. This phenomenon is stimulated by increased production of ROS during postharvest storage and reduced activity of antioxidant enzymes in avocado [67] and other plant species [30,68]. For this reason, the status of the antioxidant and energy balance plays a critical role in protecting cellular membranes and enhancing tolerance to chilling conditions [48]. In this sense, GABA can induce the expression of antioxidant enzymes or have indirect antioxidant effects by modulating metabolic pathways related to oxidative stress [48]. The potential of GABA treatment has been studied as an effective tool to positively protect cell membranes from lipid peroxidation in blood orange and loquat fruit [69,70]. This effect has been related to an improvement of the antioxidant balance, increasing the activity of the antioxidant enzymes in fruit treated with GABA at preharvest [71] or postharvest [69,72]. This maintenance of antioxidant balance combined with an increase in the GABA-shunt pathway in GABA-treated fruit has been shown to provide extra energy in cells to address cold stress [48].

The 1-MCP has been studied in avocados and has been observed to mitigate membrane damage in avocado fruit through the reduction in EL, reducing, in turn, the prevalence of physiological mesocarp discoloration (gray flesh) and internal CI [27,28]. These results were coincident with those obtained in this study, where it could be observed that treatments with 1-MCP alone or in combination with GABA significantly ($p < 0.05$) reduced avocado CI during storage (Figure 5B).

These results can be visually verified in Figure 6, where the effectiveness of the different treatments assayed on CI was also observed. GABA plays an important role in reducing CI symptoms through better maintenance of cell membrane integrity in plant products such as peach [20], banana [73], and zucchini [74]. For these reasons, the increased effect combining both technologies may be related to the additive benefits observed with the combined treatment.

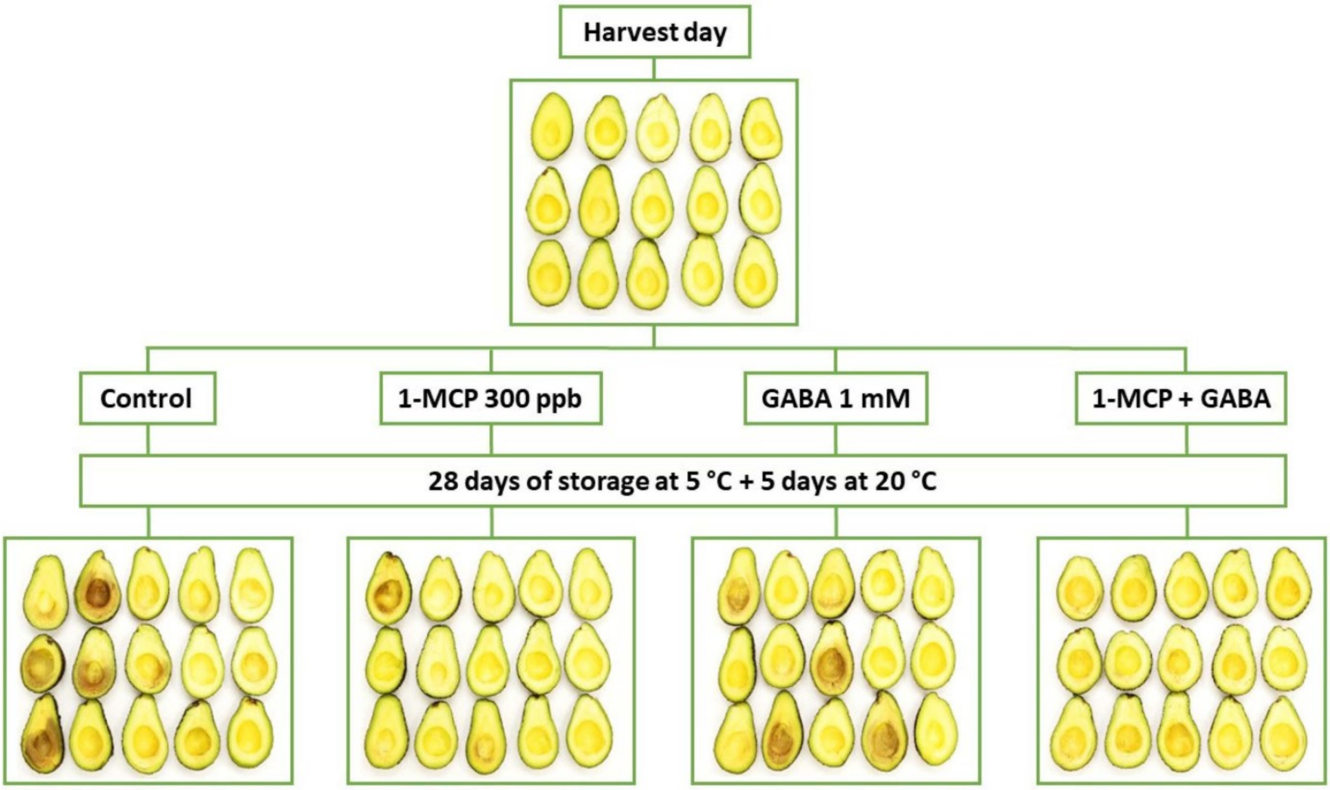

Figure 6. Internal visual aspect of Hass avocado at harvest day and avocado treated with distilled water (control), 1-MCP at 0.3 μL L^{-1}, GABA at 1 mM, and the combination of 1-MCP and GABA (M+G) after 28 days of storage after 5 °C plus 5 days at 20 °C.

4. Conclusions

In conclusion, the results of this study suggested the significant potential of combined applications of 1-methylcyclopropene and γ-aminobutyric acid in enhancing the overall quality and extending the shelf life of Hass avocados. The results indicated that the combined application of 1-methylcyclopropene and γ-aminobutyric acid impacted with synergistic effects, resulting in improved preservation of fruit weight, firmness, and respiratory rate regulation. Additionally, the treatments influenced biochemical parameters contributing to the maintenance of fruit quality attributes and delaying external color changes, enhancing visual appeal and marketability. Notably, the reduction in electrolyte leakage and chilling injury highlights the role of these treatments in enhancing membrane integrity and minimizing physiological disorders during storage. Overall, the results suggested that the combined application of 1-methylcyclopropene and γ-aminobutyric acid could be an effective postharvest management strategy for Hass avocados, offering growers and stakeholders a viable approach to optimize fruit quality and extend market availability.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/foods13162485/s1: Figure S1: Preliminary experiment. Weight loss (%) evolution of 'Hass' avocado; Figure S2: Preliminary experiment. Chilling injury symptoms (0–10 scale) of 'Hass' avocado; Figure S3: Evolution of skin external colour CIE *hue** (A) and ΔE (B) of 'Hass' avocado.

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

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References

1. Sánchez-Quezada, V.; Campos-Vega, R.; Loarca-Piña, G. Prediction of the physicochemical and nutraceutical characteristics of 'Hass' avocado seeds by correlating the physicochemical avocado fruit properties according to their ripening state. *Plant Foods Hum. Nutr.* **2021**, *76*, 311–318. [CrossRef]
2. Bhuyan, D.J.; Alsherbiny, M.A.; Perera, S.; Low, M.; Basu, A.; Devi, O.A.; Barooah, M.S.; Li, C.G.; Papoutsis, K. The odyssey of bioactive compounds in avocado (*Persea americana*) and their health benefits. *Antioxidants* **2019**, *8*, 426. [CrossRef]
3. Bill, M.; Sivakumar, D.; Thompson, A.K.; Korsten, L. Avocado fruit quality management during the postharvest supply chain. *Food Rev. Int.* **2014**, *30*, 169–202. [CrossRef]
4. Yahia, E.M.; Fonseca, J.M.; Kitinoja, L. Chapter 2—Postharvest losses and waste. In *Postharvest Technology of Perishable Horticultural Commodities*; Yahia, E.M., Ed.; Woodhead Publishing: Cambridge, UK, 2019; pp. 43–69. ISBN 9780128132760.
5. Karoney, E.M.; Molelekoa, T.; Bill, M.; Siyoum, N.; Korsten, L. Global research network analysis of fresh produce postharvest technology: Innovative trends for loss reduction. *Postharvest Biol. Technol.* **2024**, *208*, 112642. [CrossRef]
6. Vargas-Ortiz, M.; Rodríguez-Jimenes, G.; Salgado-Cervantes, M.; Pallet, D. Minimally Processed Avocado Through Flash Vacuum-Expansion: Its Effect in Major Physicochemical Aspects of the Puree and Stability on Storage. *J. Food Process. Pres.* **2017**, *41*, e12988. [CrossRef]
7. Arpaia, M.L.; Collin, S.; Sievert, J.; Obenland, D. 'Hass' avocado quality as influenced by temperature and ethylene prior to and during final ripening. *Postharvest Biol. Technol.* **2018**, *140*, 76–84. [CrossRef]
8. Magwaza, L.S.; Tesfay, S.Z. A review of destructive and non-destructive methods for determining avocado fruit maturity. *Food Bioprocess Technol.* **2015**, *8*, 1995–2011. [CrossRef]
9. Chirinos, R.; Ramon, K.; Mendoza, M.; Figueroa-Merma, A.; Pacheco-Ávalos, A.; Campos, D.; Pedreschi, R. Effect of Prolonged Cold Storage on the Dynamics of the Enzymatic and Non-Enzymatic Antioxidant System in the Mesocarp of Avocado (*Persea americana*) cv. Hass: Relationship with Oxidative Processes. *Horticulturae* **2022**, *8*, 880. [CrossRef]
10. Yahia, E.M.; Woolf, A.B. Avocado (*Persea americana* Mill.). In *Postharvest Biology and Technology of Tropical and Subtropical Fruits*; Yahia, E.M., Ed.; Woodhead Publishing: Cambridge, UK, 2011; pp. 125–186. ISBN 9781845697341.
11. Woolf, A.B.; Arpaia, M.L.; Defilippi, B.G.; Bower, J.P. Subtropical fruits: Avocados. In *Controlled and Modified Atmospheres for Fresh and Fresh-Cut Produce*; Gil, M.I., Beaudry, R., Eds.; Academic Press: San Diego, CA, USA, 2020; pp. 389–397. ISBN 9780128045992.
12. Zhang, J.; Ma, Y.; Dong, C.; Terry, L.A.; Watkins, C.B.; Yu, Z.; Cheng, Z.M.M. Meta-analysis of the effects of 1-methylcyclopropene (1-MCP) treatment on climacteric fruit ripening. *Hortic. Res.* **2020**, *7*, 208. [CrossRef] [PubMed]
13. Kruger, F.J.; Volschenk, G.O. Ripening patterns of South African export "Hass" avocado hold-back samples from commercial 1-methylcyclopropene (SmartFreshSM) applications. In Proceedings of the VIII World Avocado Congress, Cairns, Australia, 5–9 September 2011.
14. Satekge, T.K.; Magwaza, L.S. Postharvest application of 1-Methylcyclopropene (1-MCP) on climacteric fruits: Factors affecting efficacy. *Int. J. Fruit Sci.* **2022**, *22*, 595–607. [CrossRef]
15. Medina-Santamarina, J.; Serrano, M.; Ruiz-Aracil, M.C.; Ilea, M.I.M.; Martínez-Romero, D.; Guillén, F. A synergistic effect based on the combination of melatonin with 1-methylcyclopropene as a new strategy to increase chilling tolerance and general quality in zucchini fruit. *Foods* **2022**, *11*, 2784. [CrossRef]
16. Lorente-Mento, J.M.; Serrano, M.; Martínez-Romero, D.; Ruiz-Aracil, M.C.; Valero, D.; Guillén, F. The Simultaneous Use of 1-Methylcyclopropene and Methyl Jasmonate Vapor as an Innovative Strategy for Reducing Chilling Injury and Maintaining Pomegranate Fruit Quality at Suboptimal Temperatures. *Foods* **2023**, *13*, 60. [CrossRef] [PubMed]
17. Perato, S.M.; Martínez-Zamora, M.G.; Salazar, S.M.; Díaz-Ricci, J.C. The elicitor AsES stimulates ethylene synthesis, induce ripening and enhance protection against disease naturally produced in avocado fruit. *Sci. Hortic.* **2018**, *240*, 288–292. [CrossRef]
18. Kinnersley, A.M.; Turano, F.J. Gamma aminobutyric acid (GABA) and plant responses to stress. *Crit. Rev. Plant Sci.* **2000**, *19*, 479–509. [CrossRef]
19. Shelp, B.J.; Bown, A.W.; Zarei, A. 4-Aminobutyrate (GABA): A metabolite and signal with practical significance. *Botany* **2017**, *95*, 1015–1032. [CrossRef]
20. Yang, A.; Cao, S.; Yang, Z.; Cai, Y.; Zheng, Y. Γ-Aminobutyric acid treatment reduces chilling injury and activates the defence response of peach fruit. *Food Chem.* **2011**, *129*, 1619–1622. [CrossRef]

21. Sheng, L.; Shen, D.; Luo, Y.; Sun, X.; Wang, J.; Luo, T.; Zeng, Y.; Xu, J.; Deng, X.; Cheng, Y. Exogenous γ-aminobutyric acid treatment affects citrate and amino acid accumulation to improve fruit quality and storage performance of postharvest citrus fruit. *Food Chem.* **2017**, *216*, 138–145. [CrossRef] [PubMed]
22. Zhou, C.; Dong, W.; Jin, S.; Liu, Q.; Shi, L.; Cao, S.; Li, S.; Chen, W.; Yang, Z. Γ-Aminobutyric acid treatment induced chilling tolerance in postharvest peach fruit by upregulating ascorbic acid and glutathione contents at the molecular level. *Front. Plant Sci.* **2022**, *13*, 1059979. [CrossRef] [PubMed]
23. Defilippi, B.G.; Ejsmentewicz, T.; Covarrubias, M.P.; Gudenschwager, O.; Campos-Vargas, R. Changes in cell wall pectins and their relation to postharvest mesocarp softening of "Hass" avocados (*Persea americana* Mill.). *Plant Physiol. Biochem.* **2018**, *128*, 142–151. [CrossRef]
24. Ramírez-Gil, J.G.; López, J.H.; Henao-Rojas, J.C. Causes of Hass Avocado Fruit Rejection in Preharvest, Harvest, and Packinghouse: Economic Losses and Associated Variables. *Agronomy* **2020**, *10*, 8. [CrossRef]
25. Martínez-Romero, D.; Serrano, M.; Carbonell, A.; Burgos, L.; Riquelme, F.; Valero, D. Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. *J. Food Sci.* **2002**, *67*, 1706–1712. [CrossRef]
26. Lombardelli, C.; Benucci, I.; Esti, M. Novel food colorants from tomatoes: Stability of carotenoid-containing chromoplasts under different storage conditions. *LWT* **2021**, *140*, 110725. [CrossRef]
27. Vu, H.T.; Scarlett, C.J.; Vuong, Q.V. Changes of phytochemicals and antioxidant capacity of banana peel during the ripening process; with and without ethylene treatment. *Sci. Hortic.* **2019**, *253*, 255–262. [CrossRef]
28. Hershkovitz, V.; Saguy, S.I.; Pesis, E. Postharvest application of 1-MCP to improve the quality of various avocado cultivars. *Postharvest Biol. Technol.* **2005**, *37*, 252–264. [CrossRef]
29. Pachón, Y.V.; Balaguera-López, H.E.; Florez-Velasco, N. Postharvest behavior and chilling injury in avocado (*Persea americana Mill*) fruit cv. Hass treated with 1-methylcyclopropene, ethylene, and intermittent warming. *Rev. Fac. Nac. Agron. Medellín* **2022**, *75*, 9895–9907. [CrossRef]
30. Maftoonazad, N.; Ramaswamy, H.S. Effect of pectin-based coating on the kinetics of quality change associated with stored avocados. *J. Food Process. Pres.* **2008**, *32*, 621–643. [CrossRef]
31. Palou, L.; Serrano, M.; Martínez-Romero, D.; Valero, D. New approaches for postharvest quality retention of table grapes. *Fresh Prod.* **2010**, *4*, 103–110.
32. Mekontso, F.N.; Duan, W.; Cisse, E.H.M.; Chen, T.; Xu, X. Alleviation of postharvest chilling injury of carambola fruit by γ-aminobutyric acid: Physiological, biochemical, and structural characterization. *Front. Nutr.* **2021**, *8*, 752583. [CrossRef]
33. Fan, Z.; Lin, B.; Lin, H.; Lin, M.; Chen, J.; Lin, Y. Γ-Aminobutyric acid treatment reduces chilling injury and improves quality maintenance of cold-stored Chinese olive fruit. *Food Chem.* **2022**, *13*, 100208. [CrossRef]
34. Rastegar, S.; Khankahdani, H.H.; Rahimzadeh, M. Effect of γ-aminobutyric acid on the antioxidant system and biochemical changes of mango fruit during storage. *J. Food Meas. Charact.* **2020**, *14*, 778–789. [CrossRef]
35. Win, N.M.; Yoo, J.; Naing, A.H.; Kwon, J.G.; Kang, I.K. 1-Methylcyclopropene (1-MCP) treatment delays modification of cell wall pectin and fruit softening in "Hwangok" and "Picnic" apples during cold storage. *Postharvest Biol. Technol.* **2021**, *180*, 111599. [CrossRef]
36. Zhang, Z.; Huber, D.J.; Rao, J. Ripening delay of mid-climacteric avocado fruit in response to elevated doses of 1-methylcyclopropene and hypoxia-mediated reduction in internal ethylene concentration. *Postharvest Biol. Technol.* **2011**, *60*, 83–91. [CrossRef]
37. Olivares, D.; García-Rojas, M.; Ulloa, P.A.; Riveros, A.; Pedreschi, R.; Campos-Vargas, R.; Meneses, C.; Defilippi, B.G. Response Mechanisms of "Hass" Avocado to Sequential 1–methylcyclopropene Applications at Different Maturity Stages during Cold Storage. *Plants* **2022**, *11*, 1781. [CrossRef] [PubMed]
38. Shelp, B.J.; Bown, A.W.; McLean, M.D. Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci.* **1999**, *4*, 446–452. [CrossRef]
39. Lum, G.B.; Brikis, C.J.; Deyman, K.L.; Subedi, S.; DeEll, J.R.; Shelp, B.J.; Bozzo, G.G. Pre-storage conditioning ameliorates the negative impact of 1-methylcyclopropene on physiological injury and modifies the response of antioxidants and γ-aminobutyrate in 'Honeycrisp' apples exposed to controlled-atmosphere conditions. *Postharvest Biol. Technol.* **2016**, *116*, 115–128. [CrossRef]
40. Aghdam, M.S.; Fard, J.R. Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (*Fragaria×anannasa* cv. Selva) by enhancing GABA shunt activity. *Food Chem.* **2017**, *221*, 1650–1657. [CrossRef] [PubMed]
41. Olivares, D.; Alvarez, E.; Véliz, D.; García-Rojas, M.; Díaz, C.; Defilippi, B.G. Effects of 1-Methylcyclopropene and Controlled Atmosphere on Ethylene Synthesis and Quality Attributes of Avocado cvs. Edranol and Fuerte. *J. Food Qual.* **2020**, *20*, 5075218. [CrossRef]
42. Fan, X.; Argenta, L.; Mattheis, J.P. Inhibition of ethylene action by 1-methylcyclopropene prolongs storage life of apricots. *Postharvest Biol. Technol.* **2000**, *20*, 135–142. [CrossRef]
43. Ruiz-Aracil, M.C.; Guillén, F.; Ilea, M.I.M.; Martínez-Romero, D.; Lorente-Mento, J.M.; Valverde, J.M. Comparative Effect of Melatonin and 1-Methylcyclopropene Postharvest Applications for Extending 'Hayward' Kiwifruit Storage Life. *Agriculture* **2023**, *13*, 806. [CrossRef]
44. Watkins, C.B. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnol. Adv.* **2006**, *24*, 389–409. [CrossRef]
45. Lwin, H.P.; Rudell, D.R.; Lee, J. Metabolism and cold chain performance of 'Chuhwangbae' Asian pears as impacted by 1-MCP treatment. *Sci. Hortic.* **2021**, *288*, 110357. [CrossRef]

46. Deyman, K.L.; Brikis, C.J.; Bozzo, G.G.; Shelp, B.J. Impact of 1-methylcyclopropene and controlled atmosphere storage on polyamine and 4-aminobutyrate levels in "Empire" apple fruit. *Front. Plant Sci.* **2014**, *5*, 81066. [CrossRef] [PubMed]
47. Hijaz, F.; Nehela, Y.; Killiny, N. Application of gamma-aminobutyric acid increased the level of phytohormones in Citrus sinensis. *Planta* **2018**, *248*, 909–918. [CrossRef] [PubMed]
48. Aghdam, M.S.; Jannatizadeh, A.; Luo, Z.; Paliyath, G. Ensuring sufficient intracellular ATP supplying and friendly extracellular ATP signaling attenuates stresses, delays senescence and maintains quality in horticultural crops during postharvest life. *Trends Food Sci. Technol.* **2018**, *76*, 67–81. [CrossRef]
49. Peng, J.; Cao, S.F.; Zheng, Y.H. Managing chilling injury in fruits. *Acta Hortic.* **2013**, *1012*, 1087–1095. [CrossRef]
50. Li, Z.; Huang, T.; Tang, M.; Cheng, B.; Peng, Y.; Zhang, X. iTRAQ-based proteomics reveals key role of γ-aminobutyric acid (GABA) in regulating drought tolerance in perennial creeping bentgrass (*Agrostis stolonifera*). *Plant Physiol. Biochem.* **2019**, *145*, 216–226. [CrossRef]
51. Hershkovitz, V.; Friedman, H.; Goldschmidt, E.E.; Pesis, E. Ethylene regulation of avocado ripening differs between seeded and seedless fruit. *Postharvest Biol. Technol.* **2010**, *56*, 138–146. [CrossRef]
52. Yang, X.; Song, J.; Campbell-Palmer, L.; Fillmore, S.; Zhang, Z. Effect of ethylene and 1-MCP on expression of genes involved in ethylene biosynthesis and perception during ripening of apple fruit. *Postharvest Biol. Technol.* **2013**, *78*, 55–66. [CrossRef]
53. Jeong, J.; Huber, D.J.; Sargent, S.A. Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (*Persea americana*) fruit. *Postharvest Biol. Technol.* **2002**, *25*, 241–256. [CrossRef]
54. Han, S.; Nan, Y.; Qu, W.; He, Y.; Ban, Q.; Lv, Y.; Rao, J. Exogenous γ-aminobutyric acid treatment that contributes to regulation of malate metabolism and ethylene synthesis in apple fruit during storage. *J. Agric. Food Chem.* **2018**, *66*, 13473–13482. [CrossRef]
55. Chen, H.; Wang, H.; Jiang, X.; Chen, C.; Cheng, Y. Effects of γ-aminobutyric acid treatment on the quality properties and chilling tolerance of guava fruit during cold storage. *Food Ferm. Indust.* **2021**, *47*, 130–136. [CrossRef]
56. Salameh, M.; Nacouzi, D.; Lahoud, G.; Riachy, I.; El Kayal, W. Evaluation of Postharvest Maturity Indices of Commercial Avocado Varieties Grown at Various Elevations Along Lebanon's Coast. *Front. Plant Sci.* **2022**, *13*, 895964. [CrossRef] [PubMed]
57. Vázquez-López, Y.; Iribe-Salazar, R.; Carrazco-Escalante, M.; Gaxiola-Camacho, S.; Corrales, J.C. Quality variables of "Hass" avocado stored in modified atmosphere packaging. *Agrociencia* **2022**, *56*, 805–829. [CrossRef]
58. Meyer, M.D.; Terry, L.A. Fatty acid and sugar composition of avocado, cv. Hass, in response to treatment with an ethylene scavenger or 1-methylcyclopropene to extend storage life. *Food Chem.* **2010**, *121*, 1203–1210. [CrossRef]
59. Niazi, Z.; Razavi, F.; Khademi, O.; Aghdam, M.S. Exogenous application of hydrogen sulfide and γ-aminobutyric acid alleviates chilling injury and preserves quality of persimmon fruit (*Diospyros kaki*, cv. Karaj) during cold storage. *Sci. Hortic.* **2021**, *285*, 110198. [CrossRef]
60. Homez-Jara, A.; Cardenas-Roa, H.; Montealegre, M.; Lim, L.T.; Corradini, M.G.; Váquiro-Herrera, H.A.; Sandoval-Aldana, A. Postharvest Treatments of Hass Avocado (*Persea americana* Mill.) and Estimation of Its Quality Using Hyperspectral Imaging (HSI). *ACS Food Sci. Technol.* **2023**, *3*, 932–944. [CrossRef]
61. Ashton, O.B.; Wong, M.; Mcghie, T.K.; Vather, R.; Wang, Y.; Requejo-Jackman, C.; Ramankutty, P.; Woolf, A.B. Pigments in avocado tissue and oil. *J. Agric. Food Chem.* **2006**, *54*, 10151–10158. [CrossRef]
62. Nunes, M.C.N. Correlations between subjective quality and physicochemical attributes of fresh fruits and vegetables. *Postharvest Biol. Technol.* **2015**, *107*, 43–54. [CrossRef]
63. Al-Shoffe, Y.; Nock, J.F.; Zhang, Y.; Watkins, C.B. Pre-and post-harvest γ-aminobutyric acid application in relation to fruit quality and physiological disorder development in 'Honeycrisp' apples. *Sci. Hortic.* **2021**, *289*, 110431. [CrossRef]
64. Saeedi, M.; Mirdehghan, S.H.; Nazoori, F.; Esmaeilizadeh, M.; Saba, M.K. Impact of calcium and γ-aminobutyric acid (GABA) on qualitative attributes and shelf life characteristics of fresh in-hull pistachio during cold storage. *Postharvest Biol. Technol.* **2022**, *187*, 111863. [CrossRef]
65. Tian, S.; Qin, G.; Li, B. Reactive oxygen species involved in regulating fruit senescence and fungal pathogenicity. *Plant Mol. Biol.* **2013**, *82*, 593–602. [CrossRef] [PubMed]
66. Montoya, M.M.; López-Rodriguez, V.; De La Plaza, J.L. An improved technique for measuring the electrical conductivity of intact fruits. *LWT-Food Sci. Technol.* **1994**, *27*, 29–33. [CrossRef]
67. Chirinos, R.; Delgado-Pariona, J.; Aguilar-Galvez, A.; Figueroa-Merma, A.; Pacheco-Ávalos, A.; Campos, D.; Pedreschi, R. Postharvest Storage Differentially Modulates the Enzymatic and Non-Enzymatic Antioxidant System of the Exocarp and Mesocarp of Hass Avocado: Implications for Disorders. *Plants* **2023**, *12*, 4008. [CrossRef] [PubMed]
68. Aghdam, M.S.; Naderi, R.; Sarcheshmeh, M.A.A.; Babalar, M. Amelioration of postharvest chilling injury in anthurium cut flowers by γ-aminobutyric acid (GABA) treatments. *Postharvest Biol. Technol.* **2015**, *110*, 70–76. [CrossRef]
69. Habibi, F.; Ramezanian, A.; Rahemi, M.; Eshghi, S.; Guillén, F.; Serrano, M.; Valero, D. Postharvest treatments with γ-aminobutyric acid, methyl jasmonate, or methyl salicylate enhance chilling tolerance of blood orange fruit at prolonged cold storage. *J. Sci. Food Agric.* **2019**, *99*, 6408–6417. [CrossRef] [PubMed]
70. Zhang, H.; Pu, J.; Liu, H.; Wang, M.; Du, Y.; Tang, X.; Luo, X.; Wang, Y.; Deng, Q. Effects of L-Cysteine and γ-Aminobutyric Acid Treatment on Postharvest Quality and Antioxidant Activity of Loquat Fruit during Storage. *Int. J. Mol. Sci.* **2023**, *24*, 10541. [CrossRef] [PubMed]

71. 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. [CrossRef]
72. Fan, Z.; Lin, B.; Lin, Y.; Chen, Y.; Chen, G.; Chen, J.; Wang, H.; Chen, Y.; Lin, H. Alleviation of chilling injury in cold-stored Chinese olive (*Canarium album* Lour.) fruit by γ-aminobutyric acid treatment in relation to ROS metabolism. *Sci. Hortic.* **2024**, *327*, 112851. [CrossRef]
73. Wang, Y.; Luo, Z.; Huang, X.; Yang, K.; Gao, S.; Du, R. Effect of exogenous γ-aminobutyric acid (GABA) treatment on chilling injury and antioxidant capacity in banana peel. *Sci. Hortic.* **2014**, *168*, 132–137. [CrossRef]
74. Palma, F.; Carvajal, F.; Jiménez-Muñoz, R.; Pulido, A.; Jamilena, M.; Garrido, D. Exogenous γ-aminobutyric acid treatment improves the cold tolerance of zucchini fruit during postharvest storage. *Plant Physiol. Biochem.* **2019**, *136*, 188–195. [CrossRef]

4.7. Publicación VII - (Transcripción literal)

VII

The application of 1-MCP in combination with GABA reduces chilling injury and extends the shelf life in tomato (cv. Conquista)

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Article

The application of 1-MCP in combination with GABA reduces chilling injury and extends the shelf life in tomato (cv. Conquista)

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Abstract: Tomatoes have a short shelf life, and refrigeration is commonly used to extend tomato quality. However, suboptimal temperatures can lead to chilling injury (CI), reducing their marketability. In this study, the combined application of 10 mM γ-aminobutyric acid (GABA) and 0.5 μL L^{-1} of 1-methylcyclopropene (1-MCP) were used as strategies to reduce postharvest CI and prolong storability during tomato commercialization. Both treatments have individually proven their effectiveness in lowering physiological disorders in tomatoes. When applied, the combined treatment resulted in the lowest CI and rot incidence levels compared with the control and individual treatments. Additionally, the combined application effectively delayed weight loss, fruit softening, respiration rate, ethylene production, and increased chlorophyll and flavonoid content. The synergistic application of these substances improved the postharvest quality during storage, reducing quality losses. For this reason, the combination of GABA and 1-MCP could be an effective tool to minimize tomato waste during commercialization by increasing resilience to cold storage and extending the overall fruit shelf life during refrigerated storage.

Keywords: Cold storage; quality ripening; *Solanum lycopersicum* L.; γ-aminobutyric acid; 1-methylcyclopropene

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1. Introduction

Tomatoes (*Solanum lycopersicum* L.) are among the most extensively cultivated and consumed horticultural crops globally, owing to their nutritional attributes and economic significance. Due to its widespread consumption and subsequent high level of commercialization, implementing postharvest technologies becomes imperative to prolong the tomato quality and shelf life. As a climacteric fruit, the ripening process of tomatoes during the postharvest stage is intricately linked to the presence of ethylene [1]. Cold storage reduces plant metabolism, resulting in lower respiration rates and ethylene production, enhancing shelf life. Nonetheless, prolonged exposure to cold storage can lead to chilling injury (CI), causing physiological issues and vulnerability to microbial impact, developing disease [2]. These factors collectively impact the synthesis of secondary metabolites, affecting bioactive components. The initiation of secondary metabolite synthesis occurs during fruit growth and persists throughout the ripening phase. Secondary metabolites include volatile organic compounds (derived from organic acids, amino acids, etc.), carotenoids, and alkaloids. Whereas research efforts have improved tomato production, maximizing profits necessitates concurrent strategies to minimize postharvest losses, prolonging fruit shelf life.

Ethylene is essential among diverse plant growth regulators, exerting influence over crucial growth and developmental processes, particularly the stages of ripening and senescence. Employing ethylene inhibitors is a viable approach to delay ripening and senescence in horticultural crops [3]. The compound 1-Methylcyclopropene (1-MCP) is a potent ethylene action inhibitor widely applied in post-harvest fruit and vegetable preservation. Postharvest application of 1-MCP in tomatoes has been demonstrated to delay fruit softening, mitigate rot incidence, increase bioactive compounds, regulate antioxidant enzyme activities, and reduce ripening and ethylene production [4]. The impact of 1-MCP on tomatoes is affected by cultivation practices, the concentration and exposure duration of 1-MCP, and the specific ripening stage [5,6]. Furthermore, 1-MCP binds to ethylene receptors during treatment, and any ethylene sensitivity restoration is attributed to new receptor sites.

The γ-aminobutyric acid (GABA), a non-protein four-carbon acid, is a signaling molecule regulating plant growth and development. It is a crucial metabolite in both primary and secondary pathways, acting as a significant intermediate in nitrogen metabolism and amino acid biosynthesis [7]. The application of exogenous GABA elevates endogenous GABA levels, enhancing GABA shunt activity, resulting in increased carbon flux through respiratory pathways and restoring redox and energy levels, thus improving postharvest marketability [8]. Postharvest GABA treatments were effective in maintaining or enhancing postharvest quality in various fruits, including bananas [9] and pomegranates [10]. Positive effects have been observed when GABA was applied as a preharvest or postharvest treatment in tomatoes, providing protection against CI, reducing fruit softening, and preserving quality parameters during cold storage. These effects are associated with higher membrane integrity, represented by a lower electrolyte leakage and malondialdehyde (MDA) accumulation. The preservation of tomato fruit membrane integrity through exogenous GABA application may be attributed to the scavenging of reactive oxygen species (ROS) by catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) enzymes [11].

The main objective of this work has been to evaluate the combined effect of GABA

and 1-MCP in reducing CI and extending the shelf life of tomatoes during cold storage and commercialization. Both compounds have individually demonstrated their effectiveness in enhancing the postharvest quality of tomatoes. However, despite the individual use of both compounds to enhance tomato postharvest quality, no previous studies have elucidated the potential additional or synergistic effects of the simultaneous application of both substances.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Tomatoes (Lycopersicon esculentum Mill.) cv. Conquista was harvested from a commercial farm in Almería, Spain. The area climate is classified as semi-arid Mediterranean, with warm, dry summers and mild winters with minimal rainfall. The tomatoes were harvested from a greenhouse in late winter (February 2021). Tomatoes were rapidly transported to the laboratory on the harvest day and selected based on the uniform fruit size and color characteristics. Tomatoes were organized into three sets of five fruits each for various treatments and sampling days (n = 3).

For the GABA treatment (Sigma-Aldrich, Madrid, España, ≥99% A2129), immersion in a 10 mM solution was conducted for 10 min. In the case of the control and treatments involving 1-MCP alone or in combination, distilled water immersions lasting 10 min were employed to ensure consistent conditions. All the immersion solutions included tween-20 (Sigma-Aldrich, Madrid, España, P1379) at a concentration of 0.05%. Following treatments, tomato fruits were allowed to air-dry at 20 ◦C for one hour before being individually placed into 130-L plastic containers per treatment, providing similar and uniform conditions for the different fruit batches. Subsequently, the containers were sealed hermetically, allowing the released 1-MCP to take effect for 24 h at 20 ◦C in 1-MCP-treated batches alone or combined with previous GABA immersions. The control fruit and GABA batches were exposed to normal air within these containers under the same temperature conditions. Applying 1-MCP treatments involved commercial tablets releasing 0.5 μL L−1 with a commercial activator solution from SmartFresh (Agro-Fresh Inc., Philadelphia, PA, USA) following the manufacturer's instructions. Following treatments, all the fruits were stored for 21 days at 4 ◦C and 90% relative humidity to induce CI, followed by an additional 7-day period at 20 ◦C to simulate typical shelf-life conditions and to assess CI progression. The optimal dose of MCP applied to these fruits was determined based on the findings of Guillén et al. [5]. In a preliminary investigation, GABA doses of 1, 5, and 10 mM were tested after 10-min immersions, with the 10 mM concentration selected as the optimal concentration for this experiment.

2.2. Postharvest Quality Parameters

The weight loss was expressed as a percentage relative to their initial weight and evaluated in each tomato individually. The results represented the mean ± SE of 5 fruits per replicate (n = 3).

Fruit firmness was individually determined with a flat plate probe with a diameter of 100 mm equipped with a TX-XT2i texture analyzer (Stable Microsystems, Godalming, UK). Two equidistant readings were taken in the equatorial region of each fruit, with the disc descending at a rate of 20 mm per minute until a 5% deformation was reached. Fruit firmness was expressed as a ratio of the applied force to the distance traveled (N mm^{-1}). The fruit firmness evaluated at harvest in tomato fruit before storage was 2.91 ± 0.14 N mm^{-1}.

Respiration and ethylene levels were calculated based on three replicates of five tomatoes (n = 3) for each treatment hermetically sealed in a 4.6 L plastic container with a rubber stopper for 30 min using the static method [12]. Subsequently, a 1 mL gas sample was extracted in duplicate from the headspace in each container. These samples were injected into a Shimadzu GC-14B gas chromatograph and a Shimadzu GC-2010 gas chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) equipped with an FID detector to determine CO_2 and ethylene concentrations, respectively. The respiration rate and ethylene production results were expressed as mg of CO_2 kg^{-1} h^{-1} and nL g^{-1} h^{-1}, respectively.

The total soluble solids (TSS) content was determined in duplicate for each replicate (n = 3) using approximately 50 g obtained from a mix of five halves of tomatoes for each replicate and homogenized. These samples were filtered through two layers of cotton cloth. The resulting juice was measured at 20 ◦C using an Atago PR-101 digital refractometer (Atago Co., Ltd., Tokyo, Japan). Results were expressed as g per 100 g^{-1}. Similarly, total acidity (TA) was assessed in each filtered sample in duplicate (n = 3) through automated titration (785 DMP Titrino, Metrohm, Herisau, Switzerland) and expressed as grams of malic acid equivalents per 100 g^{-1}. These parameters were assessed at harvest with a TSS and TA of 5.26 ± 0.16 and 1.29 ± 0.06 values, respectively.

Color measurements were conducted for each fruit using a Minolta colorimeter (CRC400, Minolta Camera Co., Kanto, Tokyo, Japan). Three color measurements were taken for each fruit at two opposing and longitudinally equidistant points and expressed as CIE *hue** (arctg *b**/*a**) and CIE *L** based on CIELab coordinates. Colour parameters were assessed at harvest, obtaining different values for CIE *L** (47.74 ± 0.40), CIE *a** (−7.48 ± 0.51), CIE *b** (24.94 ± 0.34), and CIE *hue** (106.59 ± 1.05).

Total polyphenols were assessed using the Folin-Ciocalteu (FC) method described by Lezoul et al. [13] for different plant tissues. For the analysis, 200 µL of each extract was taken in duplicate (n = 3) and combined with 300 µL of 50 mM phosphate buffer solution, 2.5 mL of FC reagent, and 2 mL of Na_2CO_3 at 1 N. After stirring and incubating in a water bath at 50 ◦C for 5 min, the extracts were centrifuged, and the absorbance was measured at 760 nm using a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan). The results were expressed as milligrams of gallic acid equivalent per 100 g^{-1} (mean ± SE).

Flavonoids were measured following theWoisky & Salatino [14] method, where 500 µL of the sample extract was mixed in duplicate with 1.5 mL of 95% methanol, 0.1 mL of 10% (m/v) AlCl3, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. After incubation in the dark at room temperature for 30 min, the absorbance was measured at 415 nm using a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan). The results were expressed in milligrams equivalents of quercetin-3-rutinoside per 100 g^{-1} referencing the quercetin-3-rutinoside calibration curve.

Carotenoid and chlorophyll content were determined by extracting a homogeneous mixture from each replicate per lot using the method detailed by Vu et al. [15]. After pigment extraction through homogenization in methanol, the samples were centrifuged, and the supernatant was measured at wavelengths of 470, 652.4, and 665.2 in a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan). The results were expressed as milligrams per 100 g^{-1} of sample for both carotenoids and chlorophylls.

Total antioxidant activity was evaluated using the ABTS assay described by Lezoul et al. [13]. A total of 2 g of sample was mixed with 10 mL of 50mMphosphate buffer solution and 6 mL of ethyl acetate. After homogenization and centrifugation, the hydrophilic and lipophilic phases were separated, and the hydrophilic (H-AA) and lipophilic (LAA) antioxidant activities were measured in duplicate for each extract. The results were expressed as milligrams of Trolox per 100 g^{-1}, referencing the Trolox calibration curve.

H-AA was determined with 890 µL of 50 mM glycine buffer solution, 30 µL of 10 mM ABTS solution, 30 µL of 1 mM H_2O_2, and 25 µL of 10 µM peroxidase. In contrast, L-AA was assessed with 30 µL of 10 mM ABTS solution, 30 µL of 1 mM H_2O_2, 25 µL of 10 µM peroxidase, and 850 µL of ethanol.

Electrolyte leakage (EL) was measured following a previously described method with some modifications regarding the temperatures used [16]. Each replicate obtained 15 disks (1 cm diameter) from the outer tomato rind with a cork borer after removing the interior matrix and rinsing with distilled water. The disks were rinsed three times with deionized water for 3 min each, then immersed in deionized water (50 mL) for 1 h at room temperature and in continuous agitation. Then, the initial electrical conductivity (C1) at 20 ◦C was recorded. The discs were subsequently heated (100 ◦C for 10 min) and tempered to ambient temperature, and conductivity (C2) was recorded. EL results were obtained with the following formula: (C1/C2) × 100.

The determination of MDA content in the tomato fresh tissue was conducted according to the method of Zhang et al. [17]. The tissue sample (5.0 g) was homogenized in 10 mL of TCA (10% trichloroacetic acid solution), centrifuged at 10,000× g for 20 min, and then 2 mL of the supernatant was combined with 2 mL of TBA (0.67% thiobarbituric acid) in duplicate (n = 3). The testing tubes were heated to 100 ◦C for 20 min, rapidly cooled, and centrifuged at 10,000× g for 10 min. The samples were finally assessed in a spectrophotometer (1900 UV/Vis, Shimadzu, Kyoto, Japan) with absorbance measured at 450, 532, and 600 nm, expressed on a fresh weight basis as µmol kg^{-1} following the formula described by Zhang et al. [17].

Surface pitting as indicative of chilling damage in tomato fruit was visually assessed according to the criteria outlined by Ding et al. [18]. The severity of symptoms was evaluated on a 0–4 point scale: 0 denoting no pitting, 1 indicating few scattered pits, 2 indicating pitting impact up to 5% of the fruit surface, 3 representing the pitting impact covering > 5 and <25% of the fruit surface, and 4 for >25% of the fruit surface affected by this disorder.

Rotten fruits were quantified based on the percentage of decayed fruits relative to the total number of fruits for each replicate (n = 3).

2.3. Statistical analysis

Data are presented as the average ± standard error (SE). The data underwent variance analysis (ANOVA). Comparative assessments of means were executed using a multiplerange test (specifically Tukey's HSD test) to identify significant differences ($p < 0.05$) among treatments for each sampling date. These differences were represented with different lowercase letters. All statistical analyses were conducted using the SPSS software package, version 22 (IBM Corp., Armonk, NY, USA).

3. Results and discussions

3.1. Effect of 1-MCP and GABA Treatments on Weight Loss and Fruit Firmness

The weight loss of tomatoes increased with storage time (Figure 1A). Tomatoes in the GABA group did not delay fruit weight loss ($p > 0.05$) compared with control fruit. In addition, 1-MCP treatment alone only delayed fruit weight loss significantly ($p < 0.05$) after 7 days of refrigerated storage plus 7 days more at 20 ◦C. However, the most effective reduction in the weight loss evolution was observed with the combination of 1-MCP and GABA, showing significant differences ($p < 0.05$) compared with the control fruit. Both substances, when applied together, exhibited a synergistic effect delaying this quality parameter, which was not only the result of an additive effect between the two technologies, considering the results obtained.

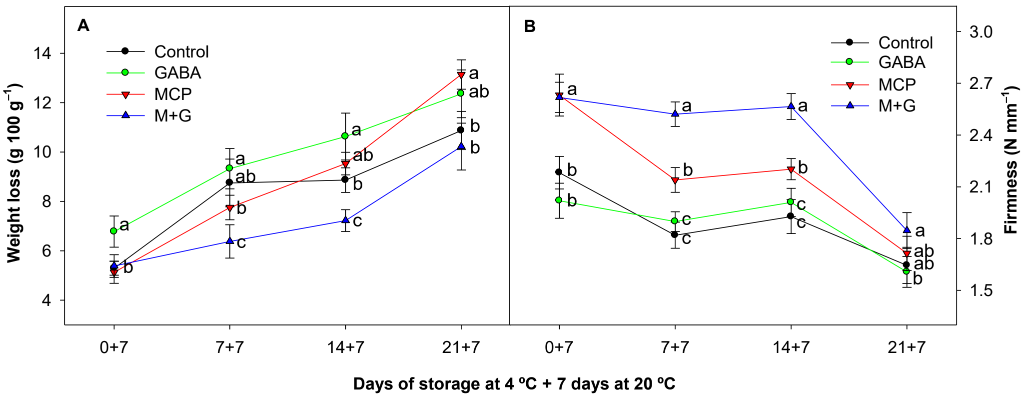

Figure 1. Evolution of weight loss (g 100 g^{-1}) (**A**) and fruit firmness (N mm^{-1}) (**B**) of tomatoes cv. Conquista treated with distilled water (Control), 1-MCP at 0.5 μL L^{-1}, GABA at 10 mM, and the combination of 1-MCP and GABA (M+G) during storage at 4 °C plus 7 days at 20 °C. Data are the mean ± SE (n = 3). Different lowercase letters show significant differences ($p < 0.05$) among treatments for each sampling date.

The fruit cultivar and the storage conditions influence fruit water loss after harvest. Water loss negatively impacts fruit quality, including weight reduction, skin wrinkling, fruit collapse, colour changes, and decreased organoleptic quality [19]. During storage, weight loss occurs due to water transpiration through the fruit surface area, respiration, and other metabolic activities. Previous studies have shown that GABA treatment can affect metabolism, increasing transpiration, although it did not negatively impact fruit visual quality [10]. Additionally, 1-MCP has been demonstrated to delay fruit water loss and the consequent weight loss by regulating cuticle formation and reducing water loss in tomatoes [20]. However, this study observed delayed weight loss only after 7 days of refrigerated storage, followed by 7 days at 20°C. Previous research has indicated that 1-MCP provides beneficial effects in reducing weight loss across different cultivars and that the effect is proportional to the concentration applied [6]. The combined treatment of 1-MCP and GABA was most effective in reducing weight loss, in agreement with the findings of different studies on different fruit species in which GABA or 1-MCP were applied separately with positive results [8].

A continuous reduction in fruit firmness was observed during storage, and tomatoes treated with 1-MCP maintained significantly higher firmness values ($p < 0.05$) compared with untreated fruits (Figure 1B). There were no significant differences ($p > 0.05$) in the firmness of GABA-treated fruit compared with untreated fruit during the shelf life period. However, the combined treatment maintained the highest fruit firmness values throughout storage, showing significant differences ($p < 0.05$) from the control fruit and, generally, with the rest of the treatments applied.

The decrease in fruit firmness could be associated with the production of climacteric ethylene in tomato fruit. The wrinkling and softening of tomato fruit, as well as the degradation of cell walls, is correlated with increased ethylene production and respiration rate in tomatoes. The significantly higher values observed in tomatoes treated with 1-MCP were in agreement with previous reports where 1-MCP treatment was related to reduced enzymatic activity, particularly polygalacturonase, in different tomato cultivars [21]. In contrast, GABA treatment did not show significant effects on firmness, in agreement with findings in apples, where GABA treatment had a limited impact on firmness values [22]. However, the combined treatment of 1-MCP and GABA synergistically maintained the highest firmness values throughout storage, which may be due to the combined effects of preserving membrane integrity and inhibiting cell wall-degrading enzymes, reducing weight loss as observed in peach [23].

3.2. Effect of Postharvest 1-MCP and GABA Treatments on Respiration Rate and Ethylene Production

The results obtained in this study indicate that treatment only with 1-MCP significantly reduced respiration rates ($p < 0.05$) compared with control fruits throughout cold storage and its subsequent shelf life period (Figure 2A). Although no significant ($p > 0.05$) effect of GABA alone on fruit respiration was observed throughout most of the storage period, by the end of the experiment, the respiration levels of GABA-treated fruit were significantly lower ($p < 0.05$) compared with control fruit. Combining 1-MCP with GABA treatment also reduced respiration, with the lowest respiration levels observed at the end of storage compared with the other treatments.

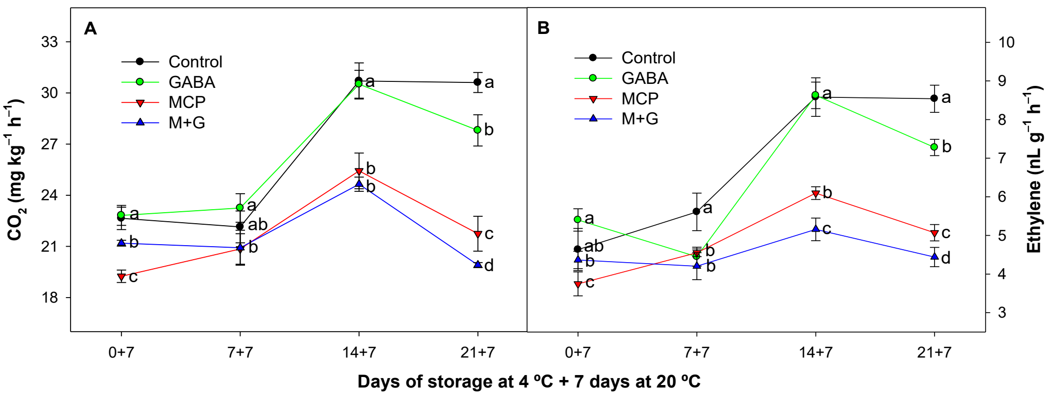

Figure 2. Evolution of respiration rate (mg CO_2 kg^{-1} h^{-1}) (**A**) and ethylene production (nL g^{-1} h^{-1}) (**B**) of tomatoes cv. Conquista treated with distilled water (Control), 1-MCP at 0.5 μL L^{-1}, GABA at 10 mM, and the combination of 1-MCP and GABA (M+G) during storage at 4 °C plus 7 days at 20 °C. Other details are the same as those in Figure 1.

In climacteric fruits, postharvest storage is characterized by increased respiration and ethylene synthesis. The effectiveness of 1-MCP in delaying the ripening process is linked to its ability to reduce the respiration rate and postpone the climacteric peak [24]. The compound 1-MCP has been shown to have an inhibitory impact on respiration rate during fruit storage, leading to a decrease in pectin hydrolysis and enzymatic activities, including polygalacturonase [21]. The reduction in respiration observed in this study supports previous findings in which the 1-MCP can decrease the respiration rate in different tomato cultivars [5], as well as in other fruits such as kiwi and avocado [25]. In addition, although GABA treatment alone did not affect respiration in most of the evaluated period, the combined treatments with 1-MCP showed a significant effect, resulting in the lowest respiration levels at the end of storage compared with the control fruit. This effect suggests that GABA may enhance the efficacy of 1-MCP in reducing respiration, although the role of GABA in fruit respiration during postharvest remains unclear. Previous research has indicated that ethylene production and respiration rates in tomato fruits treated with 5 mM GABA were significantly lower compared with those in the control batch and fruits treated with 20 mM GABA [11]. Furthermore, dipping in exogenous GABA effectively reduced the respiratory rate in apples [26]. Following these observations, Ansari et al. [27] suggested that the GABA metabolic pathway in the cytosol and mitochondrial matrix displayed a crucial connection between GABA, photosynthesis, and plant respiration.

As observed in the respiration rate, 1-MCP treatment, both in combination and alone, was very effective in reducing ethylene production with significantly lower values ($p < 0.05$) compared with untreated and GABA-treated tomato fruits (Figure 2B). GABA-treated fruit only displayed reduced ethylene levels after 7 and 21 days of storage, with lower ethylene levels for fruits treated with GABA than for control fruit. However, the lowest ethylene levels were obtained with the combination of both treatments, showing significant differences ($p < 0.05$), reducing ethylene production by half at the end of storage compared with control fruits (Figure 2B).

In tomatoes, the initiation of ripening is controlled by increased ethylene production. The 1-MCP blocks ethylene binding to its receptor, disrupting the signal transduction necessary to initiate ripening [28]. Hoeberichts et al. [29] found that ethylene perception is essential for activating genes related to tomato ripening and the corresponding physiological changes, even at later stages of the ripening process. The extent of ripening delay caused by 1-MCP has been shown to depend on the internal ethylene levels in tomato fruits [30]. Our results support these findings, demonstrating that 1-MCP, whether applied alone or in combination, significantly reduces ethylene production in tomato fruits. These effects agreed with previous studies on tomatoes, where several combinations of 1-MCP concentration, maturation stages, temperature, and application periods were tested, showing similar results [5,21]. Additionally, ethylene levels were lower for GABA-treated fruit than the control fruit, although the effect was less pronounced and erratic than when 1-MCP was applied alone. However, combining both treatments resulted in the lowest ethylene levels, reducing production by half by the end of storage compared with the control fruits. These findings suggest a synergistic effect between GABA and 1-MCP in reducing ethylene production. We previously reported the effect of GABA on reducing the climacteric peak in avocados [25], although it did not affect the rest of the storage period. Similarly, Han et al. [31] reported reduced apple ethylene production after GABA treatments. On the contrary, GABA has been shown to stimulate ethylene biosynthesis by increasing 1-aminocyclopropane-1-carboxylic acid (ACC) synthase transcript levels in sunflower tissues, acting as a signal transducer [32]. This indicates that the relationship between GABA and ethylene biosynthesis can vary across plant species, highlighting the complexity of GABA's role in ethylene regulation.

3.3. *Effect of 1-MCP and GABA Treatments on TSS and TA*

Pectin is converted into simpler sugars during fruit ripening, increasing total soluble solids during storage. The control fruit exhibited higher TSS values on days 7 and 14 of storage than those treated with 1-MCP (Figure 3A).

However, fruits treated with GABA, both alone or in combination with 1-MCP, displayed significantly higher TSS levels ($p < 0.05$) compared with the control and the fruit batches treated only with 1-MCP. Considering the observed increase in TSS in GABA-treated fruits, along with the reduced respiration observed earlier combining GABA + 1-MCP, an increased GABA content may activate the GABA shunt, enhancing ATP production and energy availability, thereby reducing the catabolism of other energy-rich substrates like sugars and organic acids, as explained by Aghdam et al. [33] for strawberries during refrigerated storage.

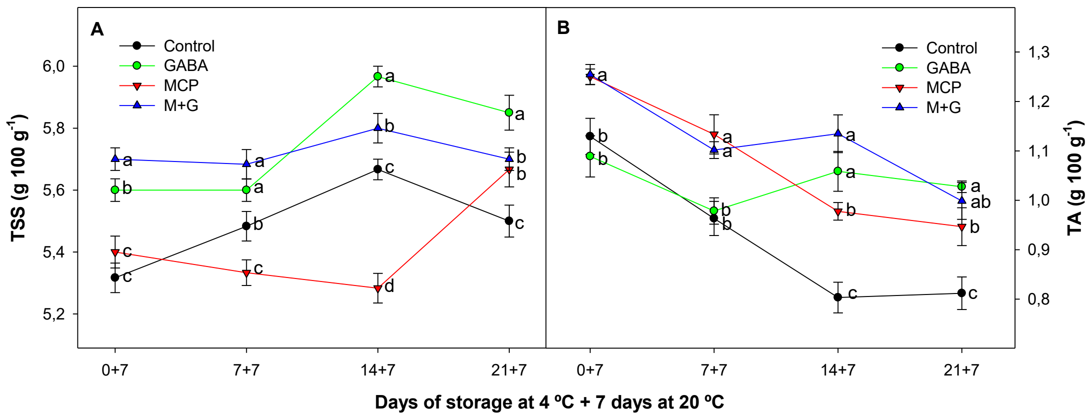

Figure 3. Evolution of total soluble solids (TSS) (g 100 g^{-1}) (**A**) and total acidity (TA) (g 100 g^{-1}) (**B**) of tomatoes cv. Conquista treated with distilled water (Control), 1-MCP at 0.5 µL L^{-1}, GABA at 10 mM, and the combination of 1-MCP and GABA (M+G) during storage at 4 °C plus 7 days at 20 °C. Other details are the same as those in Figure 1.

The ripening process affects TA, changing fruit flavor during storage. Although these tomatoes were not evaluated by a trained sensory panel, they were assessed by the research team throughout the experiment, and no unusual flavors or aromas related to the applied treatments were detected. In this study, 1-MCP treatments, both alone and in combination with GABA, successfully maintained higher TA levels in tomato fruit throughout the storage period, with significant differences ($p < 0.05$) compared with the control fruit. Furthermore, GABA treatments applied independently also preserved TA levels by the end of the storage period, showing significantly higher values ($p < 0.05$) than the control group. Fruit acidity is mainly due to the different organic acids that are used during the respiration process. These organic acids accumulate during fruit development and are used as respiratory substrates during ripening, leading to a decline in acidity post-harvest. The reduced TA observed in tomatoes could be linked to increased respiration and ripening rates, where organic acids are metabolized during respiration. In this study, 1-MCP treatments were found to reduce ethylene production, which correlates with lower fruit metabolism and respiration, thereby delaying the breakdown of organic acids, as pre- viously reported in tomatoes [5,6]. Moreover, the GABA shunt is the primary pathway for citrate breakdown, which is crucial for maintaining intracellular metabolic redox balance in mitochondria [33,34]. GABA treatments have been shown to increase TA levels in blood orange by inhibiting the loss of intracellular acidity and regulating mitochondrial energy and organic acid metabolism [35]. This effect is significant during storage at suboptimal temperatures, where the energy balance of cells requires additional energy to maintain plant tissue integrity. Therefore, it is reasonable to conclude that simultaneously applying 1-MCP and GABA is more effective in delaying the reduction of TA compared with 1-MCP alone, primarily due to the additive effects of these substances.

3.4. Effect of Postharvest 1-MCP and GABA Treatments on CIE hue and CIE L* colour*

In the present study, 1-MCP applications effectively delayed ripening-associated colour changes in tomatoes throughout the entire storage period, showing significant differences ($p < 0.05$) compared with the control group (Figure 4).

CIE *hue** values for control and GABA-treated batches declined rapidly, with final CIE *hue** values around 54◦ and 52◦, respectively, without significant differences ($p > 0.05$) between them (Figure 4A). Additionally, tomatoes treated with 1-MCP, either alone or in combination with GABA, maintained higher CIE *hue** values during the whole storage period, reaching approximately 62◦ at the end of the experiment. The evolution of the CIE *L** parameter followed a similar trend to the CIE *hue** parameter for all tomato batches. However, the combined treatment exhibited significantly ($p < 0.05$) higher values compared with other treatments, indicating a synergistic effect (Figure 4B). CIE *L** values are associated with reduced weight loss and a slower ripening pattern in fruits and vegetables. In our results, delaying weight loss and enhancing fruit firmness may have contributed to maintaining skin brightness, thereby preventing tissue oxidation. The brightness of the epidermis is linked to the structure of the cuticle, which plays a role in postharvest fruit water loss [36]. In this regard, the combined treatment was the most effective in delaying tomato weight loss and fruit firmness. However, the combination of treatments did not exert a synergistic or additive effect on the CIE *hue** parameter compared to the 1-MCP treatment alone. These findings are consistent with the known ability of 1-MCP to maintain fruit color by delaying pigment degradation and slowing the ripening process [28]. Additionally, previous experiments have shown that GABA-treated mangoes and strawberries did not delay color evolution compared with control batches throughout storage [37]. This lack of effect on color parameters agreed with our results, as GABA-treated batches did not differ significantly ($p > 0.05$) from the control group. As the storage period increased, the brightness of the tomato fruit diminished due to the progression of red color development during ripening.

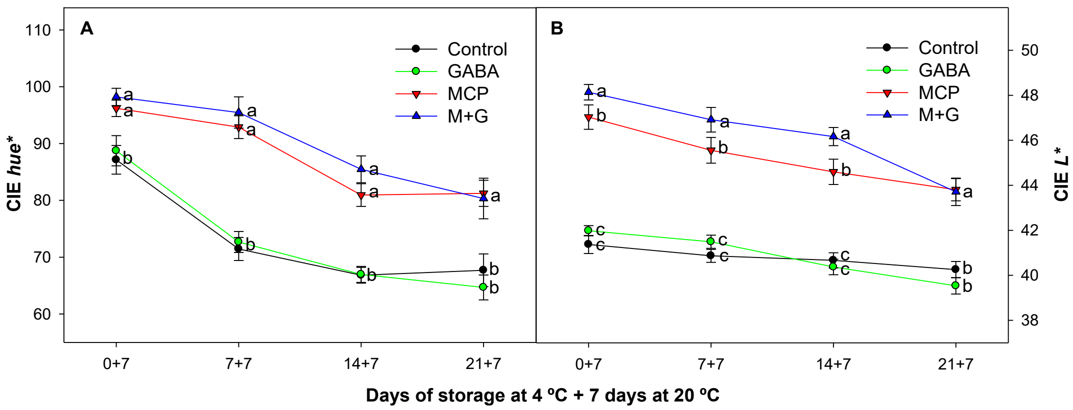

Figure 4. Evolution of CIE *hue** (**A**) and CIE *L** (**B**) values of tomatoes cv. Conquista treated with distilled water (Control), 1-MCP at 0.5 μL L^{-1}, GABA at 10 mM, and the combination of 1-MCP and GABA (M+G) during storage at 4 °C plus 7 days at 20 °C. Other details are the same as those in Figure 1.

3.5. Effect of Postharvest 1-MCP and GABA Treatments on Bioactive Compounds and Antioxidant Activity

The total polyphenol content (TPC) increased during storage for all batches studied. However, at the beginning of the storage period, fruit batches treated with GABA and 1-MCP, individually or in combination, delayed TPC compared with control batches (Figure 5A).

Although total polyphenols increased as ripening progressed, a significant delay was observed in fruits treated with 1-MCP during the early storage stages, while the control showed a faster increase. Overall, TPC was higher in tomato fruit treated only with GABA at the end of the storage period, whereas the combined treatment displayed the lowest TPC levels. A similar trend was observed with total flavonoid content (TFC) in GABA-treated fruit, which exhibited the highest levels of flavonoids when GABA was applied alone (Figure 5B). Combining 1-MCP and GABA resulted in significantly higher flavonoid levels ($p < 0.05$) than the rest of the treated batches. Interestingly, most batches treated with 1-MCP alone and the control batches displayed the lowest levels of flavonoids during the final storage period.

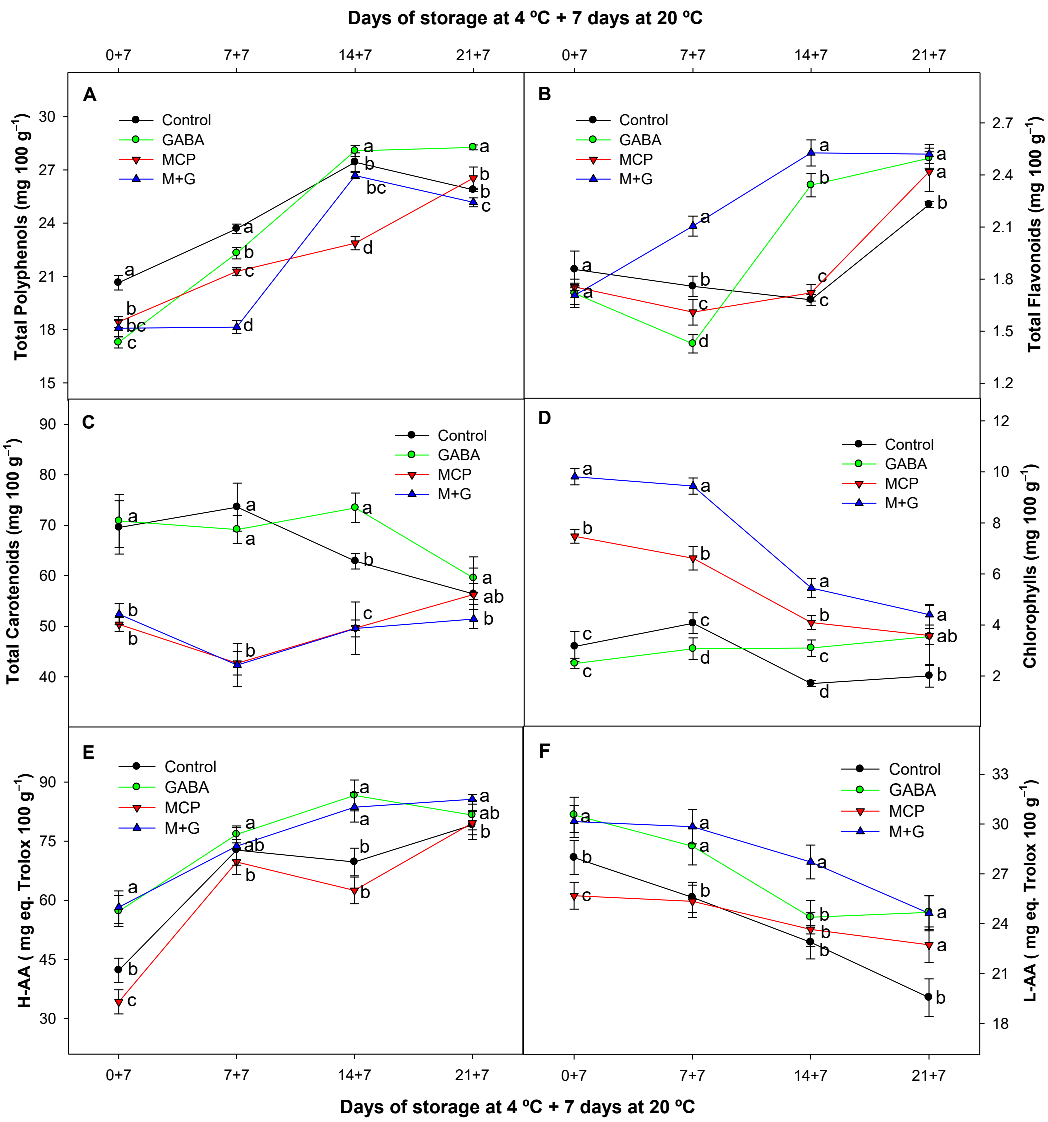

Figure 5. Evolution of total polyphenol content (TPC) (mg 100 g^{-1}) (**A**), total flavonoid content (TFC) (mg 100 g^{-1}) (**B**), total carotenoids content (TCC) (mg 100 g^{-1}) (**C**), chlorophyll content (mg 100 g^{-1}) (**D**), hydrophilic antioxidant activity (H-AA) (mg eq. Trolox 100 g^{-1}) (**E**) and lipophilic antioxidant activity (L-AA) (mg eq. Trolox 100 g^{-1}) (**F**) of tomatoes cv. Conquista treated with distilled water (Control), 0.5 μL L^{-1} 1-MCP, 10 mM GABA, and the combination of 1-MCP and GABA (M+G) during storage at 4 °C plus 7 days at 20 °C. Other details are the same as those in Figure 1.

Total carotenoid accumulation was generally delayed in fruits treated with 1-MCP, whether alone or combined with GABA (Figure 5C). Similarly, these batches exhibited higher chlorophyll content ($p < 0.05$) (Figure 5D). In contrast, the control and fruit batches treated only with GABA showed increased carotenoid accumulation and decreased chlorophyll content compared to those batches containing 1-MCP from the beginning of the experiment. The 1-MCP treatments were effective alone or when combined with GABA, preventing chlorophyll degradation compared to the other treatments. However, the combined treatment exhibited the highest chlorophyll values compared to these separately applied. The lowest chlorophyll content was observed for the control fruit during the final storage period, and these differences were statistically significant ($p <$ 0.05) compared with the rest of the treatments. GABA treatment alone did not delay the chlorophyll content evolution, obtaining similar values to those observed for the control fruit.

The H-AA levels observed were higher than those reported for L-AA in this study (Figure 5E,F). H-AA increased during storage, whereas L-AA displayed a decreased pattern.

For GABA-treated batches without 1-MCP, these two antioxidant parameters followed a similar pattern to those observed for TPC (Figure 5A) and TFC (Figure 5B). Combined treatments displayed a higher antioxidant level for both antioxidant parameters than the control fruit and batches treated only with 1-MCP, reaching the highest values for L-AA compared to the rest of the reported fruit batches. In addition, the GABA-positive effect increasing the H-AA and L-AA coincided with the accumulation of TPC and TFC observed in GABA-treated fruits. The lowest values in the lipophilic antioxidant phase were observed in the control fruit at the end of the storage period. At the same time, this decline was delayed for the GABA and 1-MCP treatments applied alone or in combination (Figure 5F).

The 1-MCP treatment was applied alone, which delayed the increase in total bioactive compounds from the beginning of the experiment. Our results agreed with those previously reported in tomatoes by different authors for TPC, TFC [38,39], and TCC [40]. Whereas the combined treatment showed the lowest accumulation of TPC, the TFC accumulated by combining GABA with 1-MCP reached the highest levels of flavonoid content. This effect was not observed in batches treated with only 1-MCP, where total polyphenols and flavonoids were delayed. For this reason, it is reasonable to assume that the GABA treatment may regulate the increased flavonoid content. Several studies have demonstrated that GABA increases flavonoid content by promoting the activity of the phenylpropanoid pathway [8]. As we did not observe essential differences in TFC between the control fruit and 1-MCP batches, the higher TFC in the combined treatment will demonstrate a synergistic effect between both compounds, in which GABA and 1-MCP could be reducing the flavonoid oxidation while GABA also stimulates flavonoid accumulation. A similar effect was reported previously by Shenglong et al. [41], who applied a combined 1-MCP treatment with a monoterpenoid with antioxidant properties (citral), reporting a positive and synergistic effect of increasing TPC and TFC during storage when both treatments were applied together.

In tomato fruit, the evolution of the chlorophyll content and other primary pigments, such as carotenoids, during ripening directly impacts color changes. Color changes regulated by ethylene are tightly associated with the chloroplast transition to chromoplasts, including carotenoid synthesis and chlorophyll degradation [42]. In our study, the 1-MCP treatment applied alone or combined with GABA delayed the chlorophyll degradation significantly ($p < 0.05$) compared to the control fruit and GABA batches. It is well known that 1-MCP reduces the production and blocks the action of ethylene by binding to the ethylene receptors in plant cells [28]. For this reason, the delayed chlorophyll degradation observed in 1-MCP treated batches could be due to the effect of reducing ethylene production observed previously in this study. Additionally, 1-MCP treatment combined with GABA was more effective in lowering ethylene production than 1-MCP applied alone, which could be why we observed higher chlorophyll maintenance with the combination of treatments. We recently reported this effect for avocados [25] with lower GABA concentrations, and it has been reported in several studies in apples or pistachios using GABA concentrations of 10 mM or 40 mM [22]. The primary mechanism associated with GABA effectiveness is its role in enhancing antioxidant activity, which helps preserve the structural integrity of membranes, particularly those of chloroplasts [43].

In our study, the antioxidant activities in tomatoes, both H-AA and L-AA, showed higher values in fruits treated with GABA alone or combined with 1-MCP. GABA has antioxidant activity and stimulates secondary metabolism, increasing the accumulation of bioactive compounds [8]. According to our results, this stimulation is supported by an increased accumulation of TFC in GABA-treated batches. Other authors reported that GABA treatments enhanced antioxidant capacity in mangoes by maintaining a balanced antioxidant level [23]. The influence of 1-MCP treatment alone on H-AA and LAA was minimal, only delaying the evolution of antioxidant activity with a limited effect. However, the combined treatment with GABA displayed a higher level of L-AA than the rest of the batches evaluated during the whole experiment. This synergistic effect could be associated with the additive effects demonstrated for both substances maintaining membrane integrity.

The 1-MCP maintains the cell membrane integrity through delayed ethylene production, which promotes fruit softening and ripening [28]. In contrast, GABA increased antioxidant activity, mitigating oxidative damage and maintaining membrane integrity [23]. This effect would also explain the highest chlorophyll values observed in our experiment, which could be related to the lower oxidative damage suffered by cell membranes.

3.6. Effect of Postharvest 1-MCP and GABA Treatments on Fruit Integrity and Oxidative Stress Marker

In this experiment, we studied the changes in membrane integrity as one of the initial physiological responses to CI in the treated fruits. After cold storage, EL increased for all tomato batches, but significant ($p < 0.05$) higher levels were found in the control fruit compared with 1-MCP or GABA-treated batches (Figure 6A). The 1-MCP and GABA treatments applied separately delayed the membrane disintegration similarly ($p > 0.05$) at the end of the experiment (17.66 ± 0.56% and 18.30 ± 0.57%, respectively) compared with significantly higher values for the control (20.41 ± 0.57%). However, GABA treatments alone controlled this parameter better than 1-MCP alone during the whole storage period. In addition, the lowest EL values were observed combining 1-MCP and GABA (15.55 ± 0.21%), showing significant ($p < 0.05$) differences compared with the rest of the batches evaluated. A similar effect was observed regarding the MDA levels because the control fruit displayed significant ($p < 0.05$) higher values of this oxidation product (Figure 6B).

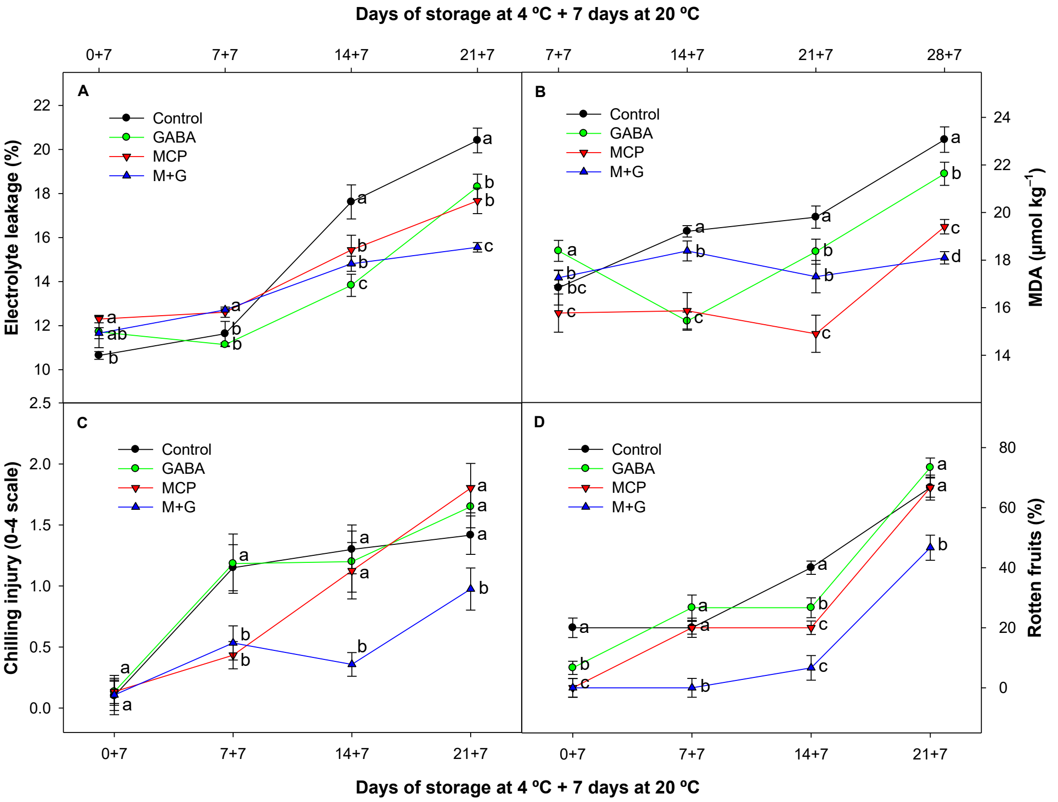

Figure 6. Evolution of electrolyte leakage (%) (**A**), malondialdehyde (MDA) content (µmol kg^{-1}) (**B**), chilling injury (0-5 scale) (**C**) and rotten fruits (%) (**D**) of tomatoes cv. Conquista treated with distilled water (Control), 1-MCP at 0.5 µL L^{-1}, GABA at 10 mM, and the combination of 1-MCP and GABA (M+G) during storage at 4 °C plus 7 days at 20 °C. Other details are the same as those in Figure 1.

MDA was better controlled by 1-MCP than by the GABA treatment, although both treatments were separately applied and were influential in delaying MDA evolution. At the end of the experiment, the final MDA concentrations obtained were significantly ($p <$ 0.05) different between fruit treatments with MDA reductions of 6.24%, 15.87%, and

21.55% for GABA, 1-MCP, and the combined treatment, respectively. These results indicated that the resilient effect was not synergistic when 1-MCP was combined with GABA in terms of MDA content because the effect was additive.

To study the resilient response of tomato fruit to cold storage, we evaluated the CI on the fruit surface of the different batches under study. CI in tomatoes was evident through several observed symptoms showing areas of yellow discoloration. In addition, affected fruits developed sunken spots along the experiment, making them more susceptible to decay and rot. These symptoms indicate significant structural damage due to suboptimal temperatures during storage. We observed an apparent effect delaying CI in 1-MCP-treated batches (alone or combined with GABA) with significantly ($p < 0.05$) lower values compared to GABA batches and the control fruit (Figure 6C). These batches did not show significant ($p > 0.05$) differences. The combined treatment delayed the appearance of CI symptoms for 14 days, whereas, when 1-MCP was applied alone, CI symptoms were delayed for only 7 days. A similar pattern was observed regarding the percentage of rotten fruit (Figure 6D).

GABA and 1-MCP treatments applied separately controlled the fungal infection after 7 days of storage at 20 ◦C with significant ($p < 0.05$) differences compared with the control fruit. Still, in general, the effectiveness of GABA or 1-MCP was weak. However, when these two different substances were applied together, the rot incidence was significantly delayed compared to the rest of the fruit batches evaluated. In fact, after 14 days of cold storage plus 7 days more at 20 ◦C, the percentage of rot incidence remained similar to that observed at the experiment's beginning. The reduction in the amount of rotten fruit after this period reached 83.35% compared with the control fruit. These results demonstrate the synergistic effect on rot incidence, comparing the reduction of rot incidence displayed by GABA (9.99%) or 1-MCP (60.01%) when applied alone for the same studied period. These findings are shown in Figure 7, in which the impact of the various treatments tested on ripening evolution can be observed.

Structural modifications in cellular membranes are among the initial physiological responses to CI. MDA content in plant tissues indicates membrane integrity in fruit subjected to cold storage conditions. In tomato fruit, increased MDA levels indicate membrane integrity loss induced by CI during cold storage, resulting in EL [44]. In this study, com- pared with the control fruit, lower levels of EL and MDA were reported for 1-MCP and GABA batches, as compared with the control fruit, which had similar effectivity. Although the 1-MCP treatment alone controlled the MDA content better, at the end of the storage, both parameters displayed lower levels with the combined treatment. Lower EL and MDA levels have also been reported in tomatoes and other fruit species, such as sweet pepper, indicating 1-MCP effectiveness in maintaining membrane integrity [28,45]. Similar observa- tions in GABA-treated fruit have been reported decreasing EL and MDA in zucchini during postharvest cold storage [46]. Reduced EL and MDA levels in the peel of GABA-treated bananas and blood oranges were associated with the maintenance of the antioxidant and energy status balance provided by GABA [9,35]. Supporting these results, we have found increased H-AA and L-AA levels in GABA-treated tomatoes compared with the control fruit associated with the GABA effect, mitigating the increase in ROS [47].

Ethylene accelerates senescence by reducing tissue integrity and increasing susceptibil- ity to CI. This tissue degradation facilitates the onset of postharvest diseases, contributing to postharvest rot. Interestingly, 1-MCP, when applied alone, shows remarkable efficacy in delaying CI for 7 days, highlighting its action as an ethylene inhibitor. However, combining 1-MCP with GABA extends this protection to 14 days, indicating a synergic pattern between the two compounds. This finding suggests that GABA may contribute to further stabilizing cell membranes or minimizing oxidative stress, thereby enhancing the protective effects of 1-MCP (Shelp et al., 2021). For this reason, a higher antioxidant balance, as we evaluated in our GABA and 1-MCP samples, could also contribute to fruit integrity, reducing rot inci- dence. The lower MDA also supports this mechanism, and EL was observed in GABA and 1-MCP-treated tomatoes. We previously observed the positive effect of combining GABA and 1-MCP in avocados on CI, and, recently, it has been proven that 1-MCP combined with antioxidant substances as essential oils can positively reduce significantly rot incidence in apricots [48]. For this reason, the synergistic effect on rot inhibition observed when applying GABA and 1-MCP could be related to the anti-senescence properties described for these substances acting together. For these reasons, postharvest treatments with 1-MCP and GABA could contribute to waste reduction in the tomato supply chain. By reducing CI symptoms and rot incidence, these treatments help maintain fruit integrity and quality during cold storage and shelf life. Several tomatoes in different fruit batches were nega- tively affected due to quality issues or premature spoilage. However, the combined effect of 1-MCP and GABA preserves essential characteristics, such as firmness and antioxidant content, enhancing tomato resilience under suboptimal storage conditions. Thus, 1-MCP and GABA treatments effectively reduce postharvest losses and improve sustainability in tomato commercialization.

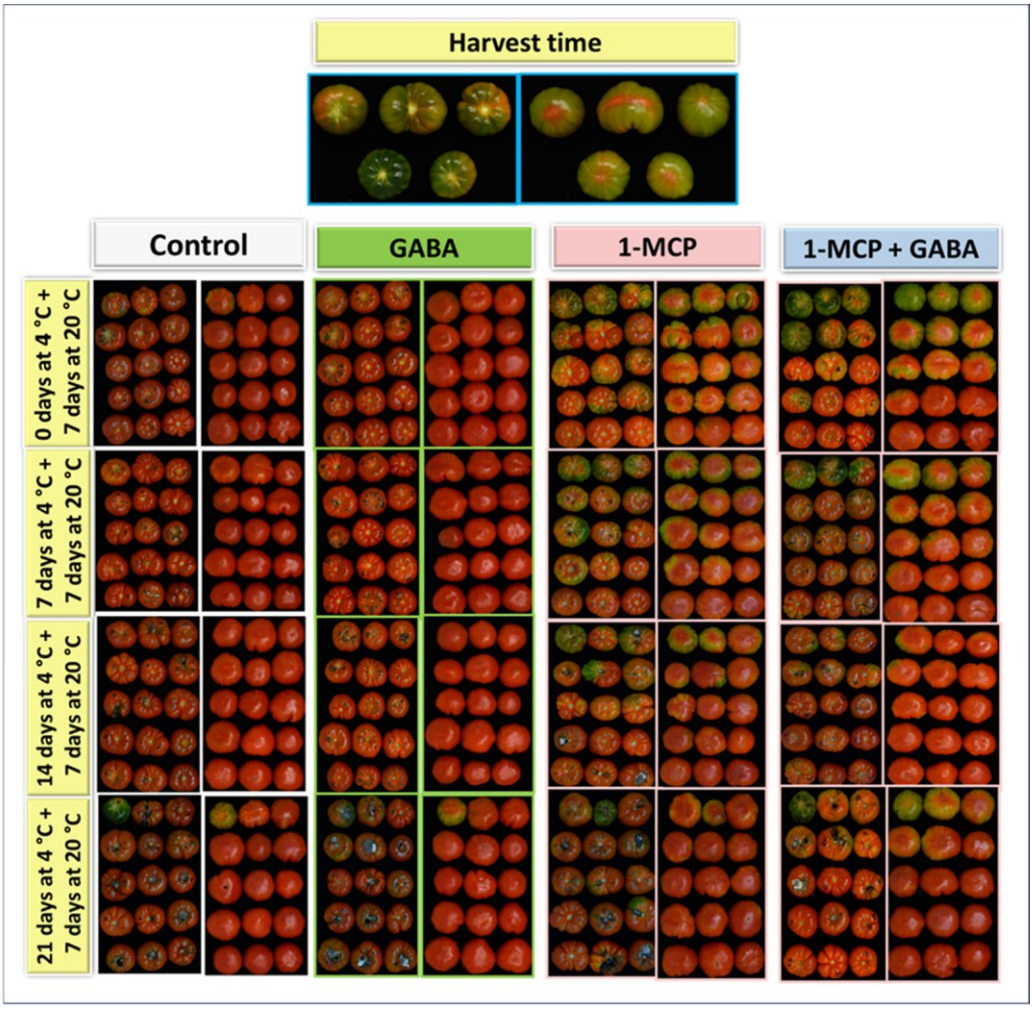

Figure 7. The external visual aspect of tomatoes cv. Conquista at harvest time and tomatoes treated with distilled water (Control), 10 mM GABA, 0.5 μL L^{-1} 1-MCP, and the combination of 1-MCP and GABA during refrigerated storage at 4 ◦C plus 7 days at 20 ◦C. Note that the tomatoes on the left and right columns are the same fruits photographed from both sides at the same period point.

4. Conclusions

Postharvest applications of γ-aminobutyric acid (GABA) and 1-methylcyclopropene (1-MCP) were effective in maintaining quality traits such as color and tissue turgor and reducing weight loss and chilling injury, mainly when both technologies were applied together. The combined treatment combined the benefits of GABA in maintaining an optimal antioxidant balance, which was associated with higher levels of bioactive compounds and a reduced metabolism with lower ethylene production and respiration rates. This reduced substrate catabolism and delayed fruit firmness evolution. Generally, 1-MCP alone exhibited positive effects in several quality parameters. However, while no overall positive effect was observed with GABA alone, it did increase antioxidant activity, which may have contributed to reducing membrane permeability, a parameter in which it showed some effectiveness. Although GABA demonstrated some specific effects on reducing metabolism or maintaining total acidity at certain sampling points, its impact was limited in comparison to 1-MCP. For these reasons, combining these two strategies could be used synergistically to maintain the postharvest quality of tomatoes stored at suboptimal temperatures, reducing fruit disorders and postharvest losses.

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References

1. Mata, C.I.; Fabre, B.; Parsons, H.T.; Hertog, M.L.; Van Raemdonck, G.; Baggerman, G.; Nicolaï, B.M. Ethylene receptors, CTRs and EIN2 target protein identification and quantification through parallel reaction monitoring during tomato fruit ripening. Front. *Plant Sci*. **2018**, *9*, 1626. [CrossRef]
2. Rai, A.; Kumari, K.; Vashistha, P. Umbrella review on chilling injuries: Post-harvest issue, cause, and treatment in tomato. *Sci. Hortic*. **2022**, *293*, 110710. [CrossRef]
3. Baswal, A.K.; Ramezanian, A. 1-Methylcyclopropene potentials in maintaining the postharvest quality of fruits, vegetables, and ornamentals: A review. *J. Food Process. Pres*. **2021**, *45*, e15129. [CrossRef]
4. Poyesh, D.S.; Terada, N.; Sanada, A.; Gemma, H.; Koshio, K. Effect of 1-MCP on ethylene regulation and quality of tomato cv. Red Ore. *Int. Food Res.* J. **2018**, *25*, 1001–1006.
5. Guillén, F.; Castillo, S.; Zapata, P.J.; Martínez-Romero, D.; Serrano, M.; Valero, D. Efficacy of 1-MCP treatment in tomato fruit. Effect of cultivar and ripening stage at harvest. *Postharvest Biol. Technol.* **2006**, 42, 235–242. [CrossRef]
6. Guillén, F.; Castillo, S.; Zapata, P.J.; Martínez-Romero, D.; Serrano, M.; Valero, D. Efficacy of 1-MCP treatment in tomato fruit: Duration and concentration of 1-MCP treatment to gain an effective delay of postharvest ripening. *Postharvest Biol. Technol.* **2007**, *43*, 23–27. [CrossRef]
7. Ramos-Ruiz, R.; Poirot, E.; Flores-Mosquera, M. GABA, a non-protein amino acid ubiquitous in food matrices. *Cogent Food Agric.* **2018**, *4*, 1534323. [CrossRef]
8. Aghdam, M.S.; Flaherty, E.J.; Shelp, B.J. γ-Aminobutyrate improves the postharvest marketability of horticultural commodities: Advances and prospects. *Front. Plant Sci.* **2022**, *13*, 884572. [CrossRef]
9. Wang, Y.; Luo, Z.; Huang, X.; Yang, K.; Gao, S.; Du, R. Effect of exogenous γ-aminobutyric acid (GABA) treatment on chilling injury and antioxidant capacity in banana peel. *Sci. Hortic.* **2014**, *168*, 132–137. [CrossRef]
10. Nazoori, F.; ZamaniBahramabadi, E.; Mirdehghan, S.H.; Rafie, A. Extending the shelf life of pomegranate (Punica granatum L.) by GABA coating application. *J. Food Meas. Charact*. **2020**, *14*, 2760–2772. [CrossRef]
11. Uluışık, S. Application of γ-aminobutyric acid treatment differently affects physicochemical characteristics of tomato fruits during post-harvest storage. *Hortic. Stud.* **2021**, *38*, 101–109. [CrossRef]
12. Martínez-Romero, D.; Serrano, M.; Carbonell, A.; Burgos, L.; Riquelme, F.; Valero, D. Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. *J. Food Sci.* **2002**, *67*, 1706–1712. [CrossRef]

13. Lezoul, N.E.H.; Belkadi, M.; Habibi, F.; Guillén, F. Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. *Molecules* **2020**, *25*, 4672. [CrossRef]
14. Woisky, R.G.; Salatino, A. Analysis of propolis: Some parameters and procedures for chemical quality control. *J. Apic. Res.* **1998**, *37*, 99–105. [CrossRef]
15. Vu, H.T.; Scarlett, C.J.; Vuong, Q.V. Changes of phytochemicals and antioxidant capacity of banana peel during the ripening process; with and without ethylene treatment. *Sci. Hortic.* **2019**, *253*, 255–262. [CrossRef]
16. Ilea, M.I.M.; Zapata, P.J.; Fernández-Picazo, C.; Díaz-Mula, H.M.; Castillo, S.; Guillén, F. Chlorogenic acid as a promising tool for mitigating chilling injury: Cold tolerance and the ripening effect on tomato fruit (*Solanum lycopersicum* L.). *Plants* **2024**, *13*, 2055. [CrossRef]
17. Zhang, M.; Liu, W.; Li, C.; Shao, T.; Jiang, X.; Zhao, H.; Ai, W. Postharvest hot water dipping and hot water forced convection treatments alleviate chilling injury for zucchini fruit during cold storage. *Sci. Hortic.* **2019**, *249*, 219–227. [CrossRef]
18. Ding, C.K.; Wang, C.; Gross, K.C.; Smith, D.L. Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. *Planta* **2002**, *214*, 895–901. [CrossRef]
19. Lufu, R.; Ambaw, A.; Opara, U.L. Water loss of fresh fruit: Influencing pre-harvest, harvest and postharvest factors. *Sci. Hortic.* **2020**, *272*, 109519. [CrossRef]
20. Wu, X.; Chen, Y.; Zhu, J.; Zhang, N.; Wei, Y.; Jiang, S.; Ye, J.; Shao, X. 1-Methylcyclopropene reduces postharvest water loss by modulating cuticle formation in tomato fruit. *Postharvest Biol. Technol.* **2023**, *206*, 112564. [CrossRef]
21. Mostofi, Y.; Toivonen, P.M.A.; Lessani, H.; Babalar, M.; Lu, C. Effects of 1-methylcyclopropene on ripening of greenhouse tomatoes at three storage temperatures. *Postharvest Biol. Technol.* **2003**, *27*, 285–292. [CrossRef]
22. Al Shoffe, Y.; Nock, J.F.; Zhang, Y.; Watkins, C.B. Pre-and post-harvest γ-aminobutyric acid application in relation to fruit quality and physiological disorder development in 'Honeycrisp' apples. *Sci. Hortic.* **2021**, *289*, 110431. [CrossRef]
23. Aghdam, M.S.; Razavi, F.; Karamneghad, F. Maintaining the postharvest nutritional quality of peach fruits by γ-Aminobutyric acid. *Iran. J. Plant Physiol.* **2016**, *5*, 1457–1463.
24. Blankenship, S.M.; Dole, J.M. 1-Methylcyclopropene: A review. *Postharvest Biol. Technol.* **2003**, *28*, 1–25. [CrossRef]
25. Ruiz-Aracil, M.C.; Valverde, J.M.; Ilea, M.I.M.; Valero, D.; Castillo, S.; Guillén, F. Innovative postharvest management for Hass avocado at the preclimacteric stage: A combined technology with GABA and 1-MCP. *Foods* **2024**, *13*, 2485. [CrossRef]
26. Li, C.; Zhu, J.; Sun, L.; Cheng, Y.; Hou, J.; Fan, Y.; Ge, Y. Exogenous γ-aminobutyric acid maintains fruit quality of apples through regulation of ethylene anabolism and polyamine metabolism. *Plant Physiol. Biochem.* **2021**, *169*, 92–101. [CrossRef]
27. Ansari, M.I.; Jalil, S.U.; Ansari, S.A.; Hasanuzzaman, M. GABA shunt: A key-player in mitigation of ROS during stress. *Plant Growth Regul.* **2021**, *94*, 131–149. [CrossRef]
28. Watkins, C.B. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnol. Adv.* **2006**, *24*, 389–409. [CrossRef]
29. Hoeberichts, F.A.; Van Der Plas, L.H.; Woltering, E.J. Ethylene perception is required for the expression of tomato ripening-related genes and associated physiological changes even at advanced stages of ripening. *Postharvest Biol. Technol.* **2002**, *26*, 125–133. [CrossRef]
30. Zhang, Z.; Huber, D.J.; Hurr, B.M.; Rao, J. Delay of tomato fruit ripening in response to 1-methylcyclopropene is influenced by internal ethylene levels. *Postharvest Biol. Technol.* **2009**, *54*, 1–8. [CrossRef]
31. Han, S.; Nan, Y.; Qu, W.; He, Y.; Ban, Q.; Lv, Y.; Rao, J. Exogenous γ-aminobutyric acid treatment that contributes to regulation of malate metabolism and ethylene synthesis in apple fruit during storage. *J. Agric. Food Chem.* **2018**, *66*, 13473–13482. [CrossRef]
32. Kathiresan, A.; Tung, P.; Chinnappa, C.C.; Reid, D.M. γ-Aminobutyric acid stimulates ethylene biosynthesis in sunflower. *Plant Physiol.* **1997**, *115*, 129–135. [CrossRef]
33. Aghdam, M.S.; Fard, J.R. Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (Fragaria × anannasa cv. Selva) by enhancing GABA shunt activity. *Food Chem.* **2017**, *221*, 1650–1657. [CrossRef]
34. Zhao, Y.; Yu, H.; Zhou, J.M.; Smith, S.M.; Li, J. Malate circulation: Linking chloroplast metabolism to mitochondrial ROS. *Trends Plant Sci.* **2020**, *25*, 446–454. [CrossRef]
35. Habibi, F.; Ramezanian, A.; Rahemi, M.; Eshghi, S.; Guillén, F.; Serrano, M.; Valero, D. Postharvest treatments with γ-aminobutyric acid, methyl jasmonate, or methyl salicylate enhance chilling tolerance of blood orange fruit at prolonged cold storage. *J. Sci. Food Agric.* **2019**, *99*, 6408–6417. [CrossRef]
36. Petit, J.; Bres, C.; Just, D.; Garcia, V.; Mauxion, J.P.; Marion, D.; Bakan, B.; Joubès, J.; Domergue, F.; Rothan, C. Analyses of tomato fruit brightness mutants uncover both cutin-deficient and cutin-abundant mutants and a new hypomorphic allele of GDSL lipase. *Plant Physiol.* **2014**, *164*, 888–906. [CrossRef]
37. Zhang, Y.; Lin, B.; Tang, G.; Chen, Y.; Deng, M.; Lin, Y.; Li, M.; He, W.; Wang, Y.; Zhang, Y.; et al. Application of γ-aminobutyric acid improves the postharvest marketability of strawberry by maintaining fruit quality and enhancing antioxidant system. *Food Chem. X* **2024**, *21*, 101252. [CrossRef]
38. Amarowicz, R.; Carle, R.; Dongowski, G.; Durazzo, A.; Galensa, R.; Kammerer, D.; Maiani, G.; Piskula, M.K. Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. *Mol. Nutr. Food Res.* **2009**, *53*, S151–S183. [CrossRef]
39. Lattanzio, V. Bioactive polyphenols: Their role in quality and storability of fruit and vegetables. J. Appl. Bot. 2003, 77, 128–146.
40. Ilic´, Z.S.; Marinkovic´, D.; Trajkovic´, R.; Šunic´, L.; Perzelan, Y.; Alkalai-Tuvia, S.; Fallik, E. Effect of 1-methylcyclopropene on the antioxidant capacity and postharvest quality of tomato fruit. *Afr. J. Biotechnol.* **2013**, *12*, 547–553. [CrossRef]

41. Shenglong, D.; Jihong, Z.; Shaoyang, C.; Shuang, M.; Li, Z. The combined effect of 1-methylcyclopropene and citral suppressed postharvest grey mould of tomato fruit by inhibiting the growth of Botrytis cinerea. *J. Phytopathol.* **2019**, *167*, 123–134. [CrossRef]
42. Barsan, C.; Sanchez-Bel, P.; Rombaldi, C.; Egea, I.; Rossignol, M.; Kuntz, M.; Zouine, M.; Latché, A.; Bouzayen, M.; Pech, J.C. Characteristics of the tomato chromoplast revealed by proteomic analysis. *J. Exp. Bot.* **2010**, *61*, 2413–2431. [CrossRef]
43. Babarabie, M.; Zarei, H.; Eskandari, A. The impact of pre-harvest treatment with gamma-aminobutyric acid (GABA) and salicylic acid on vase life and post-harvest traits of tuberose cut flowers. *Acta Sci. Pol. Hortorum Cultus* **2019**, *18*, 83–92. [CrossRef]
44. Sevillano, L.; Sanchez-Ballesta, M.T.; Romojaro, F.; Flores, F.B. Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. *J. Sci. Food Agric.* **2009**, *89*, 555–573. [CrossRef]
45. Li, X.; Yun, J.; Fan, X.; Xing, Y.; Tang, Y. Effect of 1-methylcyclopropene and modified atmosphere packaging on chilling injury and antioxidative defensive mechanism of sweet pepper. *Afr. J. Biotechnol*. **2011**, *10*, 6581–6589.
46. Palma, F.; Carvajal, F.; Jiménez-Muñoz, R.; Pulido, A.; Jamilena, M.; Garrido, D. Exogenous γ-aminobutyric acid treatment improves the cold tolerance of zucchini fruit during postharvest storage. *Plant Physiol. Biochem*. **2019**, *136*, 188–195. [CrossRef]
47. Shelp, B.J.; Aghdam, M.S.; Flaherty, E.J. γ-Aminobutyrate (GABA) regulated plant defense: Mechanisms and opportunities. *Plants* **2021**, *10*, 1939. [CrossRef]
48. Luo, Y.; Zhang, S.; Su, J.; Cao, Z.; Wang, X.; Shen, W.; Li, T.; Ge, X. Studies on the effect of cinnamon essential oil-micelles combined with 1-MCP/PVA film on postharvest preservation of apricots. *Food Control* **2024**, *162*, 110420. [CrossRef]

5. RESULTADOS Y DISCUSIÓN

5. RESULTADOS Y DISCUSIÓN

5.1. Efecto de las aplicaciones precosecha con elicitores en cereza

Los productores de cerezas deben hacer frente a diferentes desafíos climáticos anualmente, específicamente el impacto de las heladas de primavera y el riesgo inherente del agrietado de la fruta. Las características agronómicas como el cuajado, la floración y la fertilización pueden ser problemáticas en el cultivo de la cereza, y las condiciones climáticas tienen un efecto importante en ellas (Campillay-Llanos et al., 2024). Entre los cambios asociados al cambio en las condiciones climáticas se encuentra el retraso de la floración o una floración irregular que reduce la apertura de las yemas y obstaculiza la polinización, lo que puede influir significativamente en el rendimiento obtenido. El aumento de las temperaturas medias anuales causado por el cambio climático modificará el desarrollo de los brotes, la floración, las etapas de desarrollo del fruto y el momento de la cosecha del fruto (Wenden et al., 2016; Garreaud et al., 2020).

En los últimos años, los productores de cerezas han sido testigos de una caída sustancial en la producción, atribuida en gran parte al impacto perjudicial de las heladas primaverales durante la etapa de floración y a la aparición del agrietado debido a las lluvias que se dan en etapas vulnerables de madurez del fruto (Rehman et al., 2015). Dado el cambio en los patrones climáticos, la implementación de nuevas estrategias y enfoques innovadores se vuelve necesario para aliviar el daño potencial de estas adversidades climáticas. Por ello, es imperativo desarrollar herramientas para adaptar la producción de la cereza a los escenarios futuros del cambio climático.

5.1.1. Resiliencia a las heladas de primavera

Las heladas primaverales son un riesgo significativo para la producción de frutas en casi todas las regiones templadas. Las cerezas se encuentran entre las primeras especies vegetales que comienzan su desarrollo en primavera y, por lo tanto, son muy susceptibles a las heladas tardías (Demirsoy et al., 2022). Las caídas repentinas de temperatura que se producen en primavera pueden provocar daños por heladas, causando una reducción del cuajado de los frutos impactando significativamente en el rendimiento general de la cereza (Kappel, 2010; Kaya y Kose, 2022).

El daño por las heladas en los árboles frutales ocurre cuando el agua en los tejidos celulares de la planta se congela, se expande y rompe las paredes celulares. Los daños causados por las heladas primaverales en los órganos reproductores suelen producir anomalías morfológicas internas y externas que afectan el desarrollo normal del fruto o incluso provocan la abscisión (Matzneller et al., 2016).

Así, los botones florales afectados por las heladas primaverales muestran un oscurecimiento general como síntoma externo inmediato. En plena floración, el primer síntoma que se observa es una decoloración marrón del pistilo, que puede extenderse al estilo y a los óvulos (Rodrigo, 2000).

En esta Tesis Doctoral la tolerancia a las heladas primaverales en cereza se estudió para los tratamientos de PUT (Publicación I) y MT (Publicación II).

La aparición de las heladas primaverales está relacionada con un estrés por frío que desencadena la síntesis de ROS. Se genera la formación de MDA como el principal producto metabólico cuando los radicales libres oxidan los lípidos en las membranas celulares. El MDA es un biomarcador del estrés oxidativo, el cual refleja la peroxidación de las membranas plasmáticas y la integridad estructural de los tejidos vegetales (Sachdev et al., 2021).

Los niveles de MDA observados en los botones florales tratados con PUT 1 mM (2021) y 10 mM (2021 y 2022) exhibieron una disminución significativa ($p < 0,05$) en el contenido de MDA en comparación con el control para las variedades 'Prime Giant' y 'Sweetheart'. No se observó un efecto dosis-dependiente en la variedad 'Prime Giant' pero si en 'Sweetheart', donde la concentración de PUT de 10 mM exhibió un impacto significativamente ($p < 0,05$) mayor en la reducción de los niveles de MDA en comparación con la concentración de 1 mM. Además, en ambos ciclos productivos estudiados, las aplicaciones de PUT redujeron el contenido de MDA con resultados similares en los dos años para la concentración de 1 mM (2021 y 2022) (Publicación I). Durante el desarrollo floral, la tolerancia a las heladas de los botones florales disminuye. Las variaciones en el contenido de MDA entre diferentes ciclos productivos podrían estar directamente asociadas con las condiciones abióticas de cada ciclo de producción de los frutos (Giné-Bordonaba et al., 2017).

De igual forma, la aplicación foliar de MT (0,01, 0,05 y 0,1 mM) antes de la cosecha redujo los niveles medios de MDA en botones florales y fue generalmente significativa ($p < 0,05$) para 'Prime Giant' y 'Sweetheart'. Estos resultados se confirmaron para la concentración de 0,01 mM en dos ciclos productivos consecutivos (2021 y 2022) para las dos variedades evaluadas. Aunque no se observó una tendencia dependiente de la concentración en general, la concentración más alta de MT tuvo un efecto más fuerte, pero solo en la variedad 'Sweetheart' (Publicación II).

La reducción del contenido de MDA en los árboles tratados con PUT y MT puede estar relacionada con una protección de los órganos florales a través de la mejora del equilibrio antioxidante en los tejidos (Mirdehghan y Rahimi, 2016; Sharma et al., 2017; Arnao y Hernández-Ruiz, 2021, Singh et al., 2023). El impacto de las heladas primaverales sobre los órganos reproductivos de las cerezas se relaciona con desórdenes internos y externos que alteran el proceso habitual de desarrollo del fruto. De hecho, los botones florales de las cerezas afectados por heladas primaverales suelen presentar un oscurecimiento del pistilo (Matzneller et al., 2016). Durante esta investigación, se produjeron heladas importantes en 2022 y 2023 en periodos cercanos a la fase de plena floración, teniendo un impacto severo en los cerezos y comprometiendo la producción. Debido al importante efecto sobre el oscurecimiento del pistilo (90-98 %), no se encontraron diferencias significativas ($p > 0,05$) relacionadas con el tratamiento precosecha de PUT y MT aplicada en los botones florales en ambos ciclos productivos (2022 y 2023) (Publicación I y II).

Con las heladas primaverales disminuye el cuajado de frutos al aumentar el porcentaje de pistilos dañados. Si el daño por frío afecta a más del 50 % de los pistilos de las flores, el árbol no puede proporcionar una cosecha óptima, ya que, en general, solo entre el 20 % y el 30 % de las flores del cerezo se convierten en frutos en algunas especies estudiadas (Chmielewski et al., 2018). Para lograr una producción óptima, es crucial que los cerezos mantengan una tasa de cuajado dentro del rango de 25 % a 40 % (Rutkowski y Łysiak, 2022). El cuajado de frutos en los cerezos se ve afectado significativamente por las condiciones climáticas, lo que representa un desafío considerable que afecta el rendimiento total. También depende de otros factores como la densidad de los botones florales, el número de flores por botón, la tasa de caída de botones, el éxito de la polinización, la nutrición y las prácticas de manejo durante la floración (Garcia-Montiel, 2010; Beppu y Kataoka, 2011).

No se observaron diferencias significativas ($p > 0,05$) entre los árboles tratados con PUT y los árboles control 'Prime Giant' en el primer período de estudio (2021). Sin embargo, los árboles 'Sweetheart' mostraron diferencias significativas ($p < 0,05$) en los porcentajes de cuajado especialmente en la concentración de PUT de 10 mM. En el siguiente ciclo de cultivo de 2022, se observaron diferencias significativas ($p < 0,05$) en los porcentajes de cuajado de frutos para ambas variedades cuando se trataron con 1 mM de PUT para ambas variedades (Publicación I).

Por otro lado, los resultados del cuajado de frutos con el tratamiento de MT mostraron un patrón general positivo en los árboles tratados en ambas variedades, con un efecto más notorio en 'Sweetheart'. Para esta variedad, en todos los ciclos productivos estudiados, los valores medios de cuajado de frutos fueron mayores en los árboles tratados con MT que en los árboles control, mostrando diferencias significativas en 2021 y 2022. Para la variedad 'Prime Giant', se observaron valores medios más altos en los árboles tratados con MT solo en 2022 (Publicación II).

En ambas variedades, el cuajado de frutos se redujo a lo largo de los ciclos de producción estudiados. Esta reducción podría estar asociada con efectos adversos en los órganos florales durante ciclos productivos consecutivos. Los efectos adversos de las heladas primaverales en 2023 disminuyeron significativamente el porcentaje de cuajado de frutos en ambas variedades, con un impacto pronunciado en la variedad 'Prime Giant'. Estos hallazgos ponen de manifiesto la variabilidad observada en los porcentajes de cuajado de los frutos entre los diferentes ciclos productivos y variedades, como se ha descrito previamente (Carrión-Antolí et al., 2022b).

El rango óptimo de cuajado (25-40 %) sólo se alcanzó en 2021 para las variedades estudiadas. El aumento en el cuajado de frutos con estos tratamientos se ha observado en otros estudios para diferentes especies vegetales. En este sentido, se ha relacionado con una mejor viabilidad de los óvulos y retención de frutos en los árboles a través de la protección de los tejidos vegetales frente al estrés abiótico (Arnao y Hernández-Ruiz, 2020; Sabir et al., 2021).

5.1.2. Reducción del agrietado inducido por la climatología

El agrietado del fruto se ve afectado por las condiciones ambientales, como las lluvias torrenciales que suceden antes y durante la cosecha. El agrietado es debido principalmente a la humedad y al porcentaje de área superficial mojada del fruto. La evolución de microgrietas a macrogrietas requiere de una humedad superficial continua (Knoche y Peschel, 2006; Winkler et al., 2020).

A lo largo de los años se han estudiado los diferentes mecanismos de acción posibles de este desorden fisiológico. Se ha postulado que el agrietado es consecuencia de una elevada presión celular debida a una alta turgencia en los tejidos, causada por la absorción osmótica de agua y el aumento del volumen de la fruta. Esto provoca un aumento en el área de la piel que supera la capacidad elástica de la cutícula (Christensen, 1973). La contracción de la piel después de un enfriamiento rápido causado por la lluvia o por una caída repentina de la temperatura también puede provocar el agrietado. Por tanto, varios autores han concluido que los aumentos en la presión tisular son responsables del incremento en la susceptibilidad al agrietado (Measham et al., 2009). También se ha considerado que las frutas con alto contenido de azúcar son más susceptibles al agrietado debido a un mayor potencial osmótico, lo que proporciona una fuerza impulsora para la absorción de agua y su movimiento a través de la piel de la fruta (Christensen, 1996; Richardson, 1998). Además, la evolución de la maduración del fruto afecta al espesor e integridad del epicarpio de la cereza, lo que influye en la incidencia del agrietado (Demirsoy y Demirsoy, 2004; Correia et al., 2028).

En esta Tesis Doctoral se ha estudiado por primera vez la relación que podría tener el estado de desarrollo de la cereza en el árbol con su susceptibilidad al agrietado. Para ello, se estudió la incidencia del agrietado en las variedades 'Prime Giant' y 'Sweetheart' acogidas en la IGP “Cerezas de la montaña de Alicante” expuestas a factores climáticos y también bajo condiciones controladas a través de inmersiones en agua destilada durante el desarrollo del fruto.

Sin embargo, las condiciones climáticas y los factores del agrietado pueden actuar sinérgicamente en condiciones de campo no controladas. Por ello, se empleó una metodología óptima para evaluar la tolerancia al agrietado entre diferentes variedades y etapas de maduración en condiciones uniformes. En este estudio se utilizó el método de inmersión en agua destilada (Christensen, 1996; Balbotín et al., 2018) para evaluar la susceptibilidad al agrietado. Cuando las cerezas intactas se sumergieron en agua, ambas variedades mostraron una menor resiliencia al agrietado de los frutos tras la inmersión en comparación con los resultados de agrietado observados en los árboles bajo condiciones climáticas naturales.

En la variedad 'Sweetheart' no coincidieron precipitaciones sustanciales entre 2020 y 2022, ya que es atípico que se produzcan precipitaciones significativas durante el mes de julio, que es el momento habitual de cosecha para esta variedad. De hecho, la ausencia de fuertes lluvias en 2022 para ambas variedades durante el desarrollo del fruto en el árbol, cuando las cerezas son más susceptibles, jugó un papel fundamental en la prevención del agrietado en el árbol en ambas variedades.

Todos los tratamientos precosecha que contenían PUT disminuyeron con éxito la incidencia de agrietado, aunque no exhibieron un efecto dependiente de la concentración en general cuando la concentración de PUT se incrementó hasta 10 mM. En la variedad 'Prime Giant', las reducciones en el agrietado en la cosecha alcanzaron el 68,5 % y el 56,2 % en comparación con los árboles control en 2020 y 2021, respectivamente. Sin embargo, este efecto fue aún más pronunciado en la cosecha con 'Sweetheart', donde se observaron reducciones en el agrietado de la fruta del 62,1 %, 71,8 % y 64,3 % en 2020, 2021 y 2023, respectivamente. Considerando los distintos estados de maduración de los frutos durante el desarrollo en árbol en ambas variedades y su correlación con el agrietado de las cerezas, los niveles más altos de agrietado de los frutos se observaron en el estado de desarrollo S1, coincidiendo con los cambios de color para la variedad 'Prime Giant'. Sin embargo, para 'Sweetheart' la mayor del agrietado se observó cuando las cerezas alcanzaron los estados de desarrollo S3 y S4 (Publicación I).

No hay estudios que examinen los efectos de la PUT y la MT en la aparición del agrietado en cerezas en el momento de la cosecha o durante el desarrollo. En el estudio con PUT, se encontró la mayor incidencia del agrietado de las cerezas en

'Prime Giant' durante 2020 en la etapa de cosecha. En particular, la aplicación de PUT (1 mM) resultó en una reducción significativa ($p < 0,05$) del agrietado de la fruta para ambas variedades. Sin embargo, el efecto más pronunciado para el tratamiento con PUT se observó en 'Prime Giant' en 2022 y para 'Sweetheart' en 2020, 2021 y 2022, demostrando la mayor reducción del agrietado de la fruta (alrededor del 75 %) en comparación con la fruta control en el momento de la cosecha. Con respecto al tratamiento con PUT en 'Sweetheart', las etapas de maduración más tempranas (S1 y S2) o las etapas de maduración más avanzadas (S5) exhibieron mayor tolerancia. Sin embargo, en 'Prime Giant', la resiliencia al agrietado del fruto se mantuvo similar después de los cambios de color durante la maduración en árbol (S1-S4) (Publicación I).

Las evaluaciones del agrietado de las cerezas con el tratamiento de MT en el árbol durante los ciclos de producción (2021-2023) mostraron un efecto mayor desde la etapa fenotípica S1 para 'Prime Giant' y S2 y S3 para 'Sweetheart'. La MT aplicada antes de la cosecha en la concentración más baja (0,01 mM), redujo significativamente la incidencia del agrietado de los frutos desde el comienzo de la maduración en el árbol. Estas aplicaciones fueron generalmente efectivas para mitigar el agrietado de las cerezas en todas las etapas de maduración y en ambas variedades en casi todos los ciclos productivos estudiados. El mayor efecto en la reducción del agrietado de cerezas en la cosecha con el tratamiento de MT 0,01 mM se observó en 2020, con reducciones del 59,45 % y 81,5 % para las variedades 'Prime Giant' y 'Sweetheart', respectivamente. Los estudios en el árbol (2020–2022) sobre 'Sweetheart' y 'Prime Giant' mostraron diferentes tolerancias al agrietado, siendo 'Prime Giant' más vulnerable a las condiciones ambientales cada año (Publicación II) y coincidiendo las dos diferentes evaluaciones del agrietado.

Las aplicaciones de MT antes de la cosecha se redujeron de cuatro a tres en 2022 y 2023. En este contexto, las cuatro aplicaciones en 2020 y 2021 mostraron un efecto más fuerte en la variedad 'Prime Giant' en la cosecha que las tres aplicaciones en 2022. Sin embargo, en 'Sweetheart', los resultados fueron similares a pesar del número de aplicaciones, ya que el efecto de resistencia de MT se mantuvo similar a lo largo de los años consecutivos estudiados (Publicación II).

En general, durante la maduración de la cereza en el árbol, se observó un aumento en la incidencia del agrietado, que se redujo mediante aplicaciones previas a la cosecha de JaMe coincidiendo con el endurecimiento del hueso (antes de S1) en ambas variedades y con el cambio de color de las cerezas (S1 para la variedad 'Prime Giant' y S2 para 'Sweetheart'). Sin embargo, este efecto disminuyó a medida que avanzaba la maduración del fruto, en particular para 'Prime Giant', posiblemente porque es una variedad más temprana que 'Sweetheart' y los estados de maduración más avanzados suelen coincidir con lluvias más intensas en comparación con las que ocurren durante la maduración de 'Sweetheart'. Cuando se evaluó la susceptibilidad al agrietado en condiciones controladas con el tratamiento de JaMe, se observó que si bien en 'Prime Giant' la susceptibilidad fue similar en todos los estados de maduración, 'Sweetheart' mostró una sensibilidad al agrietado diferente dependiendo del estado de maduración con una mayor incidencia en el estado S3. Así, tanto los estados de maduración más tempranos como los más tardíos mostraron una menor incidencia de agrietado en condiciones controladas (Publicación III).

El agrietado de la fruta es un desorden fisiológico multifactorial, y por esta razón, es muy difícil determinar las razones específicas que afectan los diferentes niveles de resistencia entre las distintas variedades (Blažek et al., 2022). En este contexto, la diferente eficacia de los tratamientos previos a la cosecha contra el agrietado inducido por la lluvia parece estar estrechamente relacionada con la etapa de maduración (Faizy et al., 2021). Además, en esta Tesis Doctoral se ha podido dilucidar que la susceptibilidad al agrietado parece depender de la variedad, lo que es consistente con los hallazgos de investigaciones anteriores (Quero-García et al., 2021). Las variedades con piel más delgada son generalmente más susceptibles al agrietado en comparación con aquellas con piel más gruesa. Se observa una menor incidencia de agrietado en cerezas cuando las células de la epidermis, hipodermis y parénquima exhiben tamaños celulares mayores en las últimas etapas de maduración, resaltando la importancia de la flexibilidad y elasticidad de la epidermis para reducir este desorden (Brüggenwirth y Knoche, 2016). Por esta razón, la maduración tardía del fruto afecta a la incidencia del impacto del agrietado en cerezas sometidas a diferentes estreses ambientales, especialmente con lluvias persistentes.

5.1.3. Calidad de la cereza en el momento de la cosecha

Las cerezas generalmente tienen un período de cosecha corto y una ventana de comercialización limitada; son frutos altamente perecederos y su vida útil es relativamente corta debido a sus tasas moderadas de actividad respiratoria y su susceptibilidad a una rápida descomposición microbiológica durante el almacenamiento. Sin embargo, con el aumento de la producción y el comercio mundial de cerezas, es necesario mantener la calidad de las cerezas durante el almacenamiento con tiempos de comercialización más prolongados (Alonso y Alique, 2006). La disminución de la calidad incluye la pérdida de humedad, el ablandamiento y la descomposición del fruto, junto con el pardeamiento del pedicelo. Como tejido más externo de la cereza, el epicarpio juega un papel importante en la protección y preservación de la vida útil de la fruta, sin embargo, la cereza tiene una piel fina que se daña fácilmente (Pullanagari y Li, 2021).

Considerando todos los desafíos que presenta la producción de cerezas, se deben abordar estrategias mediante la combinación de buenas prácticas de manejo y la aplicación de tecnologías adecuadas que puedan maximizar el rendimiento, sin comprometer la calidad y la sostenibilidad (Martínez-Romero et al., 2006).

Los tratamientos precosecha con PUT y JaMe retrasaron la evolución de la firmeza de la fruta en el árbol en todas las variedades estudiadas, mostrando valores medios más altos en la cosecha en cuatro ciclos productivos consecutivos (Publicación I y III).

Para el tratamiento con PUT, las diferencias significativas ($p < 0,05$) se observaron en 2021 para las variedades 'Prime Giant' y 'Sweetheart' y en 2023 también para 'Sweetheart' (Publicación I). En diferentes frutas climatéricas se ha descrito una mayor firmeza de la fruta tratada con PUT a concentraciones de entre 1 y 3 mM (Shanbehpour et al., 2020; Singh et al., 2022). La mejora de la firmeza debido a las PAs se atribuye a su efecto en la reticulación de moléculas pécticas dentro de la pared celular. Este proceso de reticulación conduce a una solidificación inmediata, proporcionando un efecto inhibidor sobre las enzimas que degradan la pared (Valero y Serrano, 2010). Además, las PAs mantienen las bio-membranas celulares, protegiendo los fosfolípidos y las proteínas de la peroxidación (Du et al., 2023).

El efecto de la MT sobre la firmeza de las cerezas podría depender de factores específicos relacionados con el año y la variedad evaluada. Los tratamientos con MT en algunos casos promovieron significativamente ($p < 0,05$) valores más altos de firmeza de los frutos, mientras que en otros no se observó este efecto. En general, no se obtuvieron resultados significativos ($p < 0,05$) con el tratamiento de MT en la firmeza en comparación con los frutos control, sólo en alguna de las anualidades estudiadas (Publicación II). Sin embargo, en estudios realizados en otras especies vegetales si se ha observado un efecto significativo de la MT en el mantenimiento de los niveles de firmeza que estuvo relacionado con el poder antioxidante de este compuesto (Madebo et al., 2021; Ali et al., 2023; Medina-Santamarina et al., 2023).

El tratamiento con JaMe incrementó de forma significativa ($p > 0,05$) los niveles de firmeza en comparación con los frutos control para las variedades 'Early Lory', 'Prime Giant' y 'Sweetheart' durante los diferentes ciclos de cultivo (Publicación III). Parece que un aumento en la firmeza está correlacionado con una reducción en la susceptibilidad al agrietado. Sin embargo, esta asociación puede estar influenciada por variedades y especies vegetales específicas (Usenik et al., 2005; Balbontín et al., 2018; Matteo et al., 2025).

El desarrollo del color se retrasó significativamente (p < 0,05) con el tratamiento de PUT en 'Prime Giant' en el momento de la cosecha en las temporadas 2020 y 2021. Sin embargo, este patrón retrasado de evolución del color no se observó (p > 0,05) en la variedad 'Sweetheart' (Publicación I). En un estudio previo, Mirdheghan y Rahimi (2016) observaron la misma discrepancia entre dos variedades diferentes de uva de mesa después de tratamientos con PUT antes de la cosecha. Por esta razón, este efecto merece una mayor investigación en otras variedades de cerezas.

La aplicación de MT 0,01 mM tuvo efectos variables sobre el color de los frutos en diferentes años y variedades. Para las variedades 'Prime Giant' y 'Sweetheart', se encontraron valores de ángulo *Hue* significativamente más altos (p < 0,05) en comparación con los frutos control en la cosecha de 2021. Estos valores asociados a las cerezas de colores rojos más claros y por tanto relacionadas con un retraso en el color (Publicación II). En otras frutas no climatéricas como la uva y la granada, se ha observado que el JaMe y la MT, pueden tener efectos opuestos sobre el color del fruto en la cosecha, dependiendo de la concentración aplicada (García-Pastor et al., 2019; Medina-Santamarina et al., 2021).

Las variedades de cerezas difieren en su color óptimo en la cosecha, pero, en general, las cerezas con un color rojo intenso son las preferidas porque están asociadas con dulzura, sabor y un alto valor nutricional (Serrano et al., 2009). En general, los tratamientos previos a la cosecha con JaMe retrasaron significativamente la evolución del color (CIE *hue**) en 'Prime Giant', 'Sweetheart' y 'Staccato' en el momento de la cosecha. Sin embargo, se observaron valores similares en 'Early Lory' tratada con JaMe en comparación con los lotes control en el momento de la cosecha.

El tratamiento precosecha con PUT 1 mM no produjo un impacto significativo (p > 0,05) en 'Prime Giant' en el momento de la cosecha con respecto a la acumulación de SST y la AT en comparación con los lotes control. Sin embargo, si se observó un efecto significativo (p < 0,05) para 'Sweetheart', lo que resultó en un retraso en la acumulación de SST en los lotes tratados con PUT durante dos ciclos de producción diferentes (2020 y 2023) (Publicación I). En otras frutas como la uva y la pera, concentraciones de 2 y 3 mM retrasaron la acumulación de azúcar y obtuvieron una mayor AT en comparación con el control (Mirdheghan y Rahimi, 2016; Singh et al., 2022).

Por otro lado, el efecto de la MT en los SST y la AT no tuvo un impacto uniforme a lo largo de los años estudiados en ambas variedades. En general, los valores medios de SST en los frutos tratados con MT mostraron un retraso en la mayoría de los ciclos productivos estudiados. Respecto a la acidez del fruto, se observaron diferentes efectos según la variedad estudiada. Aunque la acumulación de AT en 'Prime Giant' se retrasó con los tratamientos de MT en comparación con los frutos control, en la variedad 'Sweetheart' se observó un nivel más alto de AT en la mayoría de las recolecciones realizadas (Publicación II). Michailidis et al. (2021) también observaron una acumulación retrasada de SST en la cosecha, pero no se encontraron diferencias relacionadas con la AT con concentraciones más altas de 0,01 mM. Sin embargo, se han descrito aumentos en los SST y la AT tras aplicar concentraciones más altas de MT que las utilizadas en este estudio (Tijero et al., 2019; Carrión-Antolí et al., 2022a). Las diferencias observadas por varios autores en los tratamientos de MT en los SST y AT podrían deberse a las variaciones en el número de aplicaciones, las concentraciones aplicadas, la etapa de madurez en el momento de la cosecha y un efecto dependiente de las variedades observadas en los estudios mencionados.

El efecto de los tratamientos precosecha con JaMe, en general, tuvo un mayor impacto en retrasar la acumulación de los SST que en afectar a la AT en comparación con los frutos control. No se encontraron diferencias significativas ($p > 0,05$) en la AT en la mayoría de las variedades y ciclos productivos estudiados para el tratamiento con JaMe, sin embargo, los valores medios de AT fueron superiores en cerezas tratadas con JaMe respecto a los frutos control. Sí se observó un efecto significativo en la mayoría de los lotes tratados con JaMe durante los cuatro ciclos productivos en el retraso de la acumulación de SST (Publicación III). Estos resultados coinciden con los obtenidos por otros autores en la variedad 'Regina', 'Sweetheart', '0900 Ziraat' y 'Bing' (Balbontín et al., 2018; Faizy et al., 2021; Saracoglu et al., 2017).

La acumulación de polifenoles está relacionada con una disminución en el CIE *hue** (Serrano et al., 2009). En general, las cerezas tratadas con PUT y MT no mostraron diferencias significativas ($p > 0,05$) de color respecto a las cerezas control (Publicación I y II). Sin embargo, los valores de CIE *hue** de las cerezas tratadas con JaMe fueron mayores en comparación con la fruta control. Estas diferencias fueron significativas para la mayoría de las variedades estudiadas, mostrando valores medios superiores en los frutos tratados con JaMe respecto a los lotes control (Publicación III).

El pardeamiento del pedicelo en las cerezas se relaciona generalmente con la senescencia y deshidratación de la fruta. La incidencia general del pardeamiento del pedicelo no fue alta en la cosecha, ya que esta condición generalmente se desarrolla durante el almacenamiento poscosecha de las cerezas (Martínez-Romero et al., 2006). Las frutas tratadas con JaMe mostraron valores medios más bajos de pardeamiento del pedicelo en el momento de la cosecha, aunque no se observaron diferencias significativas ($p > 0,05$) a excepción de la variedad 'Prime Giant' en la temporada 2019 (Publicación III). Sin embargo, se ha descrito el efecto del JaMe en la reducción de la oxidación de la clorofila al modificar el mecanismo de defensa antioxidante, preservando así el color verde de los tejidos (Hewedy et al., 2023; Swain et al., 2023).

5.2. Impacto de las aplicaciones poscosecha

De acuerdo con los resultados positivos obtenidos con elicitores en precosecha de cereza, en esta Tesis Doctoral se amplió el estudio de sus efectos sobre la poscosecha de diferentes especies vegetales. El objetivo fue evaluar la eficacia de estos compuestos sobre la calidad y vida útil de los distintos productos vegetales durante el almacenamiento.

5.2.1. Efecto de la aplicación de melatonina en clavel

La deshidratación compromete la vida útil de todas las especies vegetales, pero especialmente la de las flores cortadas. En el estudio realizado sobre el clavel 'Báltico', el PF de las flores aumentó del día 0 al día 5 debido al desarrollo y apertura de los pétalos. Los claveles con tallos sumergidos en las soluciones de MT más concentradas (0,1 y 1 mM) permanecieron con un PF constante, ≈ 107 % con respecto al estado inicial, durante más tiempo que el resto de los tratamientos. Sin embargo, todos los tratamientos con MT retrasaron significativamente ($p < 0,05$) la evolución del PF de las flores. Además, los claveles en soluciones de MT 0,1 mM mostraron la menor pérdida de peso en comparación con el resto de los tratamientos durante todo el experimento. De hecho, el porcentaje de PF observado en el día 21 del almacenamiento para el control fue de 66,05 ± 5,47 y de 97,27 ± 0,55 % para las flores expuestas a la solución de MT 0,1 mM (Publicación IV).

La absorción de la solución en florero (ASF) fue alta y sin cambios significativos durante los primeros días de vida de todas las flores de clavel. Todas las concentraciones mantuvieron una ASF más alta durante el almacenamiento en comparación con los claveles control. La ASF disminuyó bruscamente después de 10 días en los claveles control y después de 15, 17 y 18 días en los tratados con MT 0,01, 1 y 0,1 mM respectivamente. Los claveles 'Báltico' tratados con MT 0,1 mM mostraron un mayor PF durante el período de vida útil, pero una ASF más retrasada en comparación con las flores control y otras concentraciones de MT empleadas (Publicación IV). La evolución de la senescencia en flores cortadas se ve afectada por el equilibrio transpiración/absorción de agua. Por esta razón, el PF disminuye cuando la transpiración supera la ASF (Ebrahimzadeh et al., 2011). Una menor transpiración de las flores en pétalos y otros tejidos podría estar afectando a la ASF probablemente a través de un mantenimiento de la actividad estomática (Mohamed et al., 2020). Un equilibrio adecuado entre la deshidratación de los órganos y el índice de absorción de agua es fundamental para mantener la turgencia celular en las flores cortadas (Solgi, 2018).

La vida útil de los claveles fue significativamente ($p < 0,05$) mayor cuando las flores se almacenaron con las diferentes soluciones de MT, en comparación con los claveles control en agua destilada. La concentración de MT 0,1 mM fue la más efectiva para extender la vida útil, alcanzando el estado 7 de senescencia a los 20 días en comparación con los 10 días alcanzados por el control. Así, los tratamientos con MT 0,1 mM duplicaron el tiempo de almacenamiento en el que las flores mantienen un valor estético. Sin embargo, no hubo dosis dependencia en el rango de concentración de MT estudiado, ya que la concentración de MT 0,1 mM condujo a una vida en florero más larga que aplicando MT a la concentración de 1 mM (Publicación IV). Varios estudios indican que la senescencia de las flores podría verse afectada por el contenido endógeno de MT. A pesar de las variaciones entre especies florales, parece que los niveles de MT disminuyen a lo largo del desarrollo, especialmente en las últimas etapas de senescencia (Murch et al., 2009; Zhao et al., 2017). En el presente estudio se demostró que los tratamientos exógenos con MT ejercieron un efecto antisenescente en claveles, aumentando la vida en florero y manteniendo la relación hídrica.

Los claveles se caracterizan por una producción autocatalítica de etileno que acelera los fenómenos de senescencia de las flores afectando también a otros órganos de la planta (Ebrahimzadeh et al., 2008). La producción de etileno fue muy baja en todas las flores estudiadas durante el período de vida útil en florero, sin observarse diferencias significativas atribuidas a los tratamientos con MT. El clavel 'Báltico' mostró un patrón de producción de etileno bajo a nivel basal sin un pico pronunciado de síntesis de etileno y sin aumentos asociados en la tasa de respiración (datos no mostrados). La tasa de respiración fue mayor en las flores control que en las tratadas con MT, coincidiendo también con etapas avanzadas de senescencia alcanzadas antes en los claveles control. De hecho, los niveles de respiración más altos se observaron en los claveles control, mientras que los valores más bajos se obtuvieron para el día 25 tras el tratamiento de MT 0,1 mM (Publicación IV).

El inicio del proceso de senescencia requiere el aporte de energía metabólica y se ha demostrado que los tratamientos poscosecha con MT mejoran la actividad de la ruta de biosíntesis del GABA incrementando el suministro de ATP y el transporte de electrones en las mitocondrias, mejorando el balance energético celular (Aghdam y Fard, 2017; Mohammadi et al., 2021). Puesto que este es el primer estudio con MT en flores, planteamos la hipótesis de que la energía adicional proporcionada por el tratamiento con MT exógena podría mantener el estado energético en los tejidos celulares del clavel 'Báltico' disminuyendo los requerimientos energéticos y la respiración.

Al inicio del experimento, el índice de integridad de las membranas de los petálos fue del 90 % y los valores disminuyeron durante el período de vida en florero para todos los tratamientos evaluados. Se encontraron valores de alrededor del 53 % en las flores tratadas con MT 0,1 mM tras 25 días de almacenamiento, coincidiendo con valores similares para las flores control en el día 15 de este estudio (Publicación IV). Los valores significativamente (p < 0,05) más altos de este parámetro podrían estar asociados con el mantenimiento de la relación de ácidos grasos insaturados/saturados de la membrana, que se ve afectada por las ROS en los pétalos y otros tejidos vegetales (Aghdam et al., 2016a,b). Se ha descrito el papel de la MT en el mantenimiento de la homeostasis redox en diferentes especies vegetales, aumentando la capacidad antioxidante y, por tanto, modulando la reparación de las proteínas dañadas por la oxidación (Aghdam et al., 2019b; Mohamed et al., 2020; Ali et al., 2023). Este efecto previamente demostrado podría ser el responsable de mantener una mayor integridad de los pétalos de las flores tratadas con MT en comparación con los claveles control.

El Contenido Total de Clorofila (CTC) en los sépalos del clavel mostró que el efecto de diferentes concentraciones de MT fue significativo (p < 0,05) a lo largo de la vida en florero de los claveles. El CTC más alto se observó cuando se aplicó la concentración de MT de 0,1 mM después de 15 días de vida en florero, mientras que los sépalos de los claveles control alcanzaron el CTC más bajo en comparación con el resto de las concentraciones de MT para el mismo periodo estudiado (Publicación IV). Los cloroplastos son las principales dianas de las ROS durante diversos tipos de estrés (Khanna-Chopra, 2012). En este sentido, el efecto de la MT en el mantenimiento del contenido de clorofila podría estar relacionado con una mejor relación hídrica a través de una estabilidad de membrana preservada, apoyada por un sistema de mayor actividad antioxidante. Esto es consistente con los estudios de otros autores, quienes observaron un mayor contenido de clorofila e integridad de la membrana con un mayor flujo de electrones fotosintético en otras especies vegetales tratadas con MT (Zhao et al., 2016; Zhao et al., 2017).

Para verificar esta relación, en este estudio se ha examinado el Contenido Total de Polifenoles (CTP) y la AAT en los claveles. Los resultados mostraron un patrón decreciente del CTP a lo largo del periodo de almacenamiento en todas las muestras de pétalos evaluadas. El CTP de los pétalos se mantuvo con valores significativamente superiores (p < 0,05) durante el almacenamiento en todas las flores tratadas con MT, pero especialmente cuando se empleó la solución de 0,1 mM (Publicación IV). De manera similar, se observaron concentraciones fenólicas más altas en diferentes especies de flores cortadas así como una extensión de su vida en florero después de tratamientos con GABA, PAs o AG (Simões et al., 2018; Mohammadi et al., 2020). El CTP más alto en las flores tratadas con MT podría atribuirse a una reducción en la actividad de PPO y a una protección frente a la oxidación de las membranas lipídicas evitando el inicio o la propagación de reacciones en cadena oxidativas, previniendo así el daño de las ROS (Aghdam et al., 2019b).

En este sentido, cuando se evaluó la AAT en las fracciones hidrofílica y lipofílica en los extractos de los pétalos, en general, ambas fracciones mostraron un patrón decreciente durante la vida en florero. Sin embargo, los valores de AAT-H y AAT-L fueron significativamente más altos ($p < 0,05$) cuando se emplearon diferentes soluciones de MT en comparación con las muestras control. Después de 10 días de almacenamiento, la AAT-H disminuyó en los claveles control y con el tratamiento de MT 0,01 mM, mientras que las concentraciones mayores de MT (0,1 mM) mantuvieron un nivel más alto de AAT-H durante un período más prolongado. Se observó un patrón similar cuando se evaluó la AAT-L, los valores fueron mayores cuando se aplicaron diferentes soluciones de MT en comparación con las flores control, aunque la concentración de MT 0,1 mM sólo mantuvo los valores más altos de AAT-L al final del período de vida en florero en comparación con otras concentraciones de MT (Publicación IV).

El ácido cítrico, las vitaminas C y B1 y los compuestos fenólicos son los principales contribuyentes a la AAT-H y los más abundantes en las plantas, mientras que los carotenoides, la vitamina E y los compuestos terpénicos son los principales antioxidantes eliminadores de radicales lipofílicos (Lezoul et al., 2020). La MT estimula el sistema enzimático antioxidante en las flores cortadas y otros tejidos, reduciendo la actividad de las enzimas oxidativas (Zhao et al., 2017; Arnao y Hernández-Ruiz, 2019). Este efecto podría contribuir al incremento del nivel antioxidante encontrado en las flores tratadas con MT.

Teniendo en cuenta los parámetros estudiados y de forma general, la mayor vida útil en florero obtenida cuando se aplicaron soluciones de MT podría atribuirse a un menor metabolismo de la flor y a una mejor relación hídrica en los tejidos florales por el mantenimiento de la estabilidad de la membrana. Además, la mayor actividad antioxidante y el contenido fenólico estimulados por los tratamientos con MT podrían modular el daño oxidativo en los tejidos del clavel aumentando el período de vida útil en florero.

5.2.2. Efecto comparativo entre el 1-metilciclopropeno y la melatonina en kiwi

Además del efecto de la MT sobre los claveles, son varios los estudios que han demostrado el potencial innovador de este compuesto como herramienta poscosecha para mantener la calidad de diferentes frutos. En el siguiente estudio realizado en kiwi, se ha comparado el efecto de la MT con uno de los tratamientos comerciales más efectivos en la conservación de fruta como es el 1-MCP.

Una de las principales causas de la pérdida de calidad en frutos es la pérdida de peso que ocurre durante su almacenamiento. La reducción del peso del fruto esta influenciado por factores como la difusión en fase de vapor, impulsada por la diferencia de presión de vapor entre los compartimentos celulares interiores y exteriores del fruto. Y este proceso también se asocia con el metabolismo del fruto y la pérdida de agua que ocurre a través de los estomas y la cutícula (Bill et al., 2014; Pachón et al., 2022). La pérdida de peso del kiwi aumentó de manera constante y gradual durante el período de almacenamiento. Los tratamientos con MT (6,59 ± 0,22) o 1-MCP (6,73 ± 0,19) fueron igual de efectivos para retrasar la pérdida de peso significativamente (p < 0,05) en comparación con la fruta control (7,69 ± 0,28) después de 28 días de almacenamiento a 2 °C + 5 días a 20 °C. El 1-MCP y la MT retrasaron la pérdida de peso, sin embargo, este efecto se redujo al final del almacenamiento, sin diferencias significativas (p > 0,05) en comparación con el lote control (Publicación V). Los tratamientos con 1-MCP y MT como tratamiento poscosecha retrasaron la pérdida de peso de diferentes productos vegetales como tomate, melocotón, manzana y calabacín (Guillén et al., 2007; Wang et al., 2020; Onik et al., 2021; Ali et al., 2023). Las pérdidas de peso poscosecha son causadas por los procesos de transpiración y respiración del fruto (Watkins, 2006), por lo que las diferencias observadas podrían estar relacionadas con una mayor integridad tisular y una menor respiración en los kiwis tratados con 1-MCP o MT (Valero y Serrano, 2010; Lim et al., 2016; Huan et al., 2020; Jiao et al., 2022).

Debido a la maduración y senescencia de la fruta, la firmeza del kiwi disminuyó durante el almacenamiento en frío, independientemente del tratamiento aplicado. Sin embargo, los tratamientos con 1-MCP o con MT fueron significativamente (p < 0,05) efectivos para retrasar el ablandamiento de la fruta en comparación con la fruta control durante el almacenamiento. Es interesante destacar que la MT, al ser una sustancia de origen natural, mostró resultados similares a los observados para la fruta tratada con 1-MCP en éste parámetro (Publicación V). Estudios similares han demostrado que las aplicaciones poscosecha de 1-MCP retrasaron las pérdidas de peso y mantuvieron la firmeza de la fruta a través de una tasa de respiración retrasada y un proceso de transpiración reducido en diferentes frutas como arándanos (Blaker Y Olmstead, 2014) y peras

(Li et al., 2022b). Además del 1-MCP, los tratamientos con MT han demostrado proporcionar una mejor integridad tisular relacionada con la actividad reducida de las enzimas que degradan la pared celular y de las ROS (Xu et al., 2020; Gong et al., 2020; Cao et al., 2021; Jiao et al., 2022; Xiong et al., 2023).

Los kiwis, una vez cosechados aumentan la tasa de respiración y en este estudio, el aumento de la respiración fue acompañado por un pico máximo a medida que avanzaba la maduración de la fruta, mostrando un patrón climatérico típico. La evolución de la respiración fue controlada significativamente ($p < 0{,}05$) en todos los lotes de fruta tratados con MT y 1-MCP, mostrando una menor producción de respiración durante el almacenamiento en comparación con el lote control. Sin embargo, al inicio de la curva de respiración, el tratamiento con MT tuvo los niveles de respiración más bajos, mientras que el 1-MCP alcanzó estos niveles al final del período de almacenamiento. Por otro lado, la producción de etileno fue muy baja, detectándose sólo en niveles basales, y no mostró diferencias significativas ($p > 0{,}05$) entre tratamientos (Publicación V). La menor respiración observada para el kiwi tratado con 1-MCP podría indicar que este compuesto está retrasando la senescencia a través de un metabolismo reducido de la fruta al unirse competitivamente al receptor de etileno (Lim et al., 2016; Liu et al., 2021a). Asimismo, varios estudios han demostrado que las aplicaciones de MT aumentan la ruta de biosíntesis del GABA, el cual estimula a su vez el contenido de sustratos inmediatos para la respiración, aumentando el estado energético de las células vegetales y conduciendo a un mejor equilibrio metabólico (Aghdam y Fard, 2017; Bhardwaj et al., 2022).

El contenido de SST aumentó gradualmente durante el almacenamiento para todos los tratamientos. En general, la cantidad de SST durante el almacenamiento no se vio afectada significativamente por los tratamientos con MT y 1-MCP. Sin embargo, en los kiwis tratados con MT se retrasó la acumulación de SST, mostrando diferencias significativas ($p < 0{,}05$) hacia el final del almacenamiento respecto a los frutos controles y los tratados con 1-MCP (Publicación V). Nuestros resultados están en consonancia con otros estudios en kiwis en los que se observó un efecto no significativo en el contenido de SST en general cuando se aplicó 1-MCP ($p > 0{,}05$) e incluso se obtuvieron valores más bajos en comparación con los kiwis control cuando se ensayaron tratamientos poscosecha de MT (Huang et al., 2019a; Wang et al., 2019b; Huan et al., 2020). En este sentido, el efecto observado con las aplicaciones de MT en el retraso de la acumulación de SST podría indicar un efecto antisenescente más fuerte que el observado en la fruta tratada con 1-MCP.

Respecto a la AT, se observaron disminuciones graduales a medida que avanzaba el almacenamiento ya que los ácidos orgánicos son sustratos para las reacciones enzimáticas del proceso de respiración (Aghdam et al., 2018). La aplicación precosecha de 1-MCP o MT mostró un efecto significativo (p < 0,05), reduciendo las pérdidas de acidez en comparación con los lotes control excepto para el final del periodo de almacenamiento. La mayor AT observada en los kiwis tratados con 1-MCP o con MT podría estar relacionada con un menor metabolismo de degradación de los ácidos orgánicos ya que el 1-MCP reduce la percepción del etileno, retrasando así la maduración del kiwi (Publicación V). Por otra parte, la MT podría estar incrementando el estado energético celular, siendo a su vez responsable de un mayor mantenimiento de la actividad mitocondrial, retrasando el catabolismo de sustratos primarios como azúcares y ácidos orgánicos, y, por ende, retrasando la maduración y la senescencia (Cao et al., 2021; Bhardwaj et al., 2022).

La clorofila es uno de los principales pigmentos responsables del color de la pulpa del kiwi y se considera un índice importante de la madurez y senescencia del kiwi (Wang et al., 2021). Cuando estudiamos la evolución del contenido de clorofila, se observó una disminución de este parámetro a lo largo del almacenamiento para todos los tratamientos estudiados. Después de 14 días de almacenamiento, el tratamiento con 1-MCP fue eficaz significativamente (p < 0,05) retrasando la pérdida de clorofila (6,18 ± 0,07 mg 100 g^{-1}) en comparación con el lote control (5,39 ± 0,09 mg 100 g^{-1}) y los kiwis tratados con MT (4,95 ± 0,08 mg 100 g^{-1}). Al inicio del experimento la fruta tratada con 1-MCP mostró un efecto positivo en el retraso de la pérdida de clorofila en los kiwis, sin embargo, este efecto se redujo a medida que avanzó el almacenamiento. Respecto a la MT, en general no se observaron diferencias significativas en el contenido de clorofilas en comparación con los kiwis control durante el experimento (Publicación V). Los pigmentos fotosintéticos como las clorofilas son los principales objetivos de las ROS, que aumentan su contenido en los tejidos vegetales a medida que se acelera la senescencia (Khanna-Chopra, 2012). Los tratamientos con 1-MCP mejoraron los niveles de enzimas protectoras (APX, POD, SOD y CAT) contra el daño oxidativo del oxígeno, retrasando la senescencia del fruto y manteniendo la vida útil del kiwi (Zhang et al., 2020). Aunque el contenido de clorofila se ha relacionado con la maduración y el ablandamiento del fruto, en este caso, la degradación de clorofila en el kiwi tratado con MT no coincidió con una mayor pérdida de peso, firmeza o mayor producción de etileno en comparación con el fruto control.

A diferencia de otras frutas, los cambios visibles en el color de la piel del kiwi no ocurren durante su maduración, pero sí se observan en la pulpa. La evaluación del color en el kiwi se expresó como CIE *hue**, que representa la evolución a tonos más oscuros, y por esta razón, este parámetro es útil para evaluar la senescencia de la fruta. Cuanto menor es el CIE *hue**, más oscuro es el tono de la pulpa. Los tratamientos de MT y 1-MCP fueron efectivos para retrasar significativamente (p <

0,05) la evolución del color en comparación con el lote control. Sin embargo, no se encontraron diferencias significativas ($p > 0,05$) entre la fruta tratada con MT y 1-MCP, excepto en el inicio del experimento que se obtuvieron valores más altos de CIE *hue** con el 1-MCP. Aunque el contenido de clorofila indica la evolución del color, en este experimento ambos parámetros no se comportaron de manera similar (Publicación V). Esto puede ser debido a la interferencia de otros pigmentos como los carotenoides que también influyen en el color del kiwi. El contenido de carotenoides mostró una tendencia decreciente durante el inicio del almacenamiento, mientras que el contenido de clorofila permaneció estable y comenzó a disminuir una vez que la maduración del kiwi estaba más avanzada (Fuke et al., 1985). Los principales cambios de color en el kiwi son causados por el pardeamiento enzimático y no enzimático de la degradación de las clorofilas y los carotenoides que disminuyen los valores CIE *hue** e influyen en la integridad de la membrana (Liu et al., 2021b). Por esta razón, el efecto del 1-MCP y la MT en el mantenimiento del contenido de clorofila y los valores CIE *hue** podrían verse afectados por la mejora de la estructura de las membranas y la firmeza de la fruta.

El almacenamiento en frío induce un aumento de las ROS en la fruta, causando daño oxidativo a las membranas celulares y aumentando los niveles de fuga de electrolitos (FE) debido a la pérdida de la integridad celular (Valenzuela et al., 2017). La FE aumentó a lo largo del almacenamiento en frío para todos los tratamientos. Sin embargo, el tratamiento con 1-MCP fue significativamente eficaz ($p < 0,05$) en retrasar este parámetro en comparación con los kiwis control. Aunque también se observó un retraso en la FE en la fruta tratada con MT, las diferencias mostradas en comparación con los frutos control fueron menores (Publicación V). El retraso observado en este parámetro con el 1-MCP y la MT podría ser debido a la mejora del equilibrio oxidativo a través del mantenimiento de la integridad de las membranas (Zhang et al 2020c; Bhardwaj et al., 2022).

Los daños por frío son desórdenes fisiológicos que se manifiestan en la fruta después de ser expuesta a temperaturas subóptimas (Biswas y Brummell, 2019). Aunque el impacto de este daño en el kiwi durante el almacenamiento a 2 °C fue bajo, aparecieron pequeñas manchas translucidas en un pequeño porcentaje de la fruta analizada. Sin embargo, la aplicación de MT y 1-MCP aumentó significativamente la tolerancia al frío ($p < 0,05$) en comparación con la fruta control (Publicación V). Los tratamientos poscosecha con MT y 1-MCP pueden aumentar la tolerancia al frío al movilizar sustratos necesarios para el metabolismo energético y al proteger las membranas celulares con la mejora del sistema antioxidante (Ali et al., 2023; Lv et al., 2023; Liu et al., 2024). En el kiwi, se ha demostrado que tanto los tratamientos con MT (Jiao et al., 2022) como con 1-MCP (Liu et al., 2021a) reducen los síntomas de daño por frío causados por temperaturas subóptimas. Una mayor firmeza de la fruta e integridad de la membrana podrían ser los principales factores que aumentan la tolerancia al frío del kiwi. Se ha comprobado el efecto sinérgico al aplicar de forma combinada MT y 1-MCP en nuestros estudios previos (Medina-Santamarina et al., 2022), sin embargo, esta combinación no mostró efectos positivos en el estudio realizado en esta Tesis Doctoral sobre los kiwis (datos no mostrados).

5.2.3. Efecto de los tratamientos 1-metilciclopropeno y ácido γ-aminobutírico sobre la conservación de aguacate y tomate

La pérdida de agua impacta negativamente en la calidad de los aguacates y tomates, provocando la reducción del peso del fruto, arrugamiento y colapso de la piel, cambios de color y disminución de la calidad organoléptica. Durante el almacenamiento del aguacate, se observó que la pérdida de peso aumentó progresivamente. Sin embargo, tanto el tratamiento con 1-MCP como con GABA lograron retrasar esta pérdida de manera significativa ($p < 0{,}05$) en comparación con los frutos control. Este efecto fue aún más marcado cuando se combinaron ambos tratamientos (GABA + 1-MCP), resultando en una reducción del 4,38 % en la pérdida de peso después de 28 días de almacenamiento en frío, en relación con los frutos control (Publicación VI). En cuanto a los efectos observados en los tomates, estos también experimentaron un incremento en la pérdida de peso durante el almacenamiento. Sin embargo, el tratamiento con GABA no fue efectivo en retrasar esta pérdida de peso de manera significativa ($p > 0{,}05$) en comparación con los frutos control (Publicación VII). Por otro lado, y en coincidencia con el aguacate, el tratamiento con 1-MCP sí logró retrasar la pérdida de peso de los tomates significativamente ($p < 0{,}05$), aunque este efecto fue evidente únicamente al inicio del periodo de consrvación. La mayor reducción en la pérdida de peso de los tomates, al igual que en los aguacates se observó cuando ambos tratamientos, GABA y 1-MCP, se aplicaron de forma simultánea, mostrando un efecto positivo sinérgico con diferencias significativas ($p < 0{,}05$) en comparación con los frutos control (Publicación VI y VII).

El 1-MCP resultó ser efectivo en ambos frutos, aguacate y tomate, en términos de reducción de la pérdida de peso durante el almacenamiento. Este efecto se asocia con el retraso del inicio de la fase climatérica respiratoria, lo que ha sido previamente observado en estudios con estos frutos (Hershkovitz et al., 2005; Guillén et al., 2007). En los tomates, el efecto del 1-MCP además se ha asociado con el mantenimiento de la cutícula externa, reduciendo la pérdida de agua durante el almacenamiento (Wu et al., 2023a). Con respecto a los tratamientos con GABA, sus efectos variaron entre las dos especies vegetales. En el aguacate, la aplicación de GABA ha mostrado una mayor actividad de las enzimas antioxidantes y la acumulación de compuestos bioactivos, lo que disminuye el daño por frío y la permeabilidad de la membrana celular, reduciendo así la pérdida de peso (Wang et al., 2014; Zarei et al., 2022). Estos efectos no fueron observados en el tomate, donde el tratamiento con GABA no logró retrasar la pérdida de peso significativamente ($p > 0,05$), aunque se ha sugerido que el GABA podría incrementar la transpiración sin afectar negativamente la calidad visual del fruto (Nazoori et al., 2020).

La combinación de ambos tratamientos, 1-MCP y GABA, fue la más efectiva en reducir la pérdida de peso tanto en aguacates como en tomates, mostrando un efecto sinérgico ya que el impacto positivo fue mayor que el efecto aditivo de ambos (Publicación VI y VII). Esto es coherente con estudios previos en diversas especies frutales, donde la aplicación de GABA o 1-MCP por separado ha mostrado resultados positivos en términos de conservación de la calidad (Aghdam et al., 2022). La sinergia observada entre ambos compuestos sugiere que la combinación de tecnologías puede ser una estrategia valiosa para extender la vida útil de los frutos y mejorar su calidad durante el almacenamiento a largo plazo.

Una mayor pérdida de peso se relaciona con una menor firmeza puesto que disminuye la turgencia celular. Por esta razón, durante el almacenamiento de los aguacates y de los tomates evaluados, se observó una disminución gradual de la firmeza en los frutos. Los tratamientos con 1-MCP fueron capaces de mantener niveles significativamente ($p < 0,05$) más altos de firmeza en comparación con los frutos control durante los primeros 14 días de almacenamiento en frío más 7 días a 20 ºC para ambas especies vegetales. Aunque el tratamiento con GABA, aplicado de manera individual, no mostró diferencias significativas ($p > 0,05$) en comparación con los frutos control en los aguacates y tomates, la combinación de 1-MCP y GABA resultó en valores de firmeza superiores, evidenciando un efecto sinérgico. Por ello, tanto en aguacates como en tomates, esta sinergia entre ambos tratamientos proporcionó una mayor firmeza a lo largo del almacenamiento en frío, en comparación con los tratamientos individuales o con los frutos control. Al relacionar ambos estudios, se puede destacar que el 1-MCP fue efectivo en todos los frutos, retrasando el ablandamiento, mientras que el GABA sólo mostró un impacto positivo cuando se aplicó en combinación con 1-MCP (Publicación VI y VII).

La capacidad del 1-MCP para mantener la firmeza en tomates y aguacates se relaciona con la regulación de la producción de etileno y la preservación de la estructura de las paredes celulares (Hershkovitz et al., 2005; Wu et al., 2023a). En ambos frutos, la pérdida de firmeza además de asociarse a las pérdidas de peso se ha relacionado con las actividades enzimáticas implicadas en el ablandamiento. Así, en estudios previos se ha demostrado la capacidad del 1-MCP para inhibir la acción de enzimas como la poligalacturonasa y pectinasa, las cuales participan en la degradación de las paredes celulares durante la maduración del fruto (Hershkovitz et al., 2005; Dias et al., 2021). Además, la producción de etileno y la tasa de respiración son factores clave que promueven el ablandamiento del fruto, lo que se ha correlacionado con una mayor actividad de poligalacturonasa y, por tanto, una mayor degradación de las paredes celulares (Jeong et al., 2004; Mostofi et al., 2003; Choi et al., 2008). En este sentido el 1-MCP al bloquear la acción del etileno retrasa el metabolismo del fruto reduciendo la actividad de estas enzimas (Watkins, 2006).

Por otro lado, el tratamiento con GABA no tuvo un impacto significativo (p > 0,05) sobre la firmeza de los frutos cuando se aplicó de forma individual, tanto en aguacates como en tomates (Publicación VI y VII), lo que coincide con los resultados de estudios previos en realizados peras y manzanas (Yu et al., 2014; Al Shoffe et al., 2021). Sin embargo, en otros frutos como el mango, el GABA sí ha mostrado una mayor efectividad para mantener la firmeza en comparación con los frutos control (Rastegar et al., 2020). Este contraste sugiere que los efectos del GABA pueden variar según la especie y las condiciones de almacenamiento, activando mecanismos como la vía de biosíntesis del GABA para mantener el metabolismo celular en condiciones de estrés (Aghdam y Fard, 2017). Por otro lado, es posible que el mantenimiento celular a nivel estructural por parte del 1-MCP pueda estar permitiendo una óptima acción del GABA, mejorando el balance antioxidante y metabólico, pudiendo retrasar así la maduración de estos frutos.

En relación con otros aspectos metabólicos, se observó que tanto los aguacates como los tomates incrementaron la respiración y la producción de etileno durante el almacenamiento poscosecha, como es de esperar en estos frutos climatéricos. En el caso del aguacate, el aumento en la producción de CO_2 se registró tras 14 días de almacenamiento, pero el tratamiento con 1-MCP redujo significativamente (p < 0,05) esta respiración en comparación con los demás tratamientos. Sin embargo, el 1-MCP en los tomates redujo la respiración durante todo el experimento. Nuevamente en este parámetro el GABA por sí sólo no redujo significativamente (p > 0,05) la tasa de respiración, sólo al final del experimento tanto en aguacates como en tomates. No obstante, en general, su combinación con 1-MCP mostró los valores medios de respiración más bajos en ambas especies vegetales a lo largo del experimento (Publicación VI y VII).

La producción de etileno fue retrasada tanto en los aguacates como en los tomates con el 1-MCP. En el caso de los aguacates tratados con 1-MCP, se consiguió retrasar el pico climatérico hasta 14 días de almacenamiento, mientras que en los frutos control y tratados solo con GABA, este pico se observó al día 7. Aunque el 1-MCP retrasó el pico de producción de etileno, no lo redujo cuantitativamente. Por el contrario, el GABA, aplicado solo, redujo significativamente ($p < 0,05$) la producción de etileno en los primeros días de almacenamiento (Publicación VI). Los tomates tratados sólo con GABA también mostraron una reducción en los niveles de etileno después de 7 y 21 días de almacenamiento en frío más 7 días a 20 ºC. De forma adicional, la combinación de ambos tratamientos aplicados simultáneamente no mejoró el efecto del 1-MCP en el aguacate, aunque sí lo hizo en el caso del tomate de forma significativa ($p < 0,05$) (Publicación VI y VII).

El tratamiento con 1-MCP redujo significativamente la tasa de respiración tanto en aguacates como en tomates, debido a su acción al bloquear los receptores de etileno, lo que desacelera los procesos fisiológicos relacionados con la maduración (Olivares et al., 2020). Este efecto ya ha sido documentado en estudios previos, donde el 1-MCP inhibió la producción de etileno y retrasó la respiración en frutos climatéricos (Hershkovitz et al., 2005; Guillén et al., 2006). En aguacates, el retraso en la tasa de respiración y la producción de etileno inducido por el 1-MCP coincide con estudios que han demostrado un pico climatérico retrasado de etileno en frutos tratados con 1-MCP (Jeong et al., 2002; Hershkovitz et al., 2005; Zhang et al., 2011). En tomates, este efecto también ha sido ampliamente estudiado, con una reducción significativa de la producción de etileno y la actividad de la enzima 1-aminociclopropano-1-carboxilato sintasa (ACC), una enzima clave en la síntesis de etileno (Zhang et al., 2009; Hoeberichts et al., 2002; Opiyo y Ying, 2005).

En cuanto al tratamiento con GABA, aunque no mostró efectos significativos ($p > 0,05$) sobre la tasa de respiración cuando se aplicó solo, su combinación con 1-MCP reveló una sinergia importante en aguacates y tomates. De hecho, redujo de forma más efectiva la tasa de respiración en ambas especies vegetales, aunque la producción de etileno sólo en los tomates. Esto sugiere que el GABA podría potenciar el efecto del 1-MCP en la poscosecha, posiblemente debido a su acción sobre el ciclo de los ácidos tricarboxílicos o ciclo de Krebs. De esta forma, el GABA contribuye a mantener el estado energético de las células, especialmente bajo condiciones de almacenamiento en frío (Aghdam et al., 2018). En este sentido, la aplicación exógena de GABA ha demostrado estimular esta vía, manteniendo la producción de ATP en condiciones de estrés (Shan et al., 2022), lo cual es crucial para reducir el impacto del frío en los frutos. Este aspecto ha de ser tenido en cuenta en estos dos estudios, ya que tanto los aguacates como los tomates fueron sometidos a temperaturas subóptimas durante un largo periodo. Además, la reducción en la producción de etileno observada en los frutos tratados con GABA coincidió con estudios previos en los que el GABA tuvo el mismo efecto en otros frutos como las manzanas (Han et al., 2018). De hecho, son diversas las especies vegetales en las que el GABA redujo la acumulación de intermediarios metabólicos como el ACC, disminuyendo la biosíntesis de etileno (Uluışık, 2021; Li et al., 2021d).

Sin embargo, también existen investigaciones en algunas especies como los girasoles que indican que el GABA puede estimular la síntesis de etileno mediante la regulación de los niveles de transcripción de la ACC sintasa (Kathiresan et al., 1997). Esto evidencia la variabilidad del papel del GABA en la regulación del etileno entre diferentes especies vegetales, lo que resalta la complejidad de su interacción con las vías de señalización del etileno.

Nuevamente en estos parámetros se observa una reducción adicional cuando se aplican el 1-MCP y el GABA como combinación de tratamientos. La capacidad del 1-MCP para bloquear los receptores de etileno y el papel del GABA en el mantenimiento del metabolismo celular bajo condiciones de estrés sugieren que, juntos, pueden mitigar de manera más efectiva los efectos adversos del almacenamiento prolongado a bajas temperaturas, como se ha demostrado con nuestros resultados.

Con respecto al nivel de SST en aguacate y en tomates, se observó un aumento gradual de este parámetro al comienzo del experimento. Sin embargo, a medida que los aguacates continuaron madurando, se observó una disminución de los SST hacia el final del almacenamiento mientras que en los tomates este parámetro siguió incrementando. El efecto de los tratamientos aplicados tanto en los aguacates como tomates mostraron un patrón similar. Todos los tratamientos que llevaban GABA, aplicado como un único tratamiento o como combinación con 1-MCP mostraron una acumulación mayor de SST tanto en aguacates como en tomates. De hecho, los valores con mayores SST se observaron en los frutos tratados con GABA únicamente. Sin embargo, cuando se aplicó únicamente 1-MCP se observaron en general los valores medios más bajos registrados en ambas especies vegetales (Publicación VI y VII).

En cuanto a la AT, en los aguacates y en los tomates también siguió un patrón similar dependiendo del tratamiento aplicado. Pese a que la evolución de este parámetro fue descendente durante el almacenamiento, los valores más bajos en general se observaron en los frutos control, y siendo significativamente (p < 0,05) inferiores que los observados en los frutos tratados con GABA únicamente. Sin embargo, los frutos tratados con 1-MCP únicamente o combinados con GABA mostraron los niveles más altos de AT, siendo significativamente (p < 0,05) superiores en los frutos expuestos a ambas sustancias (1-MCP + GABA). Este hecho indicó un retraso más efectivo en el metabolismo cuando ambas sustancias son aplicadas conjuntamente (Publicación VI y VII).

En los aguacates y tomates, el incremento inicial y la posterior disminución de los SST hacia el final del almacenamiento se atribuye al proceso de maduración, donde los azúcares son metabolizados o convertidos en otros compuestos (Guillén et al., 2007; Meyer y Terry, 2010; Vázquez-López et al., 2022). El efecto del 1-MCP sobre el contenido de los SST ha sido ampliamente estudiado en frutas y hortalizas. El 1-MCP al retrasar el metabolismo celular, reduce la despolimerización del almidón en los frutos climatéricos contribuyendo a la menor acumulación de SST (Watkins, 2006). Los frutos tratados con GABA mostraron niveles más altos de SST en comparación con los tratados sólo con 1-MCP. Este hecho podría atribuirse a que el GABA actúa estimulando la síntesis de sustratos metabólicos mejorando la producción de ATP, lo que reduce el catabolismo de los azúcares (Aghdam et al., 2017) que experimentarían una acumulación. Este comportamiento fue similar en los tomates donde el tratamiento con GABA, sólo o en combinación con 1-MCP, mostró niveles más altos de SST que los frutos control y los tratados sólo con 1-MCP (Publicación VI y VII). Sin embargo, la verificación de esta hipótesis precisa de futuras investigaciones.

Asimismo, en frutos climatéricos el consumo de los ácidos orgánicos como sustratos durante la respiración provoca el descenso de la AT. Los frutos tratados con 1-MCP, tanto en aguacates como en tomates, mantuvieron niveles más altos de AT debido a la reducción de la respiración y el metabolismo inducido por la inhibición de los receptores de etileno, lo que retrasa la descomposición de los ácidos orgánicos (Wu et al., 2020; Guillén et al., 2006; 2007; Wang et al., 2010). La capacidad del GABA para preservar la acidez también se ha documentado en otras especies frutales, como la naranja sanguina, donde inhibió la pérdida de acidez intracelular regulando el metabolismo de los ácidos orgánicos (Habibi et al., 2019). Este efecto fue más evidente cuando el 1-MCP se combinó con GABA, ya que ambos compuestos juntos proporcionaron una mayor estabilidad metabólica. Estoy podría explicarse por los efectos sinérgicos de ambos tratamientos, como ya se ha observado en parámetros previos. El 1-MCP redujo la producción de etileno y la respiración, lo que ralentizó el uso de los ácidos orgánicos como sustratos respiratorios. Por su parte, el GABA puedo mantener el equilibrio energético de las células bajo condiciones de almacenamiento en frío, evitando la descomposición rápida de los ácidos y manteniendo los niveles de acidez (Niazi et al., 2021). La combinación de estos dos tratamientos fue la más efectiva en la conservación de la acidez para ambas especies, proporcionando un efecto aditivo que contribuyó a retrasar la maduración y mantener la calidad poscosecha (Publicación VI y VII).

El cambio de color en los aguacates 'Hass' durante la maduración ocurre inicialmente debido a una reducción en la concentración de clorofila, seguido por el aumento en la presencia de la antocianina cianidina 3-O-glucósido. En los tomates ocurre un proceso similar, pero además de la acumulación de polifenoles, se produce una acumulación significativa de carotenoides, que es la que contribuye principalmente al desarrollo del color rojo característico.

En ambos frutos, los valores del parámetro CIE *L**, que mide la luminosidad del fruto, disminuyeron durante el almacenamiento, reflejando el oscurecimiento de la piel asociada a la maduración. Sin embargo, los tratamientos con GABA y 1-MCP, tanto individualmente como en combinación, retrasaron esta pérdida de luminosidad. En los aguacates, los frutos control mostraron los valores más bajos de CIE *L**, mientras que aquellos tratados con GABA o 1-MCP mantuvieron niveles de luminosidad más altos durante el almacenamiento. Del mismo modo, en los tomates, los frutos tratados con la combinación de GABA y 1-MCP mostraron valores significativamente más altos (p < 0,05) de CIE *L** en comparación con los frutos control y tratados sólo con GABA o 1-MCP. Este comportamiento sugirió un efecto positivo de ambos tratamientos a la hora de preservar el brillo de la piel en ambos frutos (Publicación VI y VII). La reducción del color CIE *L** está relacionada con desarrollo del color rojo durante la maduración (Tilahum et al., 2017) y con las pérdidas de peso del fruto (Nunes, 2015). Por tanto, una evolución menor de este parámetro estaría indicando un retraso en la maduración de los frutos.

En los aguacates, la pérdida de luminosidad fue más rápida en los frutos control, mientras que los frutos tratados con GABA y 1-MCP lograron retrasar esta pérdida de brillo. Del mismo modo, en los tomates, el tratamiento combinado de GABA y 1-MCP mostró resultados similares al retrasar la pérdida de luminosidad, lo cual se asocia con una mejor conservación de la estructura de la piel, probablemente relacionada con una menor pérdida de agua (Petit et al., 2014). Este patrón similar en ambos frutos sugiere que tanto GABA como 1-MCP, solos o en combinación, ayudan a ralentizar la degradación de los pigmentos y la pérdida de brillo superficial, lo que implica un retraso general en la maduración (Ashton et al., 2006; Nunes, 2015).

En cuanto al color CIE *Croma** en los aguacates, que mide la intensidad del color, los frutos tratados con 1-MCP y la combinación de GABA y 1-MCP presentaron valores más altos en comparación con los frutos control. Esto sugiere un retraso en la evolución del color asociado con la maduración. Por otro lado, en los tomates, el parámetro CIE *hue**, que mide el tono del color, mostró una rápida disminución en los frutos control y tratados solo con GABA, sin diferencias significativas (p > 0,05) entre estos grupos. De igual forma, la combinación de 1-MCP con GABA no mostró un efecto sinérgico ni aditivo en el parámetro CIE *hue** en comparación con los valores observados aplicando únicamente 1-MCP, el cual mantuvo el mayor retraso de este parámetro (Publicación VI y VII).

En cuanto a la intensidad del color (CIE *Croma**), el tratamiento con 1-MCP fue muy efectivo en los aguacates, donde el retraso en la evolución del color está asociado a la disminución de la actividad de la clorofilasa y la producción de etileno, que regulan el cambio de color en los frutos climatéricos (Homez-Jara et al., 2023). Aunque no se ha evaluado previamente el efecto de GABA en el color de los aguacates, los resultados sugieren que este tratamiento podría estar contribuyendo al retraso del cambio de color, al igual que se ha observado en otros frutos como los cítricos (Al Shoffe et al., 2021).

En los tomates, el parámetro CIE *hue** mostró una rápida disminución en los frutos control y tratados solo con GABA, lo que indica una evolución más rápida hacia el color rojo de maduración. Esto concuerda con estudios previos en los que el GABA no fue efectivo en retrasar la evolución del color en frutos como mangos y fresas (Rastegar et al., 2020; Zhang et al., 2024). La combinación de 1-MCP y GABA no mostró un efecto sinérgico en este parámetro, lo que sugirió que, en tomates, el 1-MCP sigue siendo el principal responsable del retraso en la degradación de los pigmentos y la preservación del color, mientras que el GABA podría estar actuando de manera secundaria en el mantenimiento de la estructura celular (Watkins, 2006).

En resumen, tanto en los aguacates como en los tomates, la combinación de GABA y 1-MCP fue la más efectiva para retrasar la pérdida de luminosidad y mantener el color, lo que sugiere un efecto positivo en la conservación poscosecha de estos frutos. Aunque el GABA no mostró un impacto significativo (p > 0,05) en la evolución del color en los tomates, su combinación con 1-MCP mejoró los resultados en términos de retención del brillo y retraso de la maduración. Esto pone de manifiesto la importancia de la interacción entre ambos tratamientos con sus diferentes propiedades en la preservación de la calidad del fruto (Al Shoffe et al., 2021; Watkins, 2006; Homez-Jara et al., 2023).

El color de estos frutos está directamente influenciado por la degradación de la clorofila y la biosíntesis de distintos pigmentos (Horváth-Mezőfi et al., 2024). En este sentido, tanto en aguacates como en tomates, el tratamiento con 1-MCP mostró una alta efectividad en retrasar la degradación de las clorofilas, comparado con las aplicaciones de GABA y los frutos control, aunque hacia el final del estudio las diferencias fueron reduciéndose (Publicación VI y VII). La efectividad del 1-MCP fue mejorada cuando se combinó con tratamientos con GABA pese a que esta sustancia por sí sola no mostró en general diferencias con respecto a los frutos control en tomates y aguacates. El retraso en la degradación de la clorofila en los lotes tratados con 1-MCP está directamente relacionado con la reducción en la producción de etileno en los frutos tratados (Watkins, 2006). El incremento de efectividad de este compuesto combinado con GABA, podría estar vinculado a la mejora en la actividad antioxidante como veremos a continuación manteniendo las membranas celulares, concretamente las de los cloroplastos (Barbarie et al., 2019; Saeedi et al., 2022).

En tomates, los polifenoles, flavonoides y carotenoides son en general los compuestos bioactivos con actividad antioxidante más importante en los frutos estudiados. Todos estos compuestos antioxidantes incrementaron a lo largo del almacenamiento de los tomates. Sin embargo, la acumulación de todos ellos fue retrasada cuando se aplicó únicamente 1-MCP, reduciendo significativamente ($p <$ 0,05) los niveles de estas sustancias con respecto a los frutos control (Publicación VII). Por otro lado, los tratamientos con GABA acumularon polifenoles totales y carotenoides de forma similar al control, es decir rápidamente. Aunque la combinación de tratamientos (1-MCP + GABA) no mostró diferencias con respecto al contenido en carotenoides de los frutos tratados sólo con 1-MCP, estimuló de forma sinérgica la acumulación de flavonoides, y contrarrestó el efecto inhibidor del 1-MCP en la biosíntesis de fenoles totales. Este efecto pudo estar relacionado con la acción del GABA en la activación de la ruta de los fenilpropanoides, que contrarrestan el efecto inhibidor del 1-MCP en la acumulación de compuestos bioactivos (Moradi et al., 2020; Aghdam et al., 2022). De forma similar, Shenglong et al. (2019) aplicaron un tratamiento combinando 1-MCP con un monoterpenoide con propiedades antioxidantes como el citral, observando un efecto positivo y sinérgico al incrementar el contenido en fenoles y flavonoides totales durante el almacenamiento de los frutos.

El incremento en los compuestos fenólicos pudo ser una de las razones por las que se observaron los mayores niveles de AAT-H y AAT-L en los tomates tratados con ambos compuestos de forma simultánea. La AAT-H aumentó durante el almacenamiento mientras que la AAT-L descendió ligeramente. En ambos parámetros, los frutos tratados con GABA solo o en combinación con 1-MCP mostraron los valores de AAT más altos en comparación con los frutos control y tratados únicamente con 1-MCP (Publicación VII). Este efecto puede ser debido a que el GABA tiene actividad antioxidante y estimula el metabolismo secundario, aumentando la acumulación de compuestos bioactivos como los compuestos fenólicos (Aghdam et al., 2022). Otros autores han observado que los tratamientos con GABA mejoraron la capacidad antioxidante en uvas y mangos al mitigar el aumento de las ROS (Rastegar et al., 2020; Shelp et al., 2021; Asgarian et al., 2022). El efecto sinérgico combinando el 1-MCP y el GABA podría estar asociado con los efectos aditivos demostrados para ambas sustancias en el mantenimiento de las paredes y de las membranas celulares respectivamente (Watkins, 2006; Aghdam et al., 2016c).

Por ello, tanto en tomates como en aguacates se estudió el efecto del GABA y el 1-MCP sobre la integridad de las membranas celulares, puesto que, en respuesta al estrés, se produce una secuencia de reacciones metabólicas oxidativas que conducen a cambios en la permeabilidad de la membrana. Estos cambios provocan disfunciones celulares, incluido el movimiento no regulado de electrolitos a través de las membranas dañadas (Valenzuela et al., 2017; Liang et al., 2020).

En los aguacates y tomates, la permeabilidad de la membrana fue evaluada en función de la salida de electrolitos. En ambas especies vegetales, el tratamiento con 1-MCP y GABA mostró un efecto positivo al reducir esta fuga de electrolitos, lo que indicó una mayor integridad de la membrana. De forma similar a los aguacates, los tratamientos individuales de GABA y 1-MCP disminuyeron significativamente la permeabilidad de las membranas en tomate en comparación con los frutos control. En ambas especies vegetales, el mayor retraso de este parámetro se observó con el tratamiento combinado (1-MCP + GABA). Con el tratamiento combinado se observaron los valores más bajos de fuga de electrolitos en los tomates (15,55 ± 0,21 %) al final del almacenamiento en frío, indicando el efecto sinérgico en la protección de la integridad de la membrana. Tanto el 1-MCP como el GABA fueron efectivos y tuvieron efectos similares cuando se aplicaron por separado en ambas especies vegetales. De forma adicional, hacia el final de ambos periodos de almacenamiento, la aplicación combinada (1-MCP + GABA) mostró el doble de efectividad reduciendo la fuga de electrolitos con respecto a la aplicación individual (Publicaciones VI y VII). Estos efectos podrían relacionarse con los niveles de MDA, ya que el nivel de esta sustancia refleja la peroxidación lipídica de las membranas plasmáticas, lo cual afecta directamente a la integridad estructural de los tejidos vegetales (Medina-Santamarina et al., 2022).

En este sentido, el contenido de MDA fue evaluado en los tomates de esta Tesis Doctoral, confirmando la efectividad en el mantenimiento de las membranas. Los niveles de MDA al final del periodo de almacenamiento fueron más bajos especialmente cuando se aplicaron 1-MCP y GABA como combinación de tratamientos. El efecto de ambas sustancias aplicadas individualmente también controló la acumulación de MDA en comparación con los tomates controles, especialmente en los lotes tratados con 1-MCP (Publicación VII).

El incremento de la fuga de electrolitos y el contenido en MDA son fenómenos frecuentemente observados y asociados al daño por frío ocurrido en los productos vegetales (Zhang et al., 2021a). Este hecho se manifestó tanto en los aguacates como en los tomates tratados con 1-MCP y GABA, aplicados de forma separada o conjunta. Los aguacates control presentaron los niveles más altos de daño por frío. En aguacate, las aplicaciones individuales con 1-MCP o con GABA redujeron los síntomas de este parámetro un 42,81 % y 20,31 %, respectivamente, en comparación al observado en los frutos control al final del periodo de almacenamiento. Además, se observó un efecto beneficioso adicional al aplicar ambos tratamientos conjuntamente reduciendo el daño por frío hasta un 61,56 % en los aguacates (Publicación VI). De igual forma, los tomates tratados con la combinación de ambas tecnologías (1-MCP + GABA) consiguieron reducir los daños por frío un 31,20 % con respecto a los frutos control. En los tomates, únicamente las aplicaciones con 1-MCP solas o en combinación con GABA resultaron efectivas en la reducción de los daños por frío durante el almacenamiento. Sin embargo, aunque este parámetro no fue retrasado por el GABA en tomates, este compuesto si redujo de forma significativa (p < 0,05) la incidencia de la pudrición en los tomates, aunque sólo tras dos semanas de almacenamiento refrigerado más una semana a 20 ºC (Publicación VII).

El aumento de la permeabilidad de membrana y el daño por frío son fenómenos asociados a la producción de ROS y a la consecuente disfunción celular (Valenzuela et al., 2017; Liang et al., 2020). El tratamiento con 1-MCP mostró una eficacia significativa en la reducción del daño por frío y de la integridad de las membranas en ambos frutos al retrasar la producción de etileno, lo que ralentiza el metabolismo y la respiración (Publicaciones VI y VII), manteniendo así la permeabilidad de las membranas (Wu et al., 2020; Zhang et al., 2021a). Por otro lado, el GABA parece desempeñar un rol protector en la integridad de la membrana al inducir la expresión de enzimas antioxidantes. También modula las vías de producción de energía y proporciona estabilidad antioxidante reduciendo así la peroxidación lipídica (Aghdam et al., 2018; Habibi et al., 2019).

Se ha observado un efecto sinérgico entre el 1-MCP y el GABA en la reducción de la desintegración de las membranas, acumulación de MDA y expresión de los daños por frío. Este efecto sugiere que ambas tecnologías poscosecha son capaces de actuar de forma conjunta mejorando la tolerancia a las bajas temperaturas y preservando así la calidad poscosecha. La combinación de ambos tratamientos, al reducir el MDA y mantener un equilibrio antioxidante óptimo, proporciona una mayor estabilidad celular y disminuye los daños estructurales como se ha observado de forma individual en otros estudios (Carrión-Antolí et al., 2024; Watkins, 2006). Así, en consonancia con estudios previos (Palma et al., 2019; Shekari et al., 2021), las propiedades particulares de ambas tecnologías interactuando combinadas de forma aditiva y sinérgica serían las responsables del mayor efecto al reducir los daños por frío, y su impacto durante el almacenamiento a bajas temperaturas.

6. CONCLUSIONES/CONCLUSIONS

6. CONCLUSIONES

En la presente Tesis Doctoral se han desarrollado diferentes estrategias con elicitores en precosecha en cerezas y en poscosecha de manera individual o combinadas con 1-metilciclopropeno para aumentar la calidad y la vida útil de claveles, kiwis, aguacates y tomates. Estos compuestos de origen natural podrían considerarse herramientas seguras y sostenibles para la conservación de los productos vegetales estudiados. A continuación, se exponen las conclusiones generales de las investigaciones realizadas:

I. Los estudios en precosecha realizados en cereza con putrescina, melatonina y jasmonato de metilo han demostrado la eficacia de estos compuestos en la reducción del agrietado de los frutos durante su maduración en el árbol. Se ha encontrado variabilidad de tolerancia al agrietado entre variedades, ciclos productivos, etapas de maduración del fruto y concentración del compuesto utilizado. Sin embargo, en todos los casos, la aplicación de estos elicitores redujo de forma significativa el agrietado durante el desarrollo en el árbol y en el momento de la cosecha. Putrescina y melatonina también han sido eficaces en la mejora de la resiliencia de las cerezas de la variedad 'Prime Giant' y 'Sweetheart' frente a las heladas primaverales. Como consecuencia, se redujo la acumulación de malondialdehído en los botones florales, lo que resultó en un aumento del cuajado de las cerezas. De este modo, estos tratamientos podrían aumentar la producción de este fruto. Sin embargo, la efectividad de los tratamientos fue menor cuanto mayor fue la gravedad de la helada a la que estuvieron expuestos los tejidos florales. Además, los tratamientos precosecha retrasaron la maduración de los frutos en el árbol en mayor o menor medida, lo que resultó en una mayor firmeza y un retraso en el desarrollo del color, los sólidos solubles y la acidez en comparación con la fruta control en el momento de la cosecha. Por ello, estos tratamientos constituyen estrategias prometedoras para adaptar los frutos al cambio climático y mitigar el estrés producido por factores abióticos, reduciendo las pérdidas económicas debidas al menor rendimiento del cultivo.

II. En clavel 'Báltico', se obtuvo una mayor vida en florero cuando se aplicaron soluciones de melatonina, lo cual se atribuyó a un menor metabolismo de la flor, una mejor relación hídrica en los tejidos florales y el mantenimiento de la estabilidad de la membrana. Además, se produjo una estimulación de la actividad antioxidante y el contenido fenólico que podría modular el daño oxidativo en los tejidos del clavel aumentando el período de vida útil. Considerando el coste de la melatonina y la absorción de la solución de florero, este tratamiento poscosecha podría ser de interés comercial ya que aumenta la vida útil hasta 10 días más cuando se usa la concentración intermedia de 0,1 mM. Por esta razón, la melatonina como tratamiento poscosecha podría ser una herramienta útil para retrasar la senescencia del clavel, aumentando el tiempo en el que conserva su valor ornamental y permitiendo así tiempos más extensos para su transporte y exportación.

III. Los tratamientos poscosecha con melatonina 0,1 mM en kiwis 'Hayward' demostraron una eficacia similar en comparación con el 1-metilciclopropeno a la concentración de 0,5 μL L^{-1}. Ambos retrasaron la maduración, disminuyeron las pérdidas de peso y la respiración y mantuvieron una mayor firmeza y acidez en el fruto. El 1-metilciclopropeno fue más efectivo para mantener el color de la pulpa y el contenido total de clorofila en comparación con los frutos tratados con melatonina. Sin embargo, los síntomas de daño por frío en los kiwis fueron menores cuando se aplicaron los tratamientos con melatonina, aunque la reducción de la fuga de electrolitos mostró valores inferiores con el 1-metilciclopropeno. Por tanto, los tratamientos poscosecha alternativos basados en soluciones de melatonina podrían considerarse en general tan efectivos como las aplicaciones con 1-metilciclopropeno.

IV. Las aplicaciones combinadas de 1-metilciclopropeno y ácido γ-aminobutírico mostraron la capacidad de aumentar la calidad general y reducir los daños por frío, extendiendo así la vida útil de los aguacates 'Hass' (1-metilciclopropeno 0,3 μL L^{-1} + ácido γ-aminobutírico 1 mM) y los tomates 'Conquista' (1-metilciclopropeno 0,5 μL L^{-1} + ácido γ-aminobutírico 10 mM). Los mejores resultados en el retraso de la maduración tanto en aguacates como en tomates se obtuvieron cuando se aplicó la combinación de ambas tecnologías para todos los parámetros estudiados. El 1-metilciclopropeno actuó principalmente reduciendo la actividad metabólica, lo que ayudó a preservar la estructura celular y a retrasar los procesos de maduración. Por otro lado, el ácido γ-aminobutírico, además de contribuir al equilibrio energético, su carácter antioxidante mantuvo la fluidez de las membranas por más tiempo, contribuyendo a la reducción de los daños por frío. La aplicación combinada de estos compuestos podría considerarse una estrategia eficaz de gestión poscosecha para los aguacates y los tomates. Este enfoque ofrecería a los productores una alternativa viable para optimizar la calidad de la fruta. A su vez, ofrece alternativas de consumo sostenibles para los consumidores, ya que estas observaciones podrían contribuir a la reducción de sustancias de origen artificial al ser combinadas con sustancias de origen natural como el ácido γ-aminobutírico.

6. CONCLUSIONS

In this Doctoral Thesis, various strategies using elicitors have been developed for preharvest applications in sweet cherries and postharvest treatments, either individually or combined with 1-methylcyclopropene, to enhance the quality and shelf life of carnations, kiwifruits, avocados, and tomatoes. These naturally derived compounds could be considered safe and sustainable tools for preserving the studied plant products. The general conclusions of the research are presented below:

I. Preharvest studies conducted on cherries using putrescine, melatonin, and methyl jasmonate demonstrated the effectiveness of these compounds in reducing fruit cracking during on-tree ripening. Variability in cracking tolerance was observed among cultivars, production cycles, ripening stages, and compound concentrations. However, in all cases, these elicitors significantly reduced cracking during on-tree development and harvest. Putrescine and melatonin enhanced resilience to spring frosts in 'Prime Giant' and 'Sweetheart' sweet cherry. Consequently, malondialdehyde accumulation was reduced in floral buds, resulting in increased sweet cherry set and potentially increasing fruit yield. However, the effectiveness of the treatments decreased with the severity of the frost exposure on floral tissues. Additionally, the preharvest treatments delayed fruit ripening on the tree to a greater or lesser degree, leading to greater firmness and a delay in the development of colour, soluble solids, and total acidity compared to the control fruit at harvest. Therefore, these treatments constitute promising strategies to adapt fruit to climate change and mitigate stress from abiotic factors, reducing economic losses due to lower crop yields.

II. In carnation 'Baltico', the extended vase life obtained with melatonin solutions is attributed to a decrease in flower metabolism, enhanced water balance in floral tissues, and stabilized cell membranes. Moreover, melatonin stimulates antioxidant activity and phenolic content, potentially modulating oxidative degradation in carnation tissues and extending shelf life. Given melatonin’s cost-effectiveness and its absorption in vase solutions, this postharvest approach can extend vase life by as much as 10 days when used at a moderate concentration of 0.1 mM. Thus, melatonin as a postharvest treatment is a promising method to decelerate senescence in carnations, extending their ornamental value and offering longer transport and export times.

III. Postharvest treatments with 0.1 mM melatonin in 'Hayward' kiwis demonstrated similar effectiveness to 1-methylcyclopropene at a concentration of 0.5 µL L^{-1}. Both treatments delayed ripening, reduced weight loss and respiration, and maintained greater fruit firmness and acidity. 1-methylcyclopropene was more effective in maintaining pulp colour and chlorophyll content than melatonin-treated fruits. However, chilling injury symptoms in kiwis were reduced with melatonin treatments, although the reduction in electrolyte leakage was lower with 1-MCP. Thus, alternative postharvest treatments based on melatonin solutions could be considered generally as effective as 1-methylcyclopropene applications.

IV. The combined application of 1-methylcyclopropene and γ-aminobutyric acid demonstrated the ability to enhance overall quality and reduce cold damage, thereby extending the shelf life of ‘Hass’ avocados (1-methylcyclopropene 0.3 µL L^{-1} + γ-aminobutyric acid 1 mM) and ‘Conquista’ tomatoes (1-methylcyclopropene 0.5 µL L^{-1} + γ-aminobutyric acid 10 mM). The best results across all studied parameters were obtained with the combination of both technologies, proving key to delaying ripening in both avocados and tomatoes. The 1-methylcyclopropene primarily reduced metabolic activity, which helped preserve cell structure and delay ripening processes. On the other hand, γ-aminobutyric acid, in addition to contributing to energy balance, maintained membrane fluidity longer due to its antioxidant properties, aiding in the reduction of chilling injury. Based on these observations, combining 1-methylcyclopropene and γ-aminobutyric acid could be considered an effective postharvest management strategy for avocados and tomatoes. This approach would provide producers with a viable alternative to optimize fruit quality. At the same time, it offers sustainable consumption alternatives for consumers, as these findings could contribute to reducing the use of artificial substances by combining them with natural compounds such as γ-aminobutyric acid.

7

FUTURAS LÍNEAS DE INVESTIGACIÓN

7. FUTURAS LÍNEAS DE INVESTIGACIÓN

Con el análisis de los resultados obtenidos en esta Tesis Doctoral surge la necesidad de estudiar y profundizar sobre futuros escenarios que no han sido abordados y que se plantean a continuación:

I. Parte de esta Tesis Doctoral ha sido financiada con un Proyecto CDTI con la cooperativa Mas de Roc para el estudio de los elicitores en la precosecha de cereza, sin embargo, en el resto de los estudios se ha realizado únicamente investigación fundamental en el laboratorio. En relación con la transferencia de conocimientos y con el objetivo de llevar a la práctica los hallazgos obtenidos en este estudio, se hace necesario implementar las tecnologías a las empresas interesadas del sector. Se podrían establecer estudios de viabilidad y de optimización de los procesos para que la aplicación de estos compuestos tenga la mayor eficacia posible.

II. Sería interesante tener una mayor comprensión de la interacción entre los diferentes mecanismos implicados en la reducción de fisiopatías y de daños por frío, así como los implicados en la mejora de la calidad de las distintas especies vegetales estudiadas. Para ello, se propone la evaluación de la actividad enzimática relacionada con el sistema de defensa, así como la expresión de genes involucrados con enzimas clave de la síntesis de etileno y de la senescencia. También se podría estudiar el efecto sobre la modificación de la pared y las membranas celulares, así como evaluar las enzimas y expresión de genes relacionados con su degradación. Para comprender el metabolismo de los elicitores aplicados, sería conveniente analizar el contenido endógeno de jasmonato de metilo, putrescina, melatonina, y ácido γ-aminobutírico, que tienen los diferentes productos vegetales antes y después de las aplicaciones precosecha y poscosecha.

III. En cuanto a los tratamientos realizados de forma combinada con ácido γ-aminobutírico y 1-MCP, sería útil realizar futuras investigaciones que incluyan el efecto de la combinación de estos tratamientos a diferentes concentraciones. En especial, sería de relevante interés reducir las dosis de 1-MCP por debajo de las utilizadas ampliamente en la industria debido al efecto sinérgico que se produce entre ambos compuestos. La sustitución parcial o completa de sustancias de origen artificial por sustancias de origen natural podría mejorar la aceptabilidad de los tratamientos en relación con las demandas actuales del consumidor. Así como ser herramientas compatibles con los objetivos de desarrollo sostenible.

IV. Aunque se han realizado pruebas sensoriales entre los investigadores involucrados en los estudios para evaluar el sabor, textura y olor de los productos de forma general, estos datos no se consideran consistentes para ser incluidos en la investigación. Por ello, sería oportuno involucrar a un panel experto que evaluara el efecto que tienen estos compuestos sobre la percepción organoléptica del producto y también realizar un estudio de la aceptación de los productos vegetales por parte de los consumidores.

V. Los elicitores utilizados en esta Tesis Doctoral como la putrescina, melatonina, jasmonato de metilo y ácido γ-aminobutírico, son potencialmente seguros por su origen natural y por su presencia inherente en las plantas. Sin embargo, no están aprobados actualmente para su uso en alimentos debido a la falta de estudios de toxicidad y de consumo significativo en humanos. Por esta razón, se hace imprescindible iniciar procesos de aprobación mediante las autoridades competentes con la realización de estudios clínicos que evalúen la seguridad a largo plazo en humanos y el impacto y los riesgos asociados al consumo frecuente de estos compuestos en la dieta. De hecho, en estos estudios se han aplicado concentraciones bajas que deberían ser seguras para su aplicación comercial en precosecha o en poscosecha de productos vegetales.

8. REFERENCIAS BIBLIOGRÁFICAS

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-A-

Abiso, E., Satheesh, N., & Hailu, A. (2015). Effect of storage methods and ripening stages on postharvest quality of tomato (*Lycopersicom esculentum* Mill) cv. Chali. *Annals: Food Science and Technology*, 16(1).

Aghdam, M. S., & Fard, J. R. (2017). Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (*Fragaria× anannasa* cv. Selva) by enhancing GABA shunt activity. *Food Chemistry*, 221, 1650-1657. doi: 10.1016/j.foodchem.2016.10.123

Aghdam, M. S., Asghari, M. R., Moradbeygi, H., Mohammadkhani, N., Mohayeji, M., & Rezapour-Fard, J. (2012). Effect of postharvest salicylic acid treatment on reducing chilling injury in tomato fruit. *Romanian Biotechnological Letters*, 17(4), 7466-73.

Aghdam, M. S., Flaherty, E. J., & Shelp, B. J. (2022). γ-Aminobutyrate improves the postharvest marketability of horticultural commodities: advances and prospects. *Frontiers in Plant Science*, 13, 884572. doi: 10.3389/fpls.2022.884572

Aghdam, M. S., Flaherty, E. J., & Shelp, B. J. (2022). γ-Aminobutyrate improves the postharvest marketability of horticultural commodities: advances and prospects. *Frontiers in Plant Science*, 13, 884572. doi: 10.3389/fpls.2022.884572

Aghdam, M. S., Jannatizadeh, A., Luo, Z., & Paliyath, G. (2018). Ensuring sufficient intracellular ATP supplying and friendly extracellular ATP signaling attenuates stresses, delays senescence and maintains quality in horticultural crops during postharvest life. *Trends in Food Science and Technology*, 76, 67-81. doi: 10.1016/j.tifs.2018.04.003

Aghdam, M. S., Jannatizadeh, A., Nojadeh, M. S., & Ebrahimzadeh, A. (2019b). Exogenous melatonin ameliorates chilling injury in cut anthurium flowers during low temperature storage. *Postharvest Biology and Technology*, 148, 184-191.

Aghdam, M. S., Jannatizadeh, A., Sheikh-Assadi, M., & Malekzadeh, P. (2016a). Alleviation of postharvest chilling injury in anthurium cut flowers by salicylic acid treatment. *Scientia Horticulturae*, 202, 70-76. doi: 10.1016/j.postharvbio.2018.11.008

Aghdam, M. S., Kakavand, F., Rabiei, V., Zaare-Nahandi, F., & Razavi, F. (2019a). γ-Aminobutyric acid and nitric oxide treatments preserve sensory and nutritional quality of cornelian cherry fruits during postharvest cold storage by delaying softening and enhancing phenols accumulation. *Scientia Horticulturae*, 246, 812-817. doi: 10.1016/j.scienta.2018.11.064

Aghdam, M. S., Naderi, R., Jannatizadeh, A., Sarcheshmeh, M. A. A., & Babalar, M. (2016b). Enhancement of postharvest chilling tolerance of anthurium cut flowers by γ-aminobutyric acid (GABA) treatments. *Scientia Horticulturae*, 198, 52-60. doi: 10.1016/j.scienta.2015.11.019

Aghdam, M. S., Razavi, F., & Karamneghad, F. (2016). Maintaining the postharvest nutritional quality of peach fruits by γ-Aminobutyric acid. *Iranian Journal of Plant Physiology*, 5(4), 1457-1463.

Aisyah, Y., Murlida, E., & Maulizar, T. A. (2022). Effect of the edible coating containing cinnamon oil nanoemulsion on storage life and quality of tomato (*Lycopersicum esculentum* Mill) fruits. *IOP Conference Series: Earth and Environmental Science*, 951, 012048. doi: 10.1088/1755-1315/951/1/012048

Akhtar, G., Rajwana, I. A., Sajjad, Y., Shehzad, M. A., Amin, M., Razzaq, K., Ullah, S., Faried, H. N., & Farooq, A. (2021). Do natural leaf extracts involve regulation at physiological and biochemical levels to extend vase life of gladiolus cut flowers?. *Scientia Horticulturae*, 282, 110042. doi: 10.1016/j.scienta.2021.110042

Al Shoffe, Y., Nock, J. F., Zhang, Y., & Watkins, C. B. (2021). Pre-and post-harvest γ-aminobutyric acid application in relation to fruit quality and physiological disorder development in 'Honeycrisp' apples. *Scientia Horticulturae*, 289, 110431. doi: 10.1016/j.scienta.2021.110431

Albornoz, K., Rosas, R., & Lopez, M. D. (2023). Influence of a preharvest melatonin application on postharvest chilling injury in basul (Ocimum basilicum L.). *Journal of Horticultural Research*, 31(2). doi: 10.2478/johr-2023-0025

Alburquerque, N., Egea, J., Burgos, L., Martínez-Romero, D., Valero, D., & Serrano, M. (2006). The influence of polyamines on apricot ovary development and fruit set. *Ann. Appl Biol.* 149(1), 27-33. doi: 10.1111/j.1744-7348.2006.00067.x

Alcaraz, M. L., & Hormaza, J. I. (2021). Fruit set in avocado: Pollen limitation, pollen load size, and selective fruit abortion. *Agronomy*, 11(8), 1603. doi: 10.3390/agronomy11081603

Al-Dairi, M., Pathare, P. B., & Al-Yahyai, R. (2021). Effect of postharvest transport and storage on color and firmness quality of tomato. *Horticulturae*, 7(7), 163. doi: 10.3390/horticulturae7070163

Ali, E. A., Sarrwy, S. M. A., & Hassan, H. S. A. (2010). Improving Canino apricot trees productivity by foliar spraying with polyamines. *Journal of Applied Sciences Research*, 26, 1359-1365.

Ali, M., Raza, M. A., Li, S., Zhou, L., Huan, C., Shuling, S., & Zheng, X. (2021). 1-MCP regulates ethanol fermentation and GABA shunt pathway involved in kiwifruit quality during postharvest storage. *Horticultural Plant Journal*, 7(1), 23-30. doi: 10.1016/j.hpj.2020.12.006

Ali, S., Nawaz, A., Naz, S., Ali, M., Ejaz, S., Azam, M., & Razzaq, K. (2023). Exogenous melatonin mitigates chilling injury in zucchini fruit by enhancing antioxidant system activity, promoting endogenous proline and GABA accumulation, and preserving cell wall stability. *Postharvest Biology and Technology*, 204, 112445. doi: 10.1016/j.postharvbio.2023.112445

Aliniaeifard, S., Falahi, Z., Dianati Daylami, S., Li, T., & Woltering, E. (2020). Postharvest spectral light composition affects chilling injury in anthurium cut flowers. *Frontiers in Plant Science*, 11, 846. doi: 10.3389/fpls.2020.00846

Alonso, J., & Alique, R. (2006). Sweet cherries. In: Hui, Y. H., Barta, J., Cano, M. P., Gusek, T. W., Sidhu, J. S., Sinha, N. K. (Eds.), Handbook of fruits and fruit processing, Blackwell Publishing. Oxford, pp. 359-367. doi: 10.1002/9780470277737

Álvarez-Herrera, J. G., Jaime-Guerrero, M., & Reyes-Medina, A. J. (2021). Effect of maturity accelerants on the postharvest behavior of avocado (*Persea americana* Mill.) cv. Lorena. *Revista Colombiana de Ciencias Hortícolas*, 15(3). doi: 10.17584/rcch.2021v15i3.13131

Amarante, C. V. T. D., Argenta, L. C., de Freitas, S. T., & Steffens, C. A. (2022). Efficiency of pre-harvest application of 1-MCP (Harvista™ 1.3 SC) to delay maturation of 'Cripps Pink' apple fruit. *Scientia Horticulturae*, 293, 110715. doi: 10.1016/j.scienta.2021.110715

Amarowicz, R., Carle, R., Dongowski, G., Durazzo, A., Galensa, R., Kammerer, D., Maiani, G., & Piskula, M. K. (2009). Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. *Molecular Nutrition and Food Research*, 53, S151-S183. doi: 10.1002/mnfr.200700486

Ansari, M. I., Jalil, S. U., Ansari, S. A., & Hasanuzzaman, M. (2021). GABA shunt: a key-player in mitigation of ROS during stress. *Plant Growth Regulation*, 94, 131-149. doi: 10.1007/s10725-021-00710-y

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., & Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science and Technology*, 80, 51-60. doi: 10.1016/j.tifs.2018.07.027

Arias, E., López-Buesa, P., & Oria, R. (2009). Extension of fresh-cut "Blanquilla" pear (*Pyrus communis* L.) shelf-life by 1-MCP treatment after harvest. *Postharvest Biology and Technology*, 54(1), 53-58. doi: 10.1016/j.postharvbio.2009.04.009

Arnao, M. B., & Hernández-Ruiz, J. (2015). Functions of melatonin in plants: a review. *Journal of Pineal Research*, 59(2), 133-150. doi: 10.1111/jpi.12253

Arnao, M. B., & Hernández-Ruiz, J. (2019). Melatonin: a new plant hormone and/or a plant master regulator?. *Trends in Plant Science*, 24(1), 38-48. doi: https://doi.org/10.1016/j.tplants.2018.10.010

Arnao, M. B., & Hernández-Ruiz, J. (2020). Melatonin in flowering, fruit set and fruit ripening. *Plant Reproduction*, 33, 77-87. doi: 10.1007/s00497-020-00388-8

Arnao, M. B., & Hernández-Ruiz, J. (2021). Melatonin as a regulatory hub of plant hormone levels and action in stress situations. *Plant Biology*, 23, 7-19. doi: 10.1111/plb.13202

Artés-Hernández, F., Martínez-Zamora, L., & Cano-Lamadrid, M. (2024). Eco-Friendly Postharvest Technologies to Preserve or Enhance the Quality and Safety of Fruit and Vegetable Products. *Foods*, 13(12), 1939. doi: 10.3390/foods13121939

Asgarian, Z. S., Karimi, R., Ghabooli, M., & Maleki, M. (2022). Biochemical changes and quality characterization of cold-stored 'Sahebi' grape in response to postharvest application of GABA. *Food Chemistry*, 373, 131401. doi: 10.1016/j.foodchem.2021.131401

Asgarian, Z. S., Karimi, R., Ghabooli, M., & Maleki, M. (2022). Biochemical changes and quality characterization of cold-stored 'Sahebi' grape in response to postharvest application of GABA. *Food Chemistry*, 373, 131401.doi: 10.1016/j.foodchem.2021.131401

Ashton, O. B., Wong, M., Mcghie, T. K., Vather, R., Wang, Y., Requejo-Jackman, C., Ramankutty, P., & Woolf, A. B. (2006). Pigments in avocado tissue and oil. *Journal of Agricultural and Food Chemistry*, 54(26), 10151-10158. doi: 10.1021/jf061809j

-B-

Babarabie, M., Zarei, H., & Eskandari, A. (2019). The impact of pre-harvest treatment with gamma-aminobutyric acid (GABA) and salicylic acid on vase life and post-harvest traits of tuberose cut flowers. *Acta Scientiarum Polonorum. Hortorum Cultus*, 18(4). doi: 10.24326/asphc.2019.4.8

Badiche-El Hilali, F., Valverde, J. M., Díaz-Mula, H., Serrano, M., Valero, D., & Castillo, S. (2023). Potential Preharvest Application of γ-Aminobutyric Acid (GABA) on Improving Quality of 'Verna' Lemon at Harvest and during Storage. *Agriculture*, 13(7), 1397. doi: 10.3390/agriculture13071397

Baek, M. W., Choi, H. R., Solomon, T., Jeong, C. S., Lee, O. H., & Tilahun, S. (2021). Preharvest methyl jasmonate treatment increased the antioxidant activity and glucosinolate contents of hydroponically grown pak choi. *Antioxidants*, 10(1), 131. doi: 10.3390/antiox10010131

Baenas, N., García-Viguera, C., & Moreno, D. A. (2014). Elicitation: a tool for enriching the bioactive composition of foods. *Molecules*, 19(9), 13541-13563. doi: 10.3390/molecules190913541

Bagheri, M., & Esna-Ashari, M. (2022). Effects of postharvest methyl jasmonate treatment on persimmon quality during cold storage. *Scientia Horticulturae*, 294, 110756. doi: 10.1016/j.scienta.2021.110756

Bal, E. (2021). Effect of melatonin treatments on biochemical quality and postharvest life of nectarines. *Journal of Food Measurement and Characterization*, 15(1), 288-295. doi: 10.1007/s11694-020-00636-5

Balbontín, C., Gutiérrez, C., Wolff, M., & Figueroa, C. R. (2018). Effect of abscisic acid and methyl jasmonate preharvest applications on fruit quality and cracking tolerance of sweet cherry. *Chilean Journal of Agricultural Research*, 78(3), 438-446. doi: 10.4067/S0718-58392018000300438

Ballen, F. H., Evans, E., & Parra-Acosta, Y. K. (2022). Consumer preferences for green skin avocados in the US market: the role of experienced quality attributes, credence attributes, and demographic factors. *Journal of Agricultural and Food Industrial Organization*, 20(1), 15-23. doi: 10.1515/jafio-2020-0006

Baris, M. E., & Uslu, A. (2009). Cut flower production and marketing in Turkey. *African Journal of Agricultural Research*, 4(9), 765-771.

Baswal, A. K., Dhaliwal, H. S., Singh, Z., Mahajan, B. V. C., & Gill, K. S. (2020). Postharvest application of methyl jasmonate, 1-methylcyclopropene and salicylic acid extends the cold storage life and maintain the quality of 'Kinnow' mandarin (*Citrus nobilis* L. X C. *deliciosa* L.) fruit. *Postharvest Biology and Technology*, 161, 111064. doi: 10.1016/j.postharvbio.2019.111064

Beppu, K., & Kataoka, I. (2011). Studies on pistil doubling and fruit set of sweet cherry in warm climate. *Journal of the Japanese Society for Horticultural Science*, 80(1), 1-13. doi: 10.2503/jjshs1.80.1

Berry, A. D., Sargent, S. A., Pereira, M. E. C., & Huber, D. J. (2015). Postharvest ripening and quality of Guatemalan-West Indian avocado hybrids under simulated commercial shipping temperatures following treatment with aqueous 1-methylcyclopropene. *HortTechnology*, 25(1), 85-89. doi: 10.21273/HORTTECH.25.1.85

Bhardwaj, R., Pareek, S., Saravanan, C., & Yahia, E. M. (2022). Contribution of pre-storage melatonin application to chilling tolerance of some mango fruit cultivars and relationship with polyamines metabolism and γ-aminobutyric acid shunt pathway. *Environmental and Experimental Botany*, 194, 104691. doi: 10.1016/j.envexpbot.2021.104691

Bill, M., Gokul, J. K., Viljoen, F., & Korsten, L. (2022). Fungal microbiome shifts on avocado fruit associated with a combination of postharvest chemical and physical interventions. *Journal of Applied Microbiology*, 133(3), 1905-1918. doi: 10.1111/jam.15693

Bill, M., Sivakumar, D., Thompson, A. K., & Korsten, L. (2014). Avocado fruit quality management during the postharvest supply chain. *Food Reviews International*, 30(3), 169-202. doi: 10.1080/87559129.2014.907304

Biswas, P., & Brummell, D. A. (2019). Chilling injury. In: Tonetto de Freitas, S., Pareek, S. (Eds.), Postharvest physiological disorders in fruits and vegetables. CRC Pess, pp. 61-88. doi: 10.1201/b22001

Blaker, K. M., & Olmstead, J. W. (2014). Effects of preharvest applications of 1-methylcyclopropene on fruit firmness in southern highbush blueberry. *Acta Horticulturae*, 1017, 71-76. doi: 10.17660/ActaHortic.2014.1017.5

Blanco, V., Blaya-Ros, P. J., Torres-Sánchez, R., & Domingo, R. (2020). Influence of regulated deficit irrigation and environmental conditions on reproductive response of sweet cherry trees. *Plants*, 9(1), 94. doi: 10.3390/plants9010094

Blando, F., & Oomah, B. D. (2019). Sweet and sour cherries: Origin, distribution, nutritional composition and health benefits. *Trends in Food Science and Technology*, *86*, 517-529. doi: 10.1016/j.tifs.2019.02.052

Blažek, J., Zelený, L., & Suran, P. (2022). Sweet cherry research world overview 2015-2017. *Horticultural Science*, 49(3), 121-146. doi: 10.17221/70/2021-HORTSCI

Brosnan, T., & Sun, D. W. (2001). Precooling techniques and applications for horticultural products — a review. *International Journal of Refrigeration*, 24(2), 154-170. doi: 10.1016/S0140-7007(00)00017-7

Brüggenwirth, M., & Knoche, M. (2016). Mechanical properties of skins of sweet cherry fruit of differing susceptibilities to cracking. *Journal of the American Society for Horticultural Science*, 141(2), 162-168. doi: 10.21273/JASHS.141.2.162

-C-

Campillay-Llanos, W., Ortega-Farias, S., & Ahumada-Orellana, L. (2024). Development and validation of phenological models for eight varieties of sweet cherry (*Prunus avium* L.) growing under Mediterranean climate condition. *Scientia Horticulturae*, 326, 112711. doi: 10.1016/j.scienta.2023.112711

Cao, S., Qu, G., Ma, C., Ba, L., Ji, N., Meng, L., Lei, J., & Wang, R. (2021). Effects of melatonin treatment on the physiological quality and cell wall metabolites in kiwifruit. *Food Science and Technology*, 42, e85421. doi: 10.1590/fst.85421

Careli-Gondim, Í., Mesquita, T. C., Vilas Boas, E. V. D. B., Caliari, M., & Soares Júnior, M. S. (2020). The effect of active coating and refrigerated storage on the quality of avocado cultivar, Quintal. *Journal of Food Science and Technology*, 57, 143-151. doi: 10.1007/s13197-019-04039-3

Carrión-Antolí, A., Badiche-El Hilali, F., Lorente-Mento, J. M., Díaz-Mula, H. M., Serrano, M., & Valero, D. (2024). Antioxidant Systems and Quality in Sweet Cherries Are Improved by Preharvest GABA Treatments Leading to Delay Postharvest Senescence. *International Journal of Molecular Sciences*, 25(1), 260. doi: 10.3390/ijms25010260

Carrión-Antolí, A., Lorente-Mento, J. M., Valverde, J. M., Castillo, S., Valero, D., & Serrano, M. (2022a). Effects of melatonin treatment on sweet cherry tree yield and fruit quality. *Agronomy*, 12(1), 3. doi: 10.3390/agronomy12010003

Carrión-Antolí, A., Martínez-Romero, D., Guillén, F., Zapata, P. J., Serrano, M., & Valero, D. (2022b). Melatonin pre-harvest treatments leads to maintenance of sweet cherry quality during storage by increasing antioxidant systems. *Frontiers in Plant Science*, 13, 863467. doi: 10.3389/fpls.2022.863467

Chai, J., Liao, B., Li, R., & Liu, Z. (2022). Changes in taste and volatile compounds and ethylene production determined the eating window of ‘Xuxiang’ and ‘Cuixiang’ kiwifruit cultivars. *Postharvest Biology and Technology*, 194, 112093. doi: 10.1016/j.postharvbio.2022.112093

Chandler, S. F., & Brugliera, F. (2011). Genetic modification in floriculture. *Biotechnology letters*, 33(2), 207-214. doi: 10.1007/s10529-010-0424-4

Chen, L., Song, J., Wang, J., Ye, M., Deng, Q., Wu, X., Wu, X., & Ren, B. (2023). Effects of methyl jasmonate fumigation on the growth and detoxification ability of *Spodoptera litura* to xanthotoxin. *Insects*, 14(2), 145. doi: 10.3390/insects14020145

Chen, M., Guo, H., Chen, S., Li, T., Li, M., Rashid, A., Xu, C., & Wang, K. (2019). Methyl jasmonate promotes phospholipid remodeling and jasmonic acid signaling to alleviate chilling injury in peach fruit. *Journal of Agricultural and Food Chemistry*, 67(35), 9958-9966. doi: 10.1021/acs.jafc.9b03853

Chezanoglou, E., Mourtzinos, I., & Goula, A. M. (2024). Sweet cherry and its by-products as sources of valuable phenolic compounds. *Trends in Food Science and Technology*, 104367. doi: 10.1016/j.tifs.2024.104367

Chirinos, R., Ramon, K., Mendoza, M., Figueroa-Merma, A., Pacheco-Ávalos, A., Campos, D., & Pedreschi, R. (2022). Effect of prolonged cold storage on the dynamics of the enzymatic and non-enzymatic antioxidant system in the mesocarp of avocado (*Persea americana*) cv. Hass: relationship with oxidative processes. *Horticulturae*, 8(10), 880. doi: 10.3390/horticulturae8100880

Chmielewski, F. M., Götz, K. P., Weber, K. C., & Moryson, S. (2018). Climate change and spring frost damages for sweet cherries in Germany. *International Journal of Biometeorology*, 62, 217-228. doi: 10.1007/s00484-017-1443-9

Choi, H. R., Baek, M. W., Tilahun, S., & Jeong, C. S. (2022). Long-term cold storage affects metabolites, antioxidant activities, and ripening and stress-related genes of kiwifruit cultivars. *Postharvest Biology and Technology*, 189, 111912. doi: 10.1016/j.postharvbio.2022.111912

Choi, S. T., & Huber, D. J. (2008). Influence of aqueous 1-methylcyclopropene concentration, immersion duration, and solution longevity on the postharvest ripening of breaker-turning tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biology and Technology*, 49(1), 147-154. doi: 10.1016/j.postharvbio.2008.01.003

Chowdhury, N. N., Islam, M. N., Jafrin, R., Rauf, A., Khalil, A. A., Emran, T. B., Aljohani, A. S. M., Alhumaydhi, F. A., Lorenzo, J. M., Shariati, M. A., & Simal-Gandara, J. (2023). Natural plant products as effective alternatives to synthetic chemicals for postharvest fruit storage management.

Critical Reviews in Food Science and Nutrition, 63(30), 10332-10350. doi: 10.1080/10408398.2022.2079112

Christensen, J. V. (1972). Cracking in cherries: IV. Physiological studies of the mechanism of cracking. *Acta Agriculturae Scandinavica*, 22(3), 153-162. doi: 10.1080/00015127209433474

Christensen, J. V. (1996). Rain-induced cracking of sweet cherries: its causes and prevention. In: Webster, A.D., Looney, N.E. (Eds.), Cherries: Crop Physiology, Production and Uses. CAB International, UK, pp. 297-327.

Collado-González, J., Piñero, M. C., Otálora, G., López-Marín, J., & Amor, F. M. D. (2021). The effect of foliar putrescine application, ammonium exposure, and heat stress on antioxidant compounds in cauliflower waste. *Antioxidants*, 10(5), 707. doi: 10.3390/antiox10050707

Collignon, T. E., Webber, K., Piasecki, J., Rahman, A. S., Mondal, A., Barbalho, S. M., & Bishayee, A. (2023). Avocado (*Persea americana* Mill) and its phytoconstituents: potential for cancer prevention and intervention. *Critical Reviews in Food Science and Nutrition*, 1-21. doi: 10.1080/10408398.2023.2260474

Collins, E. J., Bowyer, C., Tsouza, A., & Chopra, M. (2022). Tomatoes: An extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. *Biology*, 11(2), 239. doi: 10.3390/biology11020239

Correia, S., Schouten, R., Silva, A. P., & Gonçalves, B. (2018). Sweet cherry fruit cracking mechanisms and prevention strategies: A review. *Scientia Horticulturae*, 240, 369-377. doi: 10.1016/j.scienta.2018.06.042

Costa, A. S., Nogueira, L. C., dos Santos, V. F., Finger, F. L., Camara, T. R., Loges, V., & Willadino, L. (2011). Characterization of symptoms of senescence and chilling injury on inflorescences of *Heliconia bihai* (L.) cv. Lobster Claw and cv. Halloween. *Postharvest Biology and Technology*, 59(1), 103-109. doi: 10.1016/j.postharvbio.2010.08.015

Crisosto, C. H., Crisosto, G. M., & Ritenour, M. A. (2002). Testing the reliability of skin color as an indicator of quality for early season 'Brooks' (*Prunus avium* L.) cherry. *Postharvest Biology and Technology*, 24(2), 147-154. doi: 10.1016/S0925-5214(01)00190-9

-D-

Dahal, J., Adhikari, D., Karki, K. B., Kafle, D. R., Chaudhary, C., & Pun, U. K. (2018). Carnation cut flower production and marketing in Nepal. *Nepalese Journal of Agricultural Sciences*, 17, 152.

Davey, M. W., Stals, E., Panis, B., Keulemans, J., & Swennen, R. L. (2005). High-throughput determination of malondialdehyde in plant tissues. *Analytical Biochemistry*, 347(2), 201-207.doi: 10.1016/j.ab.2005.09.041

David, S., Levin, E., Fallik, E., Alkalai-Tuvia, S., Foolad, M. R., & Lers, A. (2022). Physiological genetic variation in tomato fruit chilling tolerance during postharvest storage. *Frontiers in Plant Science*, 13, 991983. doi: 10.3389/fpls.2022.991983

De Martino, G., Vizovitis, K., Botondi, R., Bellincontro, A., & Mencarelli, F. (2006). 1-MCP controls ripening induced by impact injury on apricots by affecting SOD and POX activities. *Postharvest Biology and Technology*, 39(1), 38-47. doi: 10.1016/j.postharvbio.2005.09.002

Debnath, B., Islam, W., Li, M., Sun, Y., Lu, X., Mitra, S., Hussain, M., Liu, S., & Qiu, D. (2019). Melatonin mediates enhancement of stress tolerance in plants. *International Journal of Molecular Sciences*, 20(5), 1040. doi: 10.3390/ijms20051040

Defilippi, B. G., Ejsmentewicz, T., Covarrubias, M. P., Gudenschwager, O., & Campos-Vargas, R. (2018). Changes in cell wall pectins and their relation to postharvest mesocarp softening of "Hass" avocados (*Persea americana* Mill.). *Plant Physiology and Biochemistry*, 128, 142-151. doi: 10.1016/j.plaphy.2018.05.018

Delgado-Vargas, V. A., Ayala-Garay, O. J., Arévalo-Galarza, M. D. L., & Gautier, H. (2023). Increased Temperature Affects Tomato Fruit Physicochemical Traits at Harvest Depending on Fruit Developmental Stage and Genotype. *Horticulturae*, 9(2), 212. doi: 10.3390/horticulturae9020212

Demirsoy, H., Demirsoy, L., & Lang, G. A. (2022). Research on spring frost damage in cherries. *Horticultural Science*, 49 (2), 89-94. doi: 0.17221/91/2021-HORTSCI

Demirsoy, L., & Demirsoy, H. (2004). The epidermal characteristics of fruit skin of some sweet cherry cultivars in relation to fruit cracking. *Pakistan Journal of Botany*, 36(4), 725-731.

Di Matteo, A., Russo, R., Graziani, G., Ritieni, A., & Di Vaio, C. (2016). Characterization of autochthonous sweet cherry cultivars (*Prunus avium* L.) of southern Italy for fruit quality, bioactive compounds and antioxidant activity. *Journal of the Science of Food and Agriculture*, 97(9), 2782-2794. doi: 10.1002/jsfa.8106

Dias, C., Ribeiro, T., Rodrigues, A. C., Ferrante, A., Vasconcelos, M. W., & Pintado, M. (2021). Improving the ripening process after 1-MCP application: Implications and strategies. *Trends in Food Science and Technology*, 113, 382-396. doi: 10.1016/j.tifs.2021.05.012

Díaz-Mula, H. M., Castillo, S., Martínez-Romero, D., Valero, D., Zapata, P. J., Guillén, F., & Serrano, M. (2009a). Sensory, nutritive and functional properties of sweet cherry as affected by cultivar and ripening stage. *Food Science and Technology International*, 15(6), 535-543. doi: 10.1177/1082013209351868

Díaz-Mula, H. M., Zapata, P. J., Guillén, F., Martínez-Romero, D., Castillo, S., Serrano, M., & Valero, D. (2009b). Changes in hydrophilic and lipophilic antioxidant activity and related bioactive compounds during postharvest storage of yellow and purple plum cultivars. *Postharvest Biology and Technology*, 51(3), 354-363. doi: 10.1016/j.postharvbio.2008.09.007

Ding, C. K., Wang, C., Gross, K. C., & Smith, D. L. (2002). Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. *Planta*, 214, 895-901. doi: 10.1007/s00425-001-0698-9

Djanaguiraman, M., Prasad, P. V. V., & Al-Khatib, K. (2011). Ethylene perception inhibitor 1-MCP decreases oxidative damage of leaves through enhanced antioxidant defense mechanisms in soybean plants grown under high temperature stress. *Environmental and Experimental Botany*, 71(2), 215-223. doi: 10.1016/j.envexpbot.2010.12.006

Du, H., Liu, D., Liu, G., Liu, H., & Kurtenbach, R. (2023). Polyamines conjugated to the bio-membranes and membrane conformations are involved in the melatonin-mediated resistance of harvested plum fruit to cold stress. *Postharvest Biology and Technology*, 204, 112480. doi: 10.1016/j.postharvbio.2023.112480

-E-

Ebrahimi, S., Pourdarbani, R., & Sabzi, S. (2024). Investigating the effects of mechanical damage on the quality properties of kiwi fruit. *Journal of Environmental Science Studies*, 9(2), 8313-8321. doi: 10.22034/jess.2023.404299.2064

Ebrahimzadeh, A., Jiménez, S., Da Silva, J. T., Satoh, S., & Lao, M. T. (2008). Post-harvest physiology of cut carnation flowers. *Fresh Produce*, 2(2), 56-71.

Ebrahimzadeh, A., Jimenez-Becker, S., Manzano-Medina, S., Jamilena-Quesada, M., & Lao-Arenas, M. T. (2011). Evaluation of ethylene production by ten Mediterranean carnation cultivars and their response to ethylene exposure. *Spanish Journal of Agricultural Research*, 9(2), 524-530. doi: 10.5424/sjar/20110902-124-10

Espín, J. C., & Tomas-Barberan, F. A. (2001). Phenolic compounds and related enzymes as determinants of fruits and vegetables quality. *Journal of the Science of Food and Agriculture*, 81, 853-876. doi: 10.1002/jsfa.885

Ezzat, A., Hegedűs, A., Szabó, S., Ammar, A., Szabó, Z., Nyéki, J., Molnár, B., & Holb, I. J. (2020). Temporal changes and correlations between quality loss parameters, antioxidant properties and enzyme activities in apricot fruit treated with methyl jasmonate and salicylic acid during cold storage and shelf-life. *Applied Sciences*, 10(22), 8071. doi: 10.3390/app10228071

F-

Faizy, A. H., Ozturk, B., Aglar, E., & Yıldız, K. (2021). Role of methyl jasmonate application regime on fruit quality and bioactive compounds of sweet cherry at harvest and during cold storage. *Journal of Food Processing and Preservation*, 45(10), e15882. doi: 10.1111/jfpp.15882

Fan, L., Shi, J., Zuo, J., Gao, L., Lv, J., & Wang, Q. (2016). Methyl jasmonate delays postharvest ripening and senescence in the non-climacteric eggplant (*Solanum melongena* L.) fruit. *Postharvest Biology and Technology*, 120, 76-83. doi: 10.1016/j.postharvbio.2016.05.010

Fariman, Z. K., & Tehranifar, A. (2011). Effect of essential oils, ethanol and methanol to extend the vase-life of carnation (*Dianthus caryophyllus* L.) flowers. *Journal of Biological and Environmental Sciences*, 5(14), 91-94.

Farooq, M., Wahid, A., & Lee, D. J. (2009). Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. *Acta Physiologiae Plantarum*, 31, 937-945. doi: 10.1007/s11738-009-0307-2

Fawole, O. A., Atukuri, J., Arendse, E., & Opara, U. O. (2020). Postharvest physiological responses of pomegranate fruit (cv. Wonderful) to exogenous putrescine treatment and effects on physico-chemical and phytochemical properties. *Food Science and Human Wellness*, 9(2), 146-161. doi: 10.1016/j.fshw.2020.02.007

Ferguson, A. R., Huang, H., & Costa, G. (2023). History of Kiwifruit: Evolution of a Global Crop. In Kiwifruit: Botany, Production and Uses, 1-15. doi: 10.1079/9781800620933.0001

Fernandez, E., Mojahid, H., Fadón, E., Rodrigo, J., Ruiz, D., Egea, J. A., Mimoun, M. B., Kodad, D., Yaacoubi, A. E., Ghrab, M., Egea, J., Benmoussa, H., Borgini, N., Elloumi, O., & Luedeling, E. (2023). Climate change impacts on winter chill in Mediterranean temperate fruit orchards. *Regional Environmental Change*, 23(1), 7. doi: 10.1007/s10113-022-02006-x

Flores, G., & Castillo, M. L. R. (2014). Influence of preharvest and postharvest methyl jasmonate treatments on flavonoid content and metabolomic enzymes in red raspberry. *Postharvest Biology and Technology*, 97, 77-82. doi: 10.1016/j.postharvbio.2014.06.009

Fuentealba, C., Vidal, J., Zulueta, C., Ponce, E., Uarrota, V., Defilippi, B. G., & Pedreschi, R. (2022). Controlled Atmosphere Storage Alleviates Hass Avocado Black Spot Disorder. *Horticulturae*, 8(5), 369. doi: 10.3390/horticulturae8050369

Fuke, Y., Sasago, K., & Matsuoka, H. (1985). Determination of chlorophylls in kiwi fruit and their changes during ripening. *Journal of Food Science*, 50(5), 1220-1223. doi: 10.1111/j.1365-2621.1985.tb10446.x

-G-

Gago, C., Guerreiro, A., Souza, M., Martins, N., Fonseca, D., Cabrita, M. J., Miguel, M. D. G., & Antunes, M. D. (2024). Effectiveness of Sodium Alginate and Carnauba Wax Nanoemulsions with Lemongrass Essential Oil on the quality of 'Hass' Avocado Fruit from early, middle, and late harvest season during prolonged cold storage. *Scientia Horticulturae*, 333, 113237. doi: 10.1016/j.scienta.2024.113237

Gao, Z. Q., Zheng, J. F., Lin, Y. Z., Xiao, Y. Y., Zeng, L. Z., & Lin, H. T. (2022). Effects of paper-containing 1-methylcyclopropene treatment on the disease resistance metabolism of harvested 'Zhaoxia' peach fruit. *Journal of Food Safety and Quality*, 13(16), 5329-5336.

Garcia-Montiel, F. G., Serrano, M., Martinez-Romero, D., & Alburquerque, N. (2010). Factors influencing fruit set and quality in different sweet cherry cultivars. *Spanish Journal of Agricultural Research*, 8(4), 1118-1128. doi: 10.5424/sjar/2010084-1238

García-Pastor, M. E., Serrano, M., Guillén, F., Castillo, S., Martínez-Romero, D., Valero, D., & Zapata, P. J. (2019). Methyl jasmonate effects on table grape ripening, vine yield, berry quality and bioactive compounds depend on applied concentration. *Scientia Horticulturae*, 247, 380-389. doi: 10.1016/j.scienta.2018.12.043

Garreaud, R. D., Boisier, J. P., Rondanelli, R., Montecinos, A., Sepúlveda, H. H., & Veloso-Aguila, D. (2020). The central Chile mega drought (2010–2018): a climate dynamics perspective. *International Journal of Climatology*, 40(1), 421-439. doi: 10.1002/joc.6219

Geremu, M. A., Welelaw, E., Tamirat, S., & Kidane, B. (2022). Associated fungal pathogens and causes of postharvest losses in the avocado value chain in Hossana town market, Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, 22(6), 20646-20667. doi: 10.18697/ajfand.111.22015

Ghalati, R. E., Shamili, M., & Homaei, A. (2020). Effect of putrescine on biochemical and physiological characteristics of guava (*Psidium guajava* L.) seedlings under salt stress. *Scientia Horticulturae*, 261, 108961. doi: 10.1016/j.scienta.2019.108961

Giné-Bordonaba, J., Echeverria, G., Ubach, D., Aguiló-Aguayo, I., López, M. L., & Larrigaudière, C. (2017). Biochemical and physiological changes during fruit development and ripening of two sweet cherry varieties with different levels of cracking tolerance. *Plant Physiology and Biochemistry*, 111, 216-225. doi: 10.1016/j.plaphy.2016.12.002

Giuggioli, N. R., Merlino, V. M., Sparacino, A., Peano, C., Borra, D., & Massaglia, S. (2023). Customer preferences heterogeneity toward avocado: a latent class approach based on the best–worst scaling choice modeling. *Agricultural and Food Economics*, 11(1), 46. doi: 10.1186/s40100-023-00289-0

Glowacz, M., Bill, M., Tinyane, P. P., & Sivakumar, D. (2017). Maintaining postharvest quality of cold stored 'Hass' avocados by altering the fatty acids content and composition with the use of natural volatile compounds–methyl jasmonate and methyl salicylate. *Journal of the Science of Food and Agriculture*, 97(15), 5186-5193. doi: 10.1002/jsfa.8400

Golding, J.B., Spohr, L., Harris, A., Satyan, S., Blades, B., Crampton, K., Bullot, M., Stathopoulos, C. & McGlasson, B. (2017). Effect of postharvest display conditions on cherry stem quality. *Acta Horticulturae,* 1161, 569-574. doi: 10.17660/ActaHortic.2017.1161.90

Gong, H. J., Fullerton, C., Billing, D., & Burdon, J. J. S. H. (2020). Retardation of 'Hayward' kiwifruit tissue zone softening during storage by 1-methylcyclopropene. *Scientia Horticulturae*, 259, 108791. doi: 10.1016/j.scienta.2019.108791

González-Hernández, A. I., Scalschi, L., Vicedo, B., Marcos-Barbero, E. L., Morcuende, R., & Camañes, G. (2022). Putrescine: A key metabolite involved in plant development, tolerance and resistance responses to stress. *International Journal of Molecular Sciences*, 23(6), 2971. doi: 10.3390/ijms23062971

Gu, S., Xu, D., Zhou, F., Feng, K., Chen, C., & Jiang, A. (2022). Repairing ability and mechanism of methyl jasmonate and salicylic acid on mechanically damaged sweet cherries. *Scientia Horticulturae*, 292, 110567. doi: 10.1016/j.scienta.2021.110567

Guillén, F., Castillo, S., Zapata, P. J., Martínez-Romero, D., Serrano, M., & Valero, D. (2007). Efficacy of 1-MCP treatment in tomato fruit: 1. Duration and concentration of 1-MCP treatment to gain an effective delay of postharvest ripening. *Postharvest Biology and Technology*, 43(1), 23-27. doi: 10.1016/j.postharvbio.2006.07.004

Guillén, F., Castillo, S., Zapata, P. J., Martínez-Romero, D., Valero, D., & Serrano, M. (2006). Efficacy of 1-MCP treatment in tomato fruit: 2. Effect of cultivar and ripening stage at harvest. *Postharvest Biology and Technology*, 42(3), 235-242. doi: 10.1016/j.postharvbio.2006.07.005

Guillén, F., Habibi, F., & Golding, J. B. (2022). Pre-and postharvest treatments with elicitors on the development of bioactive compounds and nutritional quality of fruit and vegetables. *Frontiers in Nutrition*, 9, 1070945. doi: 10.3389/fnut.2022.1070945

Guru, A., Dwivedi, P., Kaur, P., & Pandey, D. K. (2022). Exploring the role of elicitors in enhancing medicinal values of plants under in vitro condition. *South African Journal of Botany*, 149, 1029-1043. doi: 10.1016/j.sajb.2021.10.014

-H-

Habibi, F., Ramezanian, A., Guillén, F., Serrano, M., & Valero, D. (2020). Blood oranges maintain bioactive compounds and nutritional quality by postharvest treatments with γ-aminobutyric acid, methyl jasmonate or methyl salicylate during cold storage. *Food Chemistry*, 306, 125634. doi: 10.1016/j.foodchem.2019.125634

Habibi, F., Ramezanian, A., Rahemi, M., Eshghi, S., Guillén, F., Serrano, M., & Valero, D. (2019). Postharvest treatments with γ-aminobutyric acid, methyl jasmonate, or methyl salicylate enhance chilling tolerance of blood orange fruit at prolonged cold storage. *Journal of the Science of Food and Agriculture*, 99(14), 6408-6417. doi: 10.1002/jsfa.9920

Han, Q. H., Liu, W., Li, H. Y., He, J. L., Guo, H., Lin, S., Li, Z., Chen, H., Liu, Y. W., Wu, D. T., Li, S. Q., & Qin, W. (2019). Extraction optimization, physicochemical characteristics, and antioxidant activities of polysaccharides from kiwifruit (*Actinidia chinensis* Planch.). *Molecules*, 24(3), 461. doi: 10.3390/molecules24030461

Han, S., Nan, Y., Qu, W., He, Y., Ban, Q., Lv, Y., & Rao, J. (2018). Exogenous γ-aminobutyric acid treatment that contributes to regulation of malate metabolism and ethylene synthesis in apple fruit during storage. *Journal of Agricultural and Food Chemistry*, 66(51), 13473-13482. doi: 10.1021/acs.jafc.8b04674

Hanif, A., Ahmad, S., Jaskani, M. J., & Ahmad, R. (2020). Papaya treatment with putrescine maintained the overall quality and promoted the antioxidative enzyme activities of the stored fruit. *Scientia Horticulturae*, 268, 109367. doi: 10.1016/j.scienta.2020.109367

Harbaugh, D. T., Nepokroeff, M., Rabeler, R. K., McNeill, J., Zimmer, E. A., & Wagner, W. L. (2010). A new lineage-based tribal classification of the family Caryophyllaceae. *International Journal of Plant Sciences*, 171(2), 185-198. doi: 10.1086/648993

Hasan, M. K., Ahammed, G. J., Yin, L., Shi, K., Xia, X., Zhou, Y., Yu, J., & Zhou, J. (2015). Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in *Solanum lycopersicum* L. *Frontiers in Plant Science*, 6, 601. doi: 10.3389/fpls.2015.00601

Hassan, F. A. S., & Fetouh, M. I. (2019). Does moringa leaf extract have preservative effect improving the longevity and postharvest quality of gladiolus cut spikes?. *Scientia Horticulturae*, 250, 287-293. doi: 10.1016/j.scienta.2019.02.059

Hassan, F. A. S., Ali, E. F., & El-Deeb, B. (2014). Improvement of postharvest quality of cut rose cv. 'First Red' by biologically synthesized silver nanoparticles. *Scientia Horticulturae*, 179, 340-348. doi: 10.1016/j.scienta.2014.09.053

He, J., & Giusti, M. M. (2010). Anthocyanins: natural colorants with health-promoting properties. *Annual Review of Food Science and Technology*, 1(1), 163-187. doi: 10.1146/annurev.food.080708.100754

Herrera-González, J. A., Bautista-Baños, S., Serrano, M., Romanazzi, G., & Gutiérrez-Martínez, P. (2021). Non-chemical treatments for the pre-and post-harvest elicitation of defense mechanisms in the fungi–avocado pathosystem. *Molecules*, 26(22), 6819. doi: 10.3390/molecules26226819

Hershkovitz, V., Saguy, S. I., & Pesis, E. (2005). Postharvest application of 1-MCP to improve the quality of various avocado cultivars. *Postharvest Biology and Technology*, 37(3), 252-264. doi: 10.1016/j.postharvbio.2005.05.003

Hewedy, O. A., Elsheery, N. I., Karkour, A. M., Elhamouly, N., Arafa, R. A., Mahmoud, G. A. E., Dawood, M. F. A., Hussein, W. E., Mansour, A., Amin, D. H., Allakhverdiev, S. I., Zivcak, M., &

Brestic, M. (2023). Jasmonic acid regulates plant development and orchestrates stress response during tough times. *Environmental and Experimental Botany*, 208, 105260. doi: 10.1016/j.envexpbot.2023.105260

Ho, T. T., Murthy, H. N., & Park, S. Y. (2020). Methyl jasmonate induced oxidative stress and accumulation of secondary metabolites in plant cell and organ cultures. *International Journal of Molecular Sciences*, 21(3), 716. doi: 10.3390/ijms21030716

Hoeberichts, F. A., Van Der Plas, L. H., & Woltering, E. J. (2002). Ethylene perception is required for the expression of tomato ripening-related genes and associated physiological changes even at advanced stages of ripening. *Postharvest Biology and Technology*, 26(2), 125-133. doi: 10.1016/S0925-5214(02)00012-1

Homez-Jara, A., Cardenas-Roa, H., Montealegre, M., Lim, L. T., Corradini, M. G., Váquiro-Herrera, H. A., & Sandoval-Aldana, A. (2023). Postharvest Treatments of Hass Avocado (*Persea americana* Mill.) and Estimation of Its Quality Using Hyperspectral Imaging (HSI). *ACS Food Science and Technology*, 3(5), 932-944. doi: 10.1021/acsfoodscitech.3c00084

Horváth-Mezőfi, Z., Baranyai, L., Nguyen, L. L. P., Dam, M. S., Ha, N. T. T., Göb, M., Sasvár, Z., Csurka, T., Zsom, T., & Hitka, G. (2024). Evaluation of Color and Pigment Changes in Tomato after 1-Methylcyclopropene (1-MCP) Treatment. *Sensors*, 24(8), 2426. doi: 10.3390/s24082426

Hosseini, C., Asghari, M., & Khezri, M. (2024). Potential of *Bacillus subtilis* and Oregano Essential Oil in Controlling Postharvest Gray Mold Caused by Botrytis cinerea and Maintaining Quality Attributes of Sweet Cherry. *Journal of Plant Growth Regulation*, 1-12. doi: 10.1007/s00344-024-11275-1

Hu, H., Zhao, H., Zhang, L., Zhou, H., & Li, P. (2021). The application of 1-methylcyclopropene preserves the postharvest quality of cabbage by inhibiting ethylene production, delaying chlorophyll breakdown and increasing antioxidant capacity. *Scientia Horticulturae*, 281, 109986. doi: 10.1016/j.scienta.2021.109986

Hu, Z., Tang, C., He, Z., Lin, J., & Ni, Y. (2017). 1-Methylcyclopropene (MCP)-containing cellulose paper packaging for fresh fruit and vegetable preservation: a review. *BioResources*, 12(1), 2234-2248. doi: 10.15376/biores.12.1.2234-2248

Huan, C., Zhang, J., Jia, Y., Jiang, T., Shen, S., & Zheng, X. (2020). Effect of 1-methylcyclopropene treatment on quality, volatile production and ethanol metabolism in kiwifruit during storage at room temperature. *Scientia Horticulturae*, 265, 109266. doi: 10.1016/j.scienta.2020.109266

Huang, B., Chen, Y. E., Zhao, Y. Q., Ding, C. B., Liao, J. Q., Hu, C., Zhou, L. J., Zhang, Z. W., Yuan, S., & Yuan, M. (2019b). Exogenous melatonin alleviates oxidative damages and protects photosystem II in maize seedlings under drought stress. *Frontiers in Plant Science*, 10, 677. doi: 10.3389/fpls.2019.00677

Huang, H., Guo, L., Wang, L., Wang, H., Ma, S., Jiang, Y., & Qu, H. (2019a). 1-Methylcyclopropene (1-MCP) slows ripening of kiwifruit and affects energy status, membrane fatty acid contents and cell membrane integrity. *Postharvest Biology and Technology*, 156, 110941. doi: 10.1016/j.postharvbio.2019.110941

Huang, L., Fu, W., Zhang, Y., Liu, X., Wang, Q., Wang, L., & Tanveer, M. (2024). The role of melatonin in regulating horticultural crop production under various abiotic stresses. *Scientia Horticulturae*, 323, 112508. doi: 10.1016/j.scienta.2023.112508

Huang, S., & Jin, S. (2024). Melatonin Interaction with Other Phytohormones in the Regulation of Abiotic Stresses in Horticultural Plants. *Antioxidants*, 13(6), 663. doi: 10.3390/antiox13060663

Huang, W., Billing, D., Cooney, J., Wang, R., & Burdon, J. (2021). The role of ethylene and abscisic acid in kiwifruit ripening during postharvest dehydration. *Postharvest Biology and Technology*, 178, 111559. doi: 10.1016/j.postharvbio.2021.111559

Huang, W., Gao, Z., Wang, L., Zhang, Y., & Fu, X. (2019a). Advances in research on cherry fruit cracking: Causes, prevention and control. *Frontiers in Plant Science*, 10, 986.

Hurtado-Fernández, E., Fernández-Gutiérrez, A., & Carrasco-Pancorbo, A. (2018). Avocado fruit—*Persea americana*. *Exotic fruits*, 37-48. doi: 10.1016/B978-0-12-803138-4.00001-0

Hussain, S. S., Ali, M., Ahmad, M., & Siddique, K. H. (2011). Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. *Biotechnology Advances*, 29(3), 300-311. doi: 10.1016/j.biotechadv.2011.01.003

Hussein, H. A. A., Alshammari, S. O., Abd El-Sadek, M. E., Kenawy, S. K., & Badawy, A. A. (2023). The promotive effect of putrescine on growth, biochemical constituents, and yield of wheat (*Triticum aestivum* L.) plants under water stress. *Agriculture*, 13(3), 587. doi: 10.3390/agriculture13030587

-I-

Ichimura, K., Takada, M., & Ogawa, K. (2022). Effects of treatments with nigerosylmaltooligosaccharide, glucose and sucrose on the vase life of cut snapdragon flowers. *Scientia Horticulturae*, 291, 110565. doi: 10.1016/j.scienta.2021.110565

Iqbal, N., Fatma, M., Gautam, H., Umar, S., Sofo, A., D'ippolito, I., & Khan, N. A. (2021). The crosstalk of melatonin and hydrogen sulfide determines photosynthetic performance by regulation of carbohydrate metabolism in wheat under heat stress. *Plants*, 10(9), 1778. doi: 10.3390/plants10091778

-J-

Jahan, M. S., Hasan, M. M., Alotaibi, F. S., Alabdallah, N. M., Alharbi, B. M., Ramadan, K. M., Bendary, E. S. A., Alshehri, D., Jabborova, D., Al-Balawi, D. A., Dessoky, E. S., Ibrahim, M. F. M., & Guo, S. (2022). Exogenous putrescine increases heat tolerance in tomato seedlings by regulating chlorophyll metabolism and enhancing antioxidant defense efficiency. *Plants*, 11(8), 1038. doi: 10.3390/plants11081038

Jalali, H., Nazoori, F., Mirdehghan, S. H., & Karimi, H. R. (2023). Pre-harvest application of GABA and CaO delays senescence and maintains of physicochemical characteristics of fresh ''Ahmad Aghaei'' pistachio during cold storage. *Journal of Stored Products Research*, 104, 102206. doi: 10.1016/j.jspr.2023.102206

Jannatizadeh, A., Aghdam, M. S., Luo, Z., & Razavi, F. (2019). Impact of exogenous melatonin application on chilling injury in tomato fruits during cold storage. *Food and Bioprocess Technology*, 12, 741-750. doi: 10.1007/s11947-019-2247-1

Jawaharlal, M., Ganga, M., Padmadevi, K., Jegadeeswari, V., & Karthikeyan, S. (2009). A technical guide on carnation. Tamil Nadu Agricultural University, Coimbatore, 1-56.

Jeong, J. W., Brecht, J. K., Huber, D. J., & Sargent, S. A. (2004). 1-Methylcyclopropene (1-MCP) for maintaining texture quality of fresh-cut tomato. *HortScience*, 39, 1359-1362. doi: 10.21273/HORTSCI.39.6.1359

Jeong, J., Huber, D. J., & Sargent, S. A. (2002). Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (*Persea americana*) fruit. *Postharvest Biology and Technology*, 25(3), 241-256. doi: 10.1016/S0925-5214(01)00184-3

Jeyasri, R., Muthuramalingam, P., Karthick, K., Shin, H., Choi, S. H., & Ramesh, M. (2023). Methyl jasmonate and salicylic acid as powerful elicitors for enhancing the production of secondary metabolites in medicinal plants: an updated review. *Plant Cell, Tissue and Organ Culture*, 153(3), 447-458. doi: 10.1007/s11240-023-02485-8

Jiang, L., Jin, P., Wang, L., Yu, X., Wang, H., & Zheng, Y. (2015). Methyl jasmonate primes defense responses against *Botrytis cinerea* and reduces disease development in harvested table grapes. *Scientia Horticulturae*, 192, 218-223. doi: 10.1016/j.scienta.2015.06.015

Jiao, J., Jin, M., Liu, H., Suo, J., Yin, X., Zhu, Q., & Rao, J. (2022). Application of melatonin in kiwifruit (*Actinidia chinensis*) alleviated chilling injury during cold storage. *Scientia Horticulturae*, 296, 110876. doi: 10.1016/j.scienta.2022.110876

Jiménez-Bolaño, D. C., Insuasty, D., Rodríguez Macías, J. D., & Grande-Tovar, C. D. (2024). Potential Use of Tomato Peel, a Rich Source of Lycopene, for Cancer Treatment. *Molecules*, 29(13), 3079. doi: 10.3390/molecules29133079

Jin, M., Jiao, J., Zhao, Q., Ban, Q., Gao, M., Suo, J., Zhu, Q., & Rao, J. (2021). Dose effect of exogenous abscisic acid on controlling lignification of postharvest kiwifruit (*Actinidia chinensis* cv. hongyang). *Food Control*, 124, 107911. doi: 10.1016/j.foodcont.2021.107911

Jürgens, A., Witt, T., & Gottsberger, G. (2003). Flower scent composition in *Dianthus* and *Saponaria* species (Caryophyllaceae) and its relevance for pollination biology and taxonomy. *Biochemical Systematics and Ecology*, 31(4), 345-357. doi: 10.1016/S0305-1978(02)00173-4

-K-

Kamakshi, K., MohanaPrasad, J., Muthamilarasan, M., & Radhakrishnan, N. (2023). Foliar application of Methyl Jasmonate Methyl Ester elicits differential antioxidant defence and expression of defence-related genes against early blight disease of tomato. *Journal of Phytopathology*, 171(7-8), 362-375. doi: 10.1111/jph.13195

Kamble, V., Narayana, C. K., Karunakaran, G., Sudhakar Rao, D. V., Sriram, S., & RH, L. (2024). Differential efficacy of storage temperature and postharvest treatment on shelf life and quality of Avocado fruit (*Persea americana* Mill.). *Plant Foods for Human Nutrition*, 1-8. doi: 10.1007/s11130-024-01207-5

Kapała, A., Szlendak, M., & Motacka, E. (2022). The anti-cancer activity of lycopene: a systematic review of human and animal studies. *Nutrients*, 14(23), 5152. doi: 10.3390/nu14235152

Kappel, F. (2010). Sweet cherry cultivars vary in their susceptibility to spring frosts. *HortScience*, 45(1), 176-177. doi: 10.21273/HORTSCI.45.1.176

Kassim, A., Workneh, T. S., & Bezuidenhout, C. N. (2013). A review on postharvest handling of avocado fruit. *African Journal of Agricultural Research*, 8(21), 2385-2402. doi: 10.5897/AJAR12.1248

Kataoka, I., Beppu, K., Sugiyama, A., & Taira, S. (1996). Enhancement of coloration of "Satohnishiki" sweet cherry fruit by postharvest irradiation with ultraviolet rays. *Environment Control in Biology*, 34(4), 313-319. doi: 10.2525/ecb1963.34.313

Kathiresan, A., Tung, P., Chinnappa, C. C., & Reid, D. M. (1997). [gamma]-Aminobutyric acid stimulates ethylene biosynthesis in sunflower. *Plant Physiology*, 115(1), 129-135. doi: 10.1104/pp.115.1.129

Kawakami, E. M., Oosterhuis, D. M., & Snider, J. L. (2010). Physiological effects of 1-methylcyclopropene on well-watered and water-stressed cotton plants. *Journal of Plant Growth Regulation*, 29, 280-288. doi: 10.1007/s00344-009-9134-3

Kaya, O., & Kose, C. (2022). Sensitivity of some sweet cherry (*Prunus avium* L.) cultivars to late spring frosts during different phenological stages following bud burst. *Theoretical and Applied Climatology*, 148(3), 1713-1725. doi: 10.1007/s00704-022-04030-7

Kelebek, H., & Selli, S. (2011). Evaluation of chemical constituents and antioxidant activity of sweet cherry (*Prunus avium* L.) cultivars. *International Journal of Food Science and Technology*, 46(12), 2530-2537. doi: 10.1111/j.1365-2621.2011.02777.x

Kesari, R., Trivedi, P. K., & Nath, P. (2010). Gene expression of pathogenesis-related protein during banana ripening and after treatment with 1-MCP. *Postharvest Biology and Technology*, 56(1), 64-70. doi: 10.1016/j.postharvbio.2009.11.012

Khalid, M., Bilal, M., & Huang, D. F. (2019). Role of flavonoids in plant interactions with the environment and against human pathogens — A review. *Journal of Integrative Agriculture*, 18(1), 211-230. doi: 10.1016/S2095-3119(19)62555-4

Khan, A. S., Singh, Z., Abbasi, N. A., & Swinny, E. E. (2008). Pre-or post-harvest applications of putrescine and low temperature storage affect fruit ripening and quality of 'Angelino' plum. *Journal of the Science of Food and Agriculture*, 88(10), 1686-1695. doi: 10.1002/jsfa.3265

Khanna, R. R., Jahan, B., Iqbal, N., Khan, N. A., AlAjmi, M. F., Rehman, M. T., & Khan, M. I. R. (2021). GABA reverses salt-inhibited photosynthetic and growth responses through its influence on NO-mediated nitrogen-sulfur assimilation and antioxidant system in wheat. *Journal of Biotechnology*, 325, 73-82. doi: 10.1016/j.jbiotec.2020.11.015

Khanna-Chopra, R. (2012). Leaf senescence and abiotic stresses share reactive oxygen species-mediated chloroplast degradation. *Protoplasma*, 249, 469-481. doi: 10.1007/s00709-011-0308-z

Khosroshahi, M. R. Z., Esna-Ashari, M., & Ershadi, A. (2007). Effect of exogenous putrescine on post-harvest life of strawberry (*Fragaria ananassa* Duch.) fruit, cultivar Selva. *Scientia Horticulturae*, 114(1), 27-32. doi: 10.1016/j.scienta.2007.05.006

Khutare, S. R., & Deshmukhs, A. S. (2023). A review on various medicinal applications of kiwi fruit. *International Journal of Science and Research Archive*, 8(2), 193-206. doi: 10.30574/ijsra.2023.8.2.0112

Kibar, H., Taş, A., & Gündoğdu, M. (2021). Evaluation of biochemical changes and quality in peach fruit: Effect of putrescine treatments and storage. *Journal of Food Composition and Analysis*, 102, 104048. doi: 10.1016/j.jfca.2021.104048

Knoche, M. Winkler, A. (2017). Chapter 7: Rain-induced cracking of sweet cherries. In: Quero-García, J., Iezzoni, A., Puławska, J., Lang, G., (Eds.), Cherries: Botany, Production and Uses. CABI, pp. 140-165. doi: 10.1079/9781780648378.0140

Knoche, M., & Peschel, S. (2006). Water on the surface aggravates microscopic cracking of the sweet cherry fruit cuticle. *Journal of the American Society for Horticultural Science*, 131(2), 192-200. doi: 10.21273/JASHS.131.2.192

Koukounaras, A., & Sfakiotakis, E. (2007). Effect of 1-MCP prestorage treatment on ethylene and CO_2 production and quality of 'Hayward' kiwifruit during shelf-life after short, medium and long term cold storage. *Postharvest Biology and Technology*, 46(2), 174-180. doi: 10.1016/j.postharvbio.2007.05.002

Kucuker, E., Aglar, E., Sakaldaş, M., Şen, F., & Gundogdu, M. (2023). Impact of postharvest putrescine treatments on phenolic compounds, antioxidant capacity, organic acid contents and some quality characteristics of fresh fig fruits during cold storage. *Plants*, 12(6), 1291. doi: 10.3390/plants12061291

Kumar, N., Gautam, A., Dubey, A. K., Ranjan, R., Pandey, A., Kumari, B., Singh, G., Mandotra, S., Chauhan, P. S., Srikrishna, S., Dutta, V., & Mallick, S. (2019). GABA mediated reduction of arsenite toxicity in rice seedling through modulation of fatty acids, stress responsive amino acids and polyamines biosynthesis. *Ecotoxicology and Environmental Safety*, 173, 15-27. doi: 10.1016/j.ecoenv.2019.02.017

Kumar, S., Korra, T., Thakur, R., Arutselvan, R., Kashyap, A. S., Nehela, Y., Chaplygin, V., Minkina, T., & Keswani, C. (2023). Role of plant secondary metabolites in defence and transcriptional regulation in response to biotic stress. *Plant Stress*, 8, 100154. doi: 10.1016/j.stress.2023.100154

Kurubaş, M. S., Sabotic, J., & Erkan, M. (2021). Effects of 1-methylcyclopropene (1-MCP) treatment on antioxidant enzymes and fruit quality parameters of cold-stored baby squash. *Turkish Journal of Agriculture and Forestry*, 45(1), 33-45. doi: 10.3906/tar-2004-112

Kuznetsov, V. V., & Shevyakova, N. I. (2007). Polyamines and stress tolerance of plants. *Plant Stress*, 1(1), 50-71.

-L-

Landrier, J. F., Breniere, T., Sani, L., Desmarchelier, C., Mounien, L., & Borel, P. (2023). Effect of tomato, tomato-derived products and lycopene on metabolic inflammation: from epidemiological data to molecular mechanisms. *Nutrition Research Reviews*, 1-43. doi: 10.1017/S095442242300029X

Lattanzio, V. (2003). Bioactive polyphenols: their role in quality and storability of fruit and vegetables. *Journal of Applied Botany*, 77(5/6), 128-146.

Lee, D. B., Lee, G. J., Kim, S. H., Kang, I. K., Choi, S. J., & Yun, H. K. (2020). Changes in fruit characteristics and expression of ripening-related genes in 'madoka' peaches treated with 1-Methylcyclopropene and Aminoethoxyvinylglycine. *Horticultural Science and Technology*, 38(1), 32-43. doi: 10.7235/HORT.20200004

Lezoul, N. E. H., Belkadi, M., Habibi, F., & Guillén, F. (2020). Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. *Molecules*, 25(20), 4672. doi: 10.3390/molecules25204672

Li, C., He, Q., Zhang, F., Yu, J., Li, C., Zhao, T., Zhang, Y., Xie, Q., Su, B., Mei, L., Zhu, S., & Chen, J. (2019b). Melatonin enhances cotton immunity to Verticillium wilt via manipulating lignin and gossypol biosynthesis. *The Plant Journal*, 100(4), 784-800. doi: 10.1111/tpj.14477

Li, C., Zhu, J., Sun, L., Cheng, Y., Hou, J., Fan, Y., & Ge, Y. (2021). Exogenous γ-aminobutyric acid maintains fruit quality of apples through regulation of ethylene anabolism and polyamine metabolism. *Plant Physiology and Biochemistry*, 169, 92-101. doi: 10.1016/j.plaphy.2021.11.008

Li, J., Zhou, X., Wei, B., Cheng, S., Zhou, Q., & Ji, S. (2019c). GABA application improves the mitochondrial antioxidant system and reduces peel browning in 'Nanguo' pears after removal from cold storage. *Food Chemistry*, 297, 124903. doi: 10.1016/j.foodchem.2019.05.177

Li, K., Liu, L., McClements, D. J., Liu, Z., Liu, X., & Liu, F. (2024a). A review of the bioactive compounds of kiwifruit: Bioactivity, extraction, processing and challenges. *Food Reviews International*, 40(3), 996-1027. doi: 10.1080/87559129.2023.2212033

Li, L., Dou, N., Zhang, H., & Wu, C. (2021c). The versatile GABA in plants. *Plant signaling & behavior*, 16(3), 1862565. doi: 10.1080/15592324.2020.1862565

Li, M., Zhang, X., Li, J., Ali, M., Wang, Y., Liu, X., Li, F. & Li, X. (2024b). GABA primes defense responses against *Botrytis cinerea* in tomato fruit by modulating ethylene and JA signaling pathways. *Postharvest Biology and Technology*, 208, 112665. doi: 10.1016/j.postharvbio.2023.112665

Li, N., Wu, X., Zhuang, W., Xia, L., Chen, Y., Wu, C., Rao, Z., Du, L., Zhao, R., Yi, M., Wan, Q., & Zhou, Y. (2021b). Tomato and lycopene and multiple health outcomes: Umbrella review. *Food Chemistry*, 343, 128396. doi: 10.1016/j.foodchem.2020.128396

Li, R., Yang, S., Wang, D., Liang, J., Huang, T., Zhang, L., & Luo, A. (2021a). Electron-beam irradiation delayed the postharvest senescence of kiwifruit during cold storage through regulating the reactive oxygen species metabolism. *Radiation Physics and Chemistry*, 189, 109717. doi: 10.1016/j.radphyschem.2021.109717

Li, S., Cheng, Y., Yan, R., Liu, Y., Huan, C., & Zheng, X. (2022a). Preharvest spray with melatonin improves postharvest disease resistance in cherry tomato fruit. *Postharvest Biology and Technology*, 193, 112055. doi: 10.1016/j.postharvbio.2022.112055

Li, S., Xu, Y., Bi, Y., Zhang, B., Shen, S., Jiang, T., & Zheng, X. (2019a). Melatonin treatment inhibits gray mold and induces disease resistance in cherry tomato fruit during postharvest. *Postharvest Biology and Technology*, 157, 110962. doi: 10.1016/j.postharvbio.2019.110962

Li, X., Li, M., Wang, L., Wang, J., Jin, P., & Zheng, Y. (2018). Methyl jasmonate primes defense responses against wounding stress and enhances phenolic accumulation in fresh-cut pitaya fruit. *Postharvest Biology and Technology*, 145, 101-107. doi: 10.1016/j.postharvbio.2018.07.001

Li, X., Yun, J., Fan, X., Xing, Y., & Tang, Y. (2011). Effect of 1-methylcyclopropene and modified atmosphere packaging on chilling injury and antioxidative defensive mechanism of sweet pepper. *African Journal of Biotechnology*, 10(34), 6581-6589.

Li, Y. L., Zhang, S. Y., Xue, Y. H., & Wang, J. R. (2022b). Effect of pre-harvest 1-methylcyclopropene application on internal browning and storage quality of Bartlett pear. *Journal of Fruit Science*, 39(11), 2141-2148. doi: 10.13925/j.cnki.gsxb.20220160

Li, Z. X., Lan, J. B., Liu, Y. Q., Qi, L. W., & Tang, J. M. (2020). Investigation of the role of AcTPR2 in kiwifruit and its response to *Botrytis cinerea* infection. *BMC Plant Biology*, 20, 1-11. doi: 10.1186/s12870-020-02773-x

Liang, S. M., Kuang, J. F., Ji, S. J., Chen, Q. F., Deng, W., Min, T., Shan, W., Chen, J. Y., & Lu, W. J. (2020). The membrane lipid metabolism in horticultural products suffering chilling injury. *Food Quality and Safety*, 4(1), 9-14. doi: 10.1093/fqsafe/fyaa001

Liao, L., Li, S., Li, Y., Huang, Z., Li, J., Xiong, B., Zhang, M., Sun, G., & Wang, Z. (2022). Pre-or post-harvest treatment with MeJA improves post-harvest storage of lemon fruit by stimulating the antioxidant system and alleviating chilling injury. *Plants*, 11(21), 2840. doi: 10.3390/plants11212840

Lieu, M. D., Phuong, T. V., Nguyen, T. T. B., Dang, T. K. T., & Nguyen, T. H. (2024). A review of preservation approaches for extending avocado fruit shelf-life. *Journal of Agriculture and Food Research*, 16, 101102. doi: 10.1016/j.jafr.2024.101102

Lim, S., Han, S. H., Kim, J., Lee, H. J., Lee, J. G., & Lee, E. J. (2016). Inhibition of hardy kiwifruit (*Actinidia aruguta*) ripening by 1-methylcyclopropene during cold storage and anticancer properties of the fruit extract. *Food Chemistry*, 190, 150-157. doi: 10.1016/j.foodchem.2015.05.085

Lin, M., Gao, Z., Wang, X., Huo, H., Mao, J., Gong, X., Chen, L., Ma, S., & Cao, Y. (2023). Eco-friendly managements and molecular mechanisms for improving postharvest quality and extending shelf life of kiwifruit: A review. *International Journal of Biological Macromolecules*, 128450. doi: 10.1016/j.ijbiomac.2023.128450

Lin, Y., Fan, L., Xia, X., Wang, Z., Yin, Y., Cheng, Y., & Li, Z. (2019). Melatonin decreases resistance to postharvest green mold on citrus fruit by scavenging defense-related reactive oxygen species. *Postharvest Biology and Technology*, 153, 21-30. doi: 10.1016/j.postharvbio.2019.03.016

Liu, B., Li, Y., Zhang, X., Liu, Y., Liu, C., Wang, H., Ren, S., Ma, F., Liang, W., & Li, C. (2022). Exogenous GABA prevents Marssonina apple blotch damage in 'Royal Gala' apple seedlings. *Scientia Horticulturae*, 299, 111005. doi: 10.1016/j.scienta.2022.111005

Liu, H., Pei, H., Jiao, J., Jin, M., Li, H., Zhu, Q., Ma, Y., & Rao, J. (2021a). 1-Methylcyclopropene treatment followed with ethylene treatment alleviates postharvest chilling injury of 'Xuxiang' kiwifruit during low-temperature storage. *Food Control*, 130, 108340. doi: 10.1016/j.foodcont.2021.108340

Liu, J., Zhang, R., Sun, Y., Liu, Z., Jin, W., & Sun, Y. (2016). The beneficial effects of exogenous melatonin on tomato fruit properties. *Scientia Horticulturae*, 207, 14-20. doi: 10.1016/j.scienta.2016.05.003

Liu, L., Huang, A., Wang, B., Zhang, H., Zheng, Y., & Wang, L. (2024). Melatonin mobilizes the metabolism of sugars, ascorbic acid and amino acids to cope with chilling injury in postharvest pear fruit. *Scientia Horticulturae*, 323, 112548. doi: 10.1016/j.scienta.2023.112548

Liu, Q., Li, X., Jin, S., Dong, W., Zhang, Y., Chen, W., Shi, L., Cao, S., & Yang, Z. (2023). γ-Aminobutyric acid treatment induced chilling tolerance in postharvest kiwifruit (*Actinidia chinensis* cv. Hongyang) via regulating ascorbic acid metabolism. *Food Chemistry*, 404, 134661. doi: 10.1016/j.foodchem.2022.134661

Liu, Y., Lv, G., Chai, J., Yang, Y., Ma, F., & Liu, Z. (2021b). The Effect of 1-MCP on the expression of carotenoid, chlorophyll degradation, and ethylene response factors in 'Qihong' kiwifruit. *Foods*, 10(12), 3017. doi: 10.3390/foods10123017

Lombardelli, C., Benucci, I., & Esti, M. (2021). Novel food colorants from tomatoes: Stability of carotenoid-containing chromoplasts under different storage conditions. *LWT*, 140, 110725. doi: 10.1016/j.lwt.2020.110725

Looney, N., & Jackson, D. (2011). Stone fruits. In Temperate and subtropical fruit production, Cambridge University Press, Cambridge, pp. 161-180. doi: 10.1079/9781845935016.0161

Lorente-Mento, J. M., Serrano, M., Martínez-Romero, D., Ruiz-Aracil, M. C., Valero, D., & Guillén, F. (2024). The Simultaneous Use of 1-Methylcyclopropene and Methyl Jasmonate Vapor as an Innovative Strategy for Reducing Chilling Injury and Maintaining Pomegranate Fruit Quality at Suboptimal Temperatures. *Foods*, 13(1), 60. doi: 10.3390/foods13010060

Lorente-Mento, J. M., Valero, D., Martínez-Romero, D., Badiche, F., Serrano, M., & Guillén, F. (2023). Preharvest multiple applications of GABA improve quality traits and antioxidant compounds of pomegranate fruit during storage. *Horticulturae*, 9(5), 534. doi: 10.3390/horticulturae9050534

Luo, A., Bai, J., Li, R., Fang, Y., Li, L., Wang, D., Zhang, L., Liang, J., Huang, T., & Kou, L. (2019). Effects of ozone treatment on the quality of kiwifruit during postharvest storage affected by *Botrytis cinerea* and *Penicillium expansum*. *Journal of Phytopathology*, 167(7-8), 470-478. doi: 10.1111/jph.12819

Lv, N., Wang, C. P., Zhou, H. T., Guo, C. J., Zhang, H. Y., & Ren, D. Y. (2023). 1-Methylcyclopropene alleviates postharvest chilling injury of snap beans by enhancing antioxidant defense system. *Food Technology and Biotechnology*, 61(3), 283-293. doi: 10.17113/ftb.61.03.23.7860

Lwin, H. P., & Lee, J. (2021). Preharvest 1-methylcyclopropene treatment effects on fruit quality attributes and targeted metabolites in 'Wonhwang' pears stored at room temperature after cold storage. *Scientia Horticulturae*, 289, 110480. doi: 10.1016/j.scienta.2021.110480

Lwin, H. P., Rudell, D. R., & Lee, J. (2021). Metabolism and cold chain performance of 'Chuhwangbae' Asian pears as impacted by 1-MCP treatment. *Scientia Horticulturae*, 288, 110357. doi: 10.1016/j.scienta.2021.110357

-M-

Ma, M., Zhu, Z., Cheng, S., Zhou, Q., Zhou, X., Kong, X., Hu, M., Yin, X., Wei, B., & Ji, S. (2020). Methyl jasmonate alleviates chilling injury by regulating membrane lipid composition in green bell pepper. *Scientia Horticulturae*, 266, 109308. doi: 10.1016/j.scienta.2020.109308

Machado, M., Costa, E. M., Silva, S., Rodriguez-Alcalá, L. M., Gomes, A. M., & Pintado, M. (2023). Understanding the Anti-Obesity Potential of an Avocado Oil-Rich Cheese through an In Vitro Co-Culture Intestine Cell Model. *Molecules*, 28(15), 5923. doi: 10.3390/molecules28155923

Madebo, M. P., Luo, S. M., Li, W. A. N. G., Zheng, Y. H., & Peng, J. I. N. (2021). Melatonin treatment induces chilling tolerance by regulating the contents of polyamine, γ-aminobutyric acid, and proline in cucumber fruit. *Journal of Integrative Agriculture*, 20(11), 3060-3074. doi: 10.1016/S2095-3119(20)63485-2

Magri, A., Cice, D., Capriolo, G., & Petriccione, M. (2022). Effects of ascorbic acid and melatonin treatments on antioxidant system in fresh-cut avocado fruits during cold storage. *Food and Bioprocess Technology*, 15(11), 2468-2482. doi: 10.1007/s11947-022-02892-3

Malik, A. U., & Zora, S. (2005). Pre-storage application of polyamines improves shelf-life and fruit quality of mango. *The Journal of Horticultural Science and Biotechnology*, 80(3), 363-369. doi: 10.1080/14620316.2005.11511945

Malik, N. A. A., Kumar, I. S., & Nadarajah, K. (2020). Elicitor and receptor molecules: orchestrators of plant defense and immunity. International Journal of Molecular Sciences, 21(3), 963. doi: 10.3390/ijms21030963

Manigo, B., & Matuginas, J. P. (2020). Effects of Pre-harvest Application of 1-Methylcyclopropene (1-MCP) on the Postharvest Quality of 'Cavendish' Banana (Musa cavendishii).

Southeastern Philippines Journal of Research and Development, 25(1), 135-152. doi: 10.53899/spjrd.v25i1.50

Manzoor, A., Bashir, M. A., Naveed, M. S., Akhtar, M. T., & Saeed, S. (2024). Postharvest Chemical Treatment of Physiologically Induced Stem End Blockage Improves Vase Life and Water Relation of Cut Flowers. *Horticulturae*, 10(3), 271. doi: 10.3390/horticulturae10030271

Mao, L. C., Wang, G. Z., Zhu, C. G., & Pang, H. Q. (2007). Involvement of phospholipase D and lipoxygenase in response to chilling stress in postharvest cucumber fruits. *Plant science*, 172(2), 400-405. doi: 10.1016/j.plantsci.2006.10.002

Martínez-Esplá, A., Zapata, P. J., Castillo, S., Guillén, F., Martínez-Romero, D., Valero, D., & Serrano, M. (2014). Preharvest application of methyl jasmonate (MeJA) in two plum cultivars. 1. Improvement of fruit growth and quality attributes at harvest. *Postharvest Biology and Technology*, 98, 98-105. doi: 10.1016/j.postharvbio.2014.07.011

Martínez-Romero, D., Alburquerque, N., Valverde, J. M., Guillén, F., Castillo, S., Valero, D., & Serrano, M. J. P. B. (2006). Postharvest sweet cherry quality and safety maintenance by Aloe vera treatment: a new edible coating. *Postharvest Biology and Technology*, 39(1), 93-100. doi: 10.1016/j.postharvbio.2005.09.006

Martínez-Romero, D., Serrano, M., Carbonell, A., Burgos, L., Riquelme, F., & Valero, D. (2002). Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. *Journal of Food Science*, 67(5), 1706-1712. doi: 10.1111/j.1365-2621.2002.tb08710.x

Mata, C. I., Magpantay, J., Hertog, M. L., Van de Poel, B., & Nicolaï, B. M. (2021). Expression and protein levels of ethylene receptors, CTRs and EIN2 during tomato fruit ripening as affected by 1-MCP. *Postharvest Biology and Technology*, 179, 111573. doi: 10.1016/j.postharvbio.2021.111573

Mateos-Fierro, Z., Garratt, M. P., Fountain, M. T., Ashbrook, K., & Westbury, D. B. (2022). Wild bees are less abundant but show better pollination behaviour for sweet cherry than managed pollinators. *Journal of Applied Entomology*, 146(4), 361-371. doi: 10.1111/jen.12984

Matteo, M., Zoffoli, J. P., & Ayala, M. (2022). Calcium sprays and crop load reduction increase fruit quality and postharvest storage in sweet cherry (*Prunus avium* L.). *Agronomy*, 12(4), 829. doi: 10.3390/agronomy12040829

Matzneller, P., Götz, K. P., & Chmielewski, F. M. (2016). Spring frost vulnerability of sweet cherries under controlled conditions. *International Journal of Biometeorology*, 60, 123-130. doi: 10.1007/s00484-015-1010-1

Mayak, S., Vaadia, Y., & Dilley, D. R. (1977). Regulation of senescence in carnation (*Dianthus caryophyllus*) by ethylene: mode of action. *Plant Physiology*, 59(4), 591-593. doi: 10.1104/pp.59.4.591

McAtee, P. A., Richardson, A. C., Nieuwenhuizen, N. J., Gunaseelan, K., Hoong, L., Chen, X., Atkinson, R. G., Burdon, J. N., David, K. M., & Schaffer, R. J. (2015). The hybrid non-ethylene and ethylene ripening response in kiwifruit (*Actinidia chinensis*) is associated with differential regulation of MADS-box transcription factors. *BMC Plant Biology*, 15, 1-16. doi: 10.1186/s12870-015-0697-9

Measham, P. F., Bound, S. A., Gracie, A. J., & Wilson, S. J. (2009). Incidence and type of cracking in sweet cherry (*Prunus avium* L.) are affected by genotype and season. *Crop and Pasture Science*, 60(10), 1002-1008. doi: 10.1071/CP08410

Medina-Santamarina, J., Guillén, F., Ilea, M. I. M., Ruiz-Aracil, M. C., Valero, D., Castillo, S., & Serrano, M. (2023). Melatonin treatments reduce chilling injury and delay ripening, leading to maintenance of quality in cherimoya fruit. *International Journal of Molecular Sciences*, 24(4), 3787. doi: https://doi.org/10.3390/ijms24043787.

Medina-Santamarina, J., Serrano, M., Lorente-Mento, J. M., García-Pastor, M. E., Zapata, P. J., Valero, D., & Guillén, F. (2021). Melatonin treatment of pomegranate trees increases crop yield and quality parameters at harvest and during storage. *Agronomy*, 11(5), 861. doi: 10.3390/agronomy11050861

Medina-Santamarina, J., Serrano, M., Ruiz-Aracil, M. C., Ilea, M. I. M., Martínez-Romero, D., & Guillén, F. (2022). A synergistic effect based on the combination of melatonin with 1-methylcyclopropene as a new strategy to increase chilling tolerance and general quality in zucchini fruit. Foods, 11(18), 2784.

Meena, M., Yadav, G., Sonigra, P., Nagda, A., Mehta, T., Swapnil, P., & Marwal, A. (2022). Role of elicitors to initiate the induction of systemic resistance in plants to biotic stress. *Plant Stress*, 5, 100103. doi: 10.1016/j.stress.2022.100103

Mekontso, F. N., Duan, W., Cisse, E. H. M., Chen, T., & Xu, X. (2021). Alleviation of postharvest chilling injury of carambola fruit by γ-aminobutyric acid: Physiological, biochemical, and structural characterization. *Frontiers in Nutrition*, 8, 752583. doi: 10.3389/fnut.2021.752583

Meng, X., Han, J., Wang, Q., & Tian, S. (2009). Changes in physiology and quality of peach fruits treated by methyl jasmonate under low temperature stress. *Food Chemistry*, 114(3), 1028-1035. doi: 10.1016/j.foodchem.2008.09.109

Meyer, M. D., & Terry, L. A. (2010). Fatty acid and sugar composition of avocado, cv. Hass, in response to treatment with an ethylene scavenger or 1-methylcyclopropene to extend storage life. *Food Chemistry*, 121(4), 1203-1210. doi: 10.1016/j.foodchem.2010.02.005

Michailidis, M., Tanou, G., Sarrou, E., Karagiannis, E., Ganopoulos, I., Martens, S., & Molassiotis, A. (2021). Pre-and post-harvest melatonin application boosted phenolic compounds accumulation and altered respiratory characters in sweet cherry fruit. *Frontiers in Nutrition*, 8, 695061. doi: 10.3389/fnut.2021.695061

Minh, N. P. (2022). Quality attributes of cantaloupe (Cucumis melo L.) fruit under pre-harvest and post-harvest treatment with methyl jasmonate and salicylic acid. *Plant Science Today*, 9(1), 52-61. doi: 10.14719/pst.1456

Mirdehghan, S. H., & Rahimi, S. (2016). Pre-harvest application of polyamines enhances antioxidants and table grape (*Vitis vinifera* L.) quality during postharvest period. *Food Chemistry*, 196, 1040-1047. doi: 10.1016/j.foodchem.2015.10.038

Mirdehghan, S. H., Rahemi, M., Castillo, S., Martínez-Romero, D., Serrano, M., & Valero, D. (2007). Pre-storage application of polyamines by pressure or immersion improves shelf-life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels. *Postharvest Biology and Technology*, 44(1), 26-33. doi: 10.1016/j.postharvbio.2006.11.010

Mohamed, I. A., Shalby, N., MA El-Badri, A., Saleem, M. H., Khan, M. N., A. Nawaz, M., Qin, M., Agami, R. A., Kuai, J., Wang, B., & Zhou, G. (2020). Stomata and xylem vessels traits improved by melatonin application contribute to enhancing salt tolerance and fatty acid composition of *Brassica napus* L. plants. *Agronomy*, 10(8), 1186. doi: 10.3390/agronomy10081186

Mohamed, M., & Saleh, M. M. S. (2013). Effect of potassium and polyamine sprays on fruit set, fruit retention, yield and fruit quality of Amhat date palm. *International Journal of Agricultural Research*, 8, 77-86. doi: 10.3923/ijar.2013.77.86

Mohammadi, M., Aelaei, M., & Saidi, M. (2020). Pre-harvest and pulse treatments of spermine, γ-and β-aminobutyric acid increased antioxidant activities and extended the vase life of gerbera cut flowers 'Stanza'. *Ornamental Horticulture*, 26(2), 306-316. doi: 10.1590/2447-536X.v26i2.2120

Mohammadi, M., Aelaei, M., & Saidi, M. (2021). Pre-harvest spray of GABA and spermine delays postharvest senescence and alleviates chilling injury of gerbera cut flowers during cold storage. *Scientific Reports*, 11(1), 14166. doi: 10.1038/s41598-021-93377-4

Mohammadi, M., Eghlima, G., & Ranjbar, M. E. (2023). Ascorbic acid reduces chilling injury in anthurium cut flowers during cold storage by increasing salicylic acid biosynthesis. *Postharvest* Biology and Technology, 201, 112359. doi: 10.1016/j.postharvbio.2023.112359

Moradi, M., Razavi, F., Rabiei, V., Soleimani Aghdam, M., & Salehi, L. (2020). Effects of gamma-aminobutyric acid treatment on postharvest chilling injury on tomato fruit. Journal of Horticultural Science, 34(2), 221-230. doi: 10.22067/jhorts4.v34i2.78718

Morales-Solis, A., Pérez-López, A., Ramírez-Guzmán, M. E., Espinosa-Solares, T., & Alia-Tejacal, I. (2024). ARIMAX Modelling: Response of Hass Avocado Respiration Rate to Environmental Factors. *Horticulturae*, 10(7), 700. doi: 10.3390/horticulturae10070700

Moreno-Pérez, A., Martínez-Ferri, E., Berg, N. V. D., & Pliego, C. (2024). Effects of Exogenous Application of Methyl Jasmonate and Salicylic Acid on the Physiological and Molecular Response of 'Dusa' Avocado to *Rosellinia necatrix*. *Plant Disease*, 108(7), 2111-2121. doi: 10.1094/PDIS-11-23-2316-RE

Mostofi, Y., Toivonen, P. M., Lessani, H., Babalar, M., & Lu, C. (2003). Effects of 1-methylcyclopropene on ripening of greenhouse tomatoes at three storage temperatures. *Postharvest Biology and Technology*, 27(3), 285-292. doi: 10.1016/S0925-5214(02)00113-8

Moysidou, A. M., Cheimpeloglou, K., Koutra, S. I., Finos, M. A., Ofrydopoulou, A., & Tsoupras, A. (2024). A Comprehensive Review on the Antioxidant and Anti-Inflammatory Bioactives of Kiwi and Its By-Products for Functional Foods and Cosmetics with Health-Promoting Properties. *Applied Sciences*, 14(14), 2076-3417. doi: 10.3390/app14145990

Mpeluza, A. S., Akinola, S. A., Shoko, T., Remize, F., & Sivakumar, D. (2023). Efficacy of Lactic Acid Bacteria as a Biocontrol Agent against Anthracnose (*Persea americana* Miller) Decay in Avocado (*Persea americana*) cv Fuerte Fruit. *Agriculture*, 13(2), 269. doi: 10.3390/agriculture13020269

Munhuweyi, K., Mpai, S., & Sivakumar, D. (2020). Extension of avocado fruit postharvest quality using non-chemical treatments. *Agronomy*, 10(2), 212. doi: 10.3390/agronomy10020212

Murch, S. J., Alan, A. R., Cao, J., & Saxena, P. K. (2009). Melatonin and serotonin in flowers and fruits of *Datura metel* L. *Journal of Pineal Research*, 47(3), 277-283. doi: 10.1111/j.1600-079X.2009.00711.x

-N-

Naeemi, M. H., Jari, S. K., Zarinnia, V., & Fatehi, F. (2022). Changes in physio-biochemical status of cut of rose (Rosa hybrida L. cv. Samurai) flowers under methyl Jasmonate, brassinosteroid, and fungal elicitor to control gray mold. *Scientia Horticulturae*, 306, 111402. doi: 10.1016/j.scienta.2022.111402

Naing, A. H., & Kim, C. K. (2020). Application of nano-silver particles to control the postharvest biology of cut flowers: A review. *Scientia Horticulturae*, 270, 109463. doi: 10.1016/j.scienta.2020.109463

Nasri, C., Halabi, Y., Hajib, A., Choukri, H., Harhar, H., Lee, L. H., Mani, V., Ming, L. C., Goh, K. W., Bouyahya, A., & Tabyaoui, M. (2023). Proximate composition, lipid and elemental profiling of eight varieties of avocado (*Persea americana*). *Scientific Reports*, 13(1), 22767. doi: 10.1038/s41598-023-50119-y

Nazoori, F., ZamaniBahramabadi, E., Mirdehghan, S. H., & Rafie, A. (2020). Extending the shelf life of pomegranate (*Punica granatum* L.) by GABA coating application. *Journal of Food Measurement and Characterization*, 14(5), 2760-2772. doi: 10.1007/s11694-020-00521-1

Ngoc, T. T. B., Thu, V. M., Hien, N. T. P., Tra, H. T. T., Anh, H. D., & Hue, T. T. (2022). Effect of exogenous melatonin on antioxidant enzyme activities and membrane lipid peroxidation in avocado fruit during ripening. *Vietnam Journal of Biotechnology*, 20(3), 495-504. doi: 10.15625/1811-4989/17191

Nian, L., Xie, Y., Zhang, H., Wang, M., Yuan, B., Cheng, S., & Cao, C. (2023). Vishniacozyma victoriae: An endophytic antagonist yeast of kiwifruit with biocontrol effect to *Botrytis cinerea*. *Food Chemistry*, 411, 135442. doi: 10.1016/j.foodchem.2023.135442

Niazi, Z., Razavi, F., Khademi, O., & Aghdam, M. S. (2021). Exogenous application of hydrogen sulfide and γ-aminobutyric acid alleviates chilling injury and preserves quality of persimmon fruit (*Diospyros kaki*, cv. Karaj) during cold storage. *Scientia Horticulturae*, 285, 110198. doi: 10.1016/j.scienta.2021.110198

Nunes, M. C. N. (2015). Correlations between subjective quality and physicochemical attributes of fresh fruits and vegetables. *Postharvest Biology and Technology*, 107, 43-54. doi: 10.1016/j.postharvbio.2015.05.001

-O-

Okobi, O. E., Odoma, V. A., Okunromade, O., Louise-Oluwasanmi, O., Itua, B., Ndubuisi, C., Ogbeifun, O. E., Nwatamole, B. C., Elimihele, T. A., Adekunle, J. O., Adekunle, A. A., Obi, C. B., & Evbayekha, E. O. (2023). Effect of Avocado Consumption on Risk factors of Cardiovascular diseases: a systematic review and Meta-analysis. *Cureus*, 15(6). doi: 10.7759%2Fcureus.41189

Olivares, D., Alvarez, E., Véliz, D., García-Rojas, M., Díaz, C., & Defilippi, B. G. (2020). Effects of 1-Methylcyclopropene and Controlled Atmosphere on Ethylene Synthesis and Quality Attributes of Avocado cvs. Edranol and Fuerte. *Journal of Food Quality*, 2020(1), 5075218. doi: 10.1155/2020/5075218

Olivares, D., García-Rojas, M., Ulloa, P. A., Riveros, A., Pedreschi, R., Campos-Vargas, R., Meneses, C., & Defilippi, B. G. (2022). Response Mechanisms of "Hass" Avocado to Sequential 1–methylcyclopropene Applications at Different Maturity Stages during Cold Storage. *Plants*, 11(13), 1781. doi: 10.3390/plants11131781

Onik, J. C., Wai, S. C., Li, A., Lin, Q., Sun, Q., Wang, Z., & Duan, Y. (2021). Melatonin treatment reduces ethylene production and maintains fruit quality in apple during postharvest storage. *Food Chemistry*, 337, 127753. doi: 10.1016/j.foodchem.2020.127753

Opiyo, A. M., & Ying, T. J. (2005). The effects of 1-methylcyclopropene treatment on the shelf life and quality of cherry tomato (*Lycopersicon esculentum var. cerasiforme*) fruit. *International Journal of Food Science and Technology*, 40(6), 665-673. doi: 10.1111/j.1365-2621.2005.00977.x

Osondu, H. A. A., Akinola, S. A., Shoko, T., & Sivakumar, D. (2022). Phenolic compounds suppress anthracnose decay by enhancing antifungal properties and biochemical defence responses in avocado fruit. *Journal of Plant Pathology*, 104(2), 711-720. doi: 10.1007/s42161-022-01085-3

Ozdemir, F., & Topuz, A. (2004). Changes in dry matter, oil content and fatty acids composition of avocado during harvesting time and post-harvesting ripening period. *Food Chemistry*, 86(1), 79-83. doi: 10.1016/j.foodchem.2003.08.012

Öztekin, Y. B., Vursavuş, K. K., & Kesilmiş, Z. (2021). Firmness classification of tomato fruits by using colour parameters. *Anadolu Journal of Agriculture Sciences*, 36(3), 511-519. doi: 10.7161/omuanajas.952786

Öztürk, B., & Yücedağ, F. (2021). Effects of methyl jasmonate on quality properties and phytochemical compounds of kiwifruit (*Actinidia deliciosa* cv. Hayward) during cold storage and shelf life. *Turkish Journal of Agriculture and Forestry*, 45(2), 154-164. doi: 10.3906/tar-2004-69

Ozturk, B., Aglar, E., Saracoglu, O., Karakaya, O., & Gun, S. (2022). Effects of GA3, CaCl2 and modified atmosphere packaging (MAP) applications on fruit quality of sweet cherry at cold storage. *International Journal of Fruit Science*, 22(1), 696-710. doi: 10.1080/15538362.2022.2113597

-P-

Pachón, Y. V., Balaguera-López, H. E., & Florez-Velasco, N. (2022). Postharvest behavior and chilling injury in avocado (*Persea americana* Mill) fruit cv. Hass treated with 1-

methylcyclopropene, ethylene, and intermittent warming. *Revista Facultad Nacional de Agronomía Medellín*, 75(2), 9895-9907. doi: 10.15446/rfnam.v75n2.98741

Palma, F., Carvajal, F., Jiménez-Muñoz, R., Pulido, A., Jamilena, M., & Garrido, D. (2019). Exogenous γ-aminobutyric acid treatment improves the cold tolerance of zucchini fruit during postharvest storage. *Plant Physiology and Biochemistry*, 136, 188-195. doi: 10.1016/j.plaphy.2019.01.023

Palma, F., Carvajal, F., Ramos, J. M., Jamilena, M., & Garrido, D. (2015). Effect of putrescine application on maintenance of zucchini fruit quality during cold storage: Contribution of GABA shunt and other related nitrogen metabolites. *Postharvest Biology and Bechnology*, 99, 131-140. doi: 10.1016/j.postharvbio.2014.08.010

Pan, L., Chen, X., Xu, W., Fan, S., Wan, T., Zhang, J., & Cai, Y. (2022). Methyl jasmonate induces postharvest disease resistance to decay caused by *Alternaria alternata* in sweet cherry fruit. *Scientia Horticulturae*, 292, 110624. doi: 10.1016/j.scienta.2021.110624

Pan, L., Zhao, X., Chen, M., Fu, Y., Xiang, M., & Chen, J. (2020). Effect of exogenous methyl jasmonate treatment on disease resistance of postharvest kiwifruit. *Food Chemistry*, 305, 125483. doi: 10.1016/j.foodchem.2019.125483

Pattyn, J., Vaughan-Hirsch, J., & Van de Poel, B. (2021). The regulation of ethylene biosynthesis: a complex multilevel control circuitry. *New Phytologist*, 229(2), 770-782. doi: 10.1111/nph.16873

Paul, V., Pandey, R., & Srivastava, G. C. (2010). Ripening of tomato (*Solanum lycopersicum* L.). Part I: 1-methylcyclopropene mediated delay at higher storage temperature. *Journal of Food Science and Technology*, 47, 519-526. doi: 10.1007/s13197-010-0090-5

Pedrosa, L. D. F., & Fabi, J. P. (2024). Dietary fiber as a wide pillar of colorectal cancer prevention and adjuvant therapy. *Critical Reviews in Food Science and Nutrition*, 64(18), 6177-6197. doi: 10.1080/10408398.2022.2164245

Pereira, A. M., de Paula Gomes, M., Freire, A. I., da Costa, L. C., dos Santos, R. M. C., & Finger, F. L. (2018). Salicylic acid reduces chilling injury in post-harvest of Bird-of-Paradise. *Revista Brasileira de Ciências Agrárias*, 13(3), 1-6. doi: 10.5039/agraria.v13i3a5558

Pereira, S., Silva, V., Bacelar, E., Guedes, F., Silva, A. P., Ribeiro, C., & Gonçalves, B. (2020). Cracking in sweet cherry cultivars early bigi and lapins: Correlation with quality attributes. *Plants*, 9(11), 1557. doi: 10.3390/plants9111557

Pérez-Burillo, S., Oliveras, M. J., Quesada, J., Rufián-Henares, J. A., & Pastoriza, S. (2018). Relationship between composition and bioactivity of persimmon and kiwifruit. *Food Research International*, 105, 461-472. doi: 10.1016/j.foodres.2017.11.022

Pesis, E., Ackerman, M., Ben-Arie, R., Feygenberg, O., Feng, X., Apelbaum, A., Goren, R., & Prusky, D. (2002). Ethylene involvement in chilling injury symptoms of avocado during cold storage. *Postharvest Biology and Technology*, 24(2), 171-181. doi: 10.1016/S0925-5214(01)00134-X

Petit, J., Bres, C., Just, D., Garcia, V., Mauxion, J. P., Marion, D., Bakan, B., Joubès, J., Domergue, F., & Rothan, C. (2014). Analyses of tomato fruit brightness mutants uncover both cutin-deficient and cutin-abundant mutants and a new hypomorphic allele of GDSL lipase. *Plant Physiology*, 164, 888-906. doi: 10.1104/pp.113.232645

Pétriacq, P., López, A., & Luna, E. (2018). Fruit decay to diseases: can induced resistance and priming help?. *Plants*, 7(4), 77. doi: 10.3390/plants7040077

Phetsirikoon, S., Ketsa, S., & Doorn, W. G. V. (2012). Chilling injury in Dendrobium inflorescences is alleviated by 1-MCP treatment. *Postharvest Biology and Technology*, 67, 144-153. doi: 10.1016/j.postharvbio.2011.12.016

Pino, S., Palma, M., Sepúlveda, Á., Sánchez-Contreras, J., Moya, M., & Yuri, J. A. (2023). Effect of rain cover on tree physiology and fruit condition and quality of ‘Rainier’, ‘Bing’ and ‘Sweetheart’ sweet cherry trees. *Horticulturae*, 9(1), 109. doi: 10.3390/horticulturae9010109

Pompodakis, N., Terry, L., Joyce, D., Papadimitriou, M., Lydakis, D., & Darras, A. (2010). Effects of storage temperature and abscisic acid treatment on the vase-life of cut 'First Red' and 'Akito' roses. *The Journal of Horticultural Science and Biotechnology*, 85(3), 253-259. doi: 10.1080/14620316.2010.11512664

Porra, R. J., Thompson, W. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 975(3), 384-394. doi: 10.1016/S0005-2728(89)80347-0

Priya, M., Sharma, L., Kaur, R., Bindumadhava, H., Nair, R. M., Siddique, K. H. M., & Nayyar, H. (2019). GABA (γ-aminobutyric acid), as a thermo-protectant, to improve the reproductive function of heat-stressed mungbean plants. *Scientific Reports*, 9(1), 7788. doi: 10.1038/s41598-019-44163-w

Probst, Y., Guan, V., & Neale, E. (2024). Avocado intake and cardiometabolic risk factors in a representative survey of Australians: a secondary analysis of the 2011–2012 national nutrition and physical activity survey. *Nutrition Journal*, 23(1), 12. doi: 10.1186/s12937-024-00915-7

Prusky, D. (2011). Reduction of the incidence of postharvest quality losses, and future prospects. *Food Security*, 3, 463-474. doi: 10.1007/s12571-011-0147-y

Prusky, D., & Romanazzi, G. (2023). Induced resistance in fruit and vegetables: A host physiological response limiting postharvest disease development. *Annual Review of Phytopathology*, 61(1), 279-300. doi: 10.1146/annurev-phyto-021722-035135

Pullanagari, R. R., & Li, M. (2021). Uncertainty assessment for firmness and total soluble solids of sweet cherries using hyperspectral imaging and multivariate statistics. *Journal of Food Engineering*, 289, 110177. doi: 10.1016/j.jfoodeng.2020.110177

-Q-

Qi, T., Ji, J., Zhang, X., Liu, L., Xu, X., Ma, K., & Gao, Y. (2022). Research progress of cold chain transport technology for storage fruits and vegetables. *Journal of Energy Storage*, 56, 105958. doi: 10.1016/j.est.2022.105958

Qi, W. J., Sheng, W. S., Peng, C., Xiaodong, M., & Yao, T. Z. (2021). Investigating into anti-cancer potential of lycopene: Molecular targets. *Biomedicine and Pharmacotherapy*, 138, 111546. doi: 10.1016/j.biopha.2021.111546

Quero-García, J., Letourmy, P., Campoy, J. A., Branchereau, C., Malchev, S., Barreneche, T., & Dirlewanger, E. (2021). Multi-year analyses on three populations reveal the first stable QTLs for tolerance to rain-induced fruit cracking in sweet cherry (*Prunus avium* L.). *Horticulture Research*, 8, 136. doi: 10.1038/s41438-021-00571-6

Quillehauquy, V., Fasciglione, M. G., Moreno, A. D., Monterubbianesi, M. G., Casanovas, E. M., Sanchez, E. E., & Yommi, A. K. (2020). Effects of cold storage duration and 1-MCP treatment on ripening and 'eating window' of 'Hayward' kiwifruit. *Journal of Berry Research*, 10(3), 419-435. doi: 10.3233/JBR-190492

-R-

Rabiei, V., Kakavand, F., Zaare-Nahandi, F., Razavi, F., & Aghdam, M. S. (2019). Nitric oxide and γ-aminobutyric acid treatments delay senescence of cornelian cherry fruits during postharvest cold storage by enhancing antioxidant system activity. *Scientia Horticulturae*, 243, 268-273. doi: 10.1016/j.scienta.2018.08.034

Rai, A., Kumari, K., & Vashistha, P. (2022). Umbrella review on chilling injuries: Post-harvest issue, cause, and treatment in tomato. *Scientia Horticulturae*, 293, 110710. doi: 10.1016/j.scienta.2021.110710

Rakbar, S., Jabbarzadeh, Z., & Barin, M. (2022). Effect of exogenous putrescine on flower growth, post-harvest quality and root mycorrhizal development of gerbera (*Gerbera jamesonii* cv. Dune) cut flowers. *South African Journal of Botany*, 150, 641-650. doi: 10.1016/j.sajb.2022.08.002

Rastegar, S., Khankahdani, H. H., & Rahimzadeh, M. (2020). Effect of γ-aminobutyric acid on the antioxidant system and biochemical changes of mango fruit during storage. *Journal of Food Measurement and Characterization*, 14(2), 778-789. doi: 10.1007/s11694-019-00326-x

Razifard, H., Ramos, A., Della Valle, A. L., Bodary, C., Goetz, E., Manser, E. J., Li, X., Zhang, L., Visa, S., Tieman, D., Knaap, E. V. D., & Caicedo, A. L. (2020). Genomic evidence for complex domestication history of the cultivated tomato in Latin America. *Molecular Biology and Evolution*, 37(4), 1118-1132. doi: 10.1093/molbev/msz297

Rehman, M. U., Rather, G. H., Dar, N. A., Mir, M. M., Iqbal, U., Mir, M. R., Fayaz, S., & Hakeem, K. R. (2015). Causes and prevention of cherry cracking: A review. In: Hakeem, K. R. (Ed.), Crop production and global environmental issues. Springer Cham, pp. 543-552. doi: 10.1007/978-3-319-23162-4

Rehman, M., Singh, Z., Khurshid, T., Malekipoor, R., & Tokala, V. Y. (2021). Preharvest spray application of methyl jasmonate promotes fruit colour and regulates quality in M7 Navel orange grown in a Mediterranean climate. *Australian Journal of Crop Science*, 15(3), 387-393. doi: 10.21475/ajcs.21.15.03.p2812

Richardson, D. G. (1998). Rain-cracking of ‘Royal Ann’ sweet cherries: fruit physiological relationships, water temperature, orchard treatments, and cracking index. *Acta Horticulturae*, 468, 677-682. doi: 10.17660/ActaHortic.1998.468.85

Rodrigo, J. (2000). Spring frosts in deciduous fruit trees—morphological damage and flower hardiness. *Scientia Horticulturae*, 85(3), 155-173. doi: 10.1016/S0304-4238(99)00150-8

Rombolà, A. D., Quartieri, M., Rodríguez-Declet, A., Minnocci, A., Sebastiani, L., & Sorrenti, G. (2023). Canopy-applied silicon is an effective strategy for reducing sweet cherry cracking. *Horticulture, Environment, and Biotechnology*, 64(3), 371-378. doi: 10.1007/s13580-022-00486-8

Romero, P., & Rose, J. K. (2019). A relationship between tomato fruit softening, cuticle properties and water availability. *Food Chemistry*, 295, 300-310. doi: 10.1016/j.foodchem.2019.05.118

Rudell, D. R., Fellman, J. K., & Mattheis, J. P. (2005). Preharvest application of methyl jasmonate to'Fuji'apples enhances red coloration and affects fruit size, splitting, and bitter pit incidence. *HortScience*, 40(6), 1760-1762. doi: 10.21273/HORTSCI.40.6.1760

Rutkowski, K., & Łysiak, G. P. (2022). Thinning methods to regulate sweet cherry crops—A review. *Applied Sciences*, 12(3), 1280. doi: 10.3390/app12031280

-S-

Saavedra, G. M., Sanfuentes, E., Figueroa, P. M., & Figueroa, C. R. (2017). Independent preharvest applications of methyl jasmonate and chitosan elicit differential upregulation of defense-related genes with reduced incidence of gray mold decay during postharvest storage of Fragaria chiloensis fruit. *International Journal of Molecular Sciences*, 18(7), 1420. doi: 10.3390/ijms18071420

Saba, M. K., & Zarei, L. (2019). Preharvest methyl jasmonate’s impact on postharvest chilling sensitivity, antioxidant activity, and pomegranate fruit quality. *Journal of Food Biochemistry*, 43(3), e12763. doi: 10.1111/jfbc.12763

Saba, M. K., Arzani, K., & Barzegar, M. (2012). Postharvest polyamine application alleviates chilling injury and affects apricot storage ability. *Journal of Agricultural and Food Chemistry*, 60(36), 8947-8953. doi: 10.1021/jf302088e

Sabir, I. A., Liu, X., Jiu, S., Whiting, M., & Zhang, C. (2021). Plant growth regulators modify fruit set, fruit quality, and return bloom in sweet cherry. *HortScience*, 56(8), 922-931. doi: 10.21273/HORTSCI15835-21

Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., & Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, 10(2), 277. doi: 10.3390/antiox10020277

Saeedi, M., Mirdehghan, S. H., Nazoori, F., Esmaeilizadeh, M., & Saba, M. K. (2022). Impact of calcium and γ-aminobutyric acid (GABA) on qualitative attributes and shelf life characteristics of fresh in-hull pistachio during cold storage. *Postharvest Biology and Technology*, 187, 111863. doi: 10.1016/j.postharvbio.2022.111863

Salazar, J., Zapata, P., Silva, C., González, M., Pacheco, I., Bastías, M., Meneses, C., Jorquera, C., Moreno, I., Shinya, P., & Infante, R. (2021). Transcriptome analysis and postharvest behavior of the kiwifruit '*Actinidia deliciosa*' reveal the role of ethylene-related phytohormones during fruit ripening. *Tree Genetics and Genomes*, 17, 1-19. doi: 10.1007/s11295-021-01493-z

Salazar-López, N. J., Domínguez-Avila, J. A., Yahia, E. M., Belmonte-Herrera, B. H., Wall-Medrano, A., Montalvo-González, E., & González-Aguilar, G. A. (2020). Avocado fruit and by-products as potential sources of bioactive compounds. *Food Research International*, 138, 109774. doi: 10.1016/j.foodres.2020.109774

Sanches, A. G., & Repolho, R. P. J. (2022). Exogenous salicylic acid preserves the quality and antioxidant metabolism of avocado 'Quintal' cultivar. *Journal of Horticulture and Postharvest Research*, 5(1), 79-92. doi: 10.22077/jhpr.2022.4820.1249

Sangprayoon, P., Supapvanich, S., Youryon, P., Wongs-Aree, C., & Boonyaritthongchai, P. (2019). Efficiency of salicylic acid or methyl jasmonate immersions on internal browning alleviation and physicochemical quality of Queen pineapple cv. "Sawi" fruit during cold storage. *Journal of Food Biochemistry*, 43(12), e13059. doi: 10.1111/jfbc.13059

Saracoglu, O., Ozturk, B., Yildiz, K., & Kucuker, E. (2017). Pre-harvest methyl jasmonate treatments delayed ripening and improved quality of sweet cherry fruits. *Scientia Horticulturae*, 226, 19-23. doi: 10.1016/j.scienta.2017.08.024

Sardar, R., Ahmed, S., & Yasin, N. A. (2022). Role of exogenously applied putrescine in amelioration of cadmium stress in *Coriandrum sativum* by modulating antioxidant system. *International Journal of Phytoremediation*, 24(9), 955-962. doi: 10.1080/15226514.2021.1985961

Sarhan, A. M., Heikal, A. A., Saadawy, F. M., El-Deen, T. M. N., & Abd Elkareem, M. K. (2023). Effect of some chemical and natural preservative solutions on vase life, water relations and some chemical composition of Dianthus caryophyllus L. cut flowers. *Advances in Horticultural Science*, 37(3), 255-269. doi: 10.36253/ahsc-14247

Sarkhosh, A., Vargas, A. I., Schaffer, B., Palmateer, A. J., Lopez, P., Soleymani, A., & Farzaneh, M. (2017). Postharvest management of anthracnose in avocado (*Persea americana* Mill.) fruit with plant-extracted oils. *Food Packaging and Shelf Life*, 12, 16-22. doi: 10.1016/j.fpsl.2017.02.001

Satekge, T. K., & Magwaza, L. S. (2022). Postharvest application of 1-Methylcyclopropene (1-MCP) on climacteric fruits: Factors affecting efficacy. *International Journal of Fruit Science*, 22(1), 595-607. doi: 10.1080/15538362.2022.2085231

Satoh, S., Nukui, H., & Inokuma, T. (2005). A method for determining the vase life of cut spray carnation flowers. *Journal of Applied Horticulture*, 7(1), 8-10. doi: 10.37855/jah.2005.v07i01.02

Sayyari, M., Babalar, M., Kalantari, S., Martínez-Romero, D., Guillén, F., Serrano, M., & Valero, D. (2011). Vapour treatments with methyl salicylate or methyl jasmonate alleviated chilling injury and enhanced antioxidant potential during postharvest storage of pomegranates. *Food Chemistry*, 124(3), 964-970. doi: 10.1016/j.foodchem.2010.07.036

Schuber, F. (1989). Influence of polyamines on membrane functions. *Biochemical Journal*, 260(1), 1. doi: 10.1042%2Fbj2600001

Schumann, C., Sitzenstock, S., Erz, L., & Knoche, M. (2020). Decreased deposition and increased swelling of cell walls contribute to increased cracking susceptibility of developing sweet cherry fruit. *Planta*, 252, 1-14. doi: 10.1007/s00425-020-03494-z

Schumann, C., Winkler, A., Brüggenwirth, M., Köpcke, K., & Knoche, M. (2019). Crack initiation and propagation in sweet cherry skin: a simple chain reaction causes the crack to 'run'. *PLoS One*, 14(7), e0219794. doi: 10.1371/journal.pone.0247692

Serna C, L., Torres V, L. S., & Ayala A, A. A. (2012). Effect of pre-and postharvest application of 1-methylcyclopropene on the maturation of yellow pitahaya (Selenicerus megalanthus Haw). *Vitae*, 19(1), 49-59. doi: 10.17533/udea.vitae.10859

Serna-Escolano, V., Martínez-Romero, D., Giménez, M. J., Serrano, M., García-Martínez, S., Valero, D., Valverde, J. M., & Zapata, P. J. (2021). Enhancing antioxidant systems by preharvest treatments with methyl jasmonate and salicylic acid leads to maintain lemon quality during cold storage. *Food Chemistry*, 338, 128044. doi: 10.1016/j.foodchem.2020.128044

Serrano, M., Díaz-Mula, H. M., Zapata, P. J., Castillo, S., Guillén, F., Martínez-Romero, D., Valverde, J. M., & Valero, D. (2009). Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. *Journal of Agricultural and Food Chemistry*, 57(8), 3240-3246. doi: 10.1021/jf803949k

Serrano, M., Guillén, F., Martínez-Romero, D., Castillo, S., & Valero, D. (2005). Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *Journal of Agricultural and Food Chemistry*, 53(7), 2741-2745. doi: 10.1021/jf0479160

Sevillano, L., Sanchez-Ballesta, M. T., Romojaro, F., & Flores, F. B. (2009). Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. *Journal of the Science of Food and Agriculture*, 89(4), 555-573. doi: 10.1002/jsfa.3468

Shah, H. M. S., Singh, Z., Hasan, M. U., Afrifa-Yamoah, E., & Woodward, A. (2023). Preharvest melatonin application alleviates red drupelet reversion, improves antioxidant potential and maintains postharvest quality of 'Elvira' blackberry. *Postharvest Biology and Technology*, 203, 112418. doi: 10.1016/j.postharvbio.2023.112418

Shahzad, R., Waqas, M., Khan, A. L., Hamayun, M., Kang, S. M., & Lee, I. J. (2015). Foliar application of methyl jasmonate induced physio-hormonal changes in *Pisum sativum* under diverse temperature regimes. *Plant Physiology and Biochemistry*, 96, 406-416. doi: 10.1016/j.plaphy.2015.08.020

Shan, Y., Zhang, D., Luo, Z., Li, T., Qu, H., Duan, X., & Jiang, Y. (2022). Advances in chilling injury of postharvest fruit and vegetable: Extracellular ATP aspects. *Comprehensive Reviews in Food Science and Food Safety*, 21(5), 4251-4273. doi: 10.1111/1541-4337.13003

Shanbehpour, F., Rastegar, S., & Ghasemi, M. (2020). Effect of preharvest application of calcium chloride, putrescine, and salicylic acid on antioxidant system and biochemical changes of two Indian jujube genotypes. *Journal of Food Biochemistry*, 44(11), e13474. doi: 10.1111/jfbc.13474

Shao, J., Huang, K., Batool, M., Idrees, F., Afzal, R., Haroon, M., Noushahi, H. A., Wu, W., Hu, Q., Lu, X., Huang, G., Aamer, M., Hassan, M. U., & El Sabagh, A. (2022). Versatile roles of polyamines in improving abiotic stress tolerance of plants. *Frontiers in Plant Science*, 13, 1003155. doi: 10.3389/fpls.2022.1003155

Sharafi, Y., Aghdam, M. S., Luo, Z., Jannatizadeh, A., Razavi, F., Fard, J. R., & Farmani, B. (2019). Melatonin treatment promotes endogenous melatonin accumulation and triggers GABA shunt pathway activity in tomato fruits during cold storage. *Scientia Horticulturae*, 254, 222-227. doi: 10.1016/j.scienta.2019.04.056

Sharma, P., Thakur, N., Mann, N. A., & Umar, A. (2024). Melatonin as plant growth regulator in sustainable agriculture. *Scientia Horticulturae*, 323, 112421. doi: 10.1016/j.scienta.2023.112421

Sharma, S., Pareek, S., Sagar, N. A., Valero, D., & Serrano, M. (2017). Modulatory effects of exogenously applied polyamines on postharvest physiology, antioxidant system and shelf life of fruits: a review. *International Journal of Molecular Sciences*, 18(8), 1789. doi: 10.3390/ijms18081789

Shekari, A., Hassani, R. N., & Aghdam, M. S. (2021). Exogenous application of GABA retards cap browning in *Agaricus bisporus* and its possible mechanism. *Postharvest Biology and Technology*, 174, 111434. doi: 10.1016/j.postharvbio.2020.111434

Shelp, B. J., Aghdam, M. S., & Flaherty, E. J. (2021). γ-Aminobutyrate (GABA) regulated plant defense: Mechanisms and opportunities. *Plants*, 10(9), 1939. doi: 10.3390/plants10091939

Sheng, L., Shen, D., Luo, Y., Sun, X., Wang, J., Luo, T., Zeng, Y., Xu, J., Deng, X., & Cheng, Y. (2017). Exogenous γ-aminobutyric acid treatment affects citrate and amino acid accumulation to improve fruit quality and storage performance of postharvest citrus fruit. *Food Chemistry*, 216, 138-145. doi: 10.1016/j.foodchem.2016.08.024

Shenglong, D., Jihong, Z., Shaoyang, C., Shuang, M., & Li, Z. (2019). The combined effect of 1-methylcyclopropene and citral suppressed postharvest grey mould of tomato fruit by inhibiting the growth of *Botrytis cinerea*. *Journal of Phytopathology*, 167(2), 123-134. doi: 10.1111/jph.12780

Shikwambana, K., Mafeo, T. P., & Mathaba, N. (2021). Effect of Postharvest Methyl Jasmonate Treatment on Early-Matured "Hass" Avocado Fruit Exocarp Colour Development during Ripening. *Agricultural Sciences*, 12(8), 875-887. doi: 10.4236/as.2021.128056

Shikwambana, K., Mathaba, N., & Mafeo, T. P. (2023). The physiological effect of fruit maturity and 1-methylcyclopropene on 'Hass' avocado fruit exocarp colour and chilling injury during ripening. *Journal of Horticulture and Postharvest Research*, 6(1), 55-76. doi: 10.22077/jhpr.2022.5538.1287

Shimizu-Yumoto, H., & Ichimura, K. (2007). Effect of relative humidity and sucrose concentration on leaf injury and vase life during sucrose pulse treatment in cut Eustoma flowers. *Horticultural Research (Japan)*, 6(2), 301-305. doi: 10.2503/hrj.6.301

Shiri, M. A., Ghasemnezhad, M., Bakhshi, D., & Sarikhani, H. (2013). Effect of postharvest putrescine application and chitosan coating on maintaining quality of table grape cv. "Shahroudi" during long-term storage. *Journal of Food Processing and Preservation*, 37(5), 999-1007. doi: 10.1111/j.1745-4549.2012.00735.x

Simões, A.N, Diniz, N. B., da Silva Vieira, M. R., Ferreira-Silva, S. L., da Silva, M. B., Minatel, I. O., & Lima, G. P. P. (2018). Impact of GA3 and spermine on postharvest quality of anthurium cut flowers (*Anthurium andraeanum*) cv. Arizona. *Scientia Horticulturae*, 241, 178-186. doi: 10.1016/j.scienta.2018.06.095

Singh, V., Jawandha, S. K., Gill, P. P. S., & Singh, D. (2022). Preharvest putrescine application extends the shelf life and maintains the pear fruit quality. *International Journal of Fruit Science*, 22(1), 514-524. doi: 10.1080/15538362.2022.2068733

Singh, V., Jawandha, S. K., Gill, P. S., & Kaur, R. (2023). Pre-storage putrescine treatment alleviates internal browning of stored pear fruit by regulating enzyme activity. *Erwerbs-Obstbau*, 65(5), 1291-1301. doi: 10.1007/s10341-023-00839-2

Sivakumar, D., & Bautista-Baños, S. (2014). A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Protection*, 64, 27-37. doi: 10.1016/j.cropro.2014.05.012

Solgi, M. (2018). The application of new environmentally friendly compounds on postharvest characteristics of cut carnation (*Dianthus caryophyllus* L.). *Brazilian Journal of Botany*, 41, 515-522. doi: 10.1007/s40415-018-0464-x

Solgi, M., Kafi, M., Taghavi, T. S., & Naderi, R. (2009). Essential oils and silver nanoparticles (SNP) as novel agents to extend vase-life of gerbera (*Gerbera jamesonii* cv. 'Dune') flowers. *Postharvest Biology and Technology*, 53(3), 155-158. doi: 10.1016/j.postharvbio.2009.04.003

Song, Y., Ren, Y., Xue, Y., Lu, D., Yan, T., & He, J. (2023). Putrescine (1, 4-Diaminobutane) enhances antifungal activity in postharvest mango fruit against *Colletotrichum gloeosporioides* through direct fungicidal and induced resistance mechanisms. *Pesticide Biochemistry and Physiology*, 195, 105581. doi: 10.1016/j.pestbp.2023.105581

Soto-Zamora, G., Yahia, E. M., Brecht, J. K., & Gardea, A. (2005). Effects of postharvest hot air treatment on the quality of "Rhapsody" tomato fruit. *Journal of Food Quality*, 28(5-6), 492-504. doi: 10.1111/j.1745-4557.2005.00051.x

Sun, B., Yan, H., Liu, N., Wei, J., & Wang, Q. (2012). Effect of 1-MCP treatment on postharvest quality characters, antioxidants and glucosinolates of Chinese kale. *Food Chemistry*, 131(2), 519-526. doi: 10.1016/j.foodchem.2011.09.016

Swain, R., Sahoo, S., Behera, M., & Rout, G. R. (2023). Instigating prevalent abiotic stress resilience in crop by exogenous application of phytohormones and nutrient. *Frontiers in Plant Science*, 14, 1104874. doi: 10.3389/fpls.2023.1104874

-T-

Tait, A., Paul, V., Sood, A., & Mowat, A. (2018). Potential impact of climate change on Hayward kiwifruit production viability in New Zealand. *New Zealand Journal of Crop and Horticultural Science*, 46(3), 175-197. doi: 10.1080/01140671.2017.1368672

Talavera, A., Gonzalez-Fernandez, J. J., Carrasco-Pancorbo, A., Olmo-García, L., & Hormaza, J. I. (2023). Avocado: Agricultural Importance and Nutraceutical Properties. In: Kole, C. (Ed.), Compendium of Crop Genome Designing for Nutraceuticals. Springer, Singapore, pp. 1-19. doi: 10.1007/978-981-19-3627-2_40-1

Taş, A., Berk, S. K., Kibar, H., & Gündoğdu, M. (2022). An in-depth study on post-harvest storage conditions depending on putrescine treatments of kiwifruit. *Journal of Food Composition and Analysis*, 111, 104605. doi: 10.1016/j.jfca.2022.104605

Thakur, M., Bhattacharya, S., Khosla, P. K., & Puri, S. (2019). Improving production of plant secondary metabolites through biotic and abiotic elicitation. *Journal of Applied Research on Medicinal and Aromatic Plants*, 12, 1-12. doi: 10.1016/j.jarmap.2018.11.004

Thole, V., Vain, P., & Martin, C. (2021). Effect of elevated temperature on tomato post-harvest properties. *Plants*, 10(11), 2359. doi: 10.3390/plants10112359

Thomas, S., Ramakrishnan, R. S., Kumar, A., Sharma, R., Tiwari, M., & Pathak, N. (2020). Putrescine as a polyamines and its role in abiotic stress tolerance: a review. *Journal of Pharmacognosy and Phytochemistry*, 9(1), 815-820.

Tijero, V., Munoz, P., & Munne-Bosch, S. (2019). Melatonin as an inhibitor of sweet cherries ripening in orchard trees. *Plant Physiology and Biochemistry*, 140, 88-95. doi: 10.1016/j.plaphy.2019.05.007

Tilahun, S., Park, D. S., Taye, A. M., & Jeong, C. S. (2017). Effects of storage duration on physicochemical and antioxidant properties of tomato (*Lycopersicon esculentum* Mill.). *Horticultural Science and Technology*, 35(1), 88-97. doi: 10.12972/kjhst.20170010

-U-

Uddin, A. J., Hashimoto, F., Shimizu, K., & Sakata, Y. (2004). Monosaccharides and chitosan sensing in bud growth and petal pigmentation in *Eustoma grandiflorum* (Raf.) Shinn. *Scientia Horticulturae*, 100(1-4), 127-138. doi: 10.1016/j.scienta.2003.08.014

Uluışık, S. (2021). Application of γ-Aminobutyric Acid Treatment Differently Affects Physicochemical Characteristics of Tomato Fruits during Post-harvest Storage. *Horticultural Studies*, 38(2), 101-109. doi: 10.16882/hortis.997921

Umeohia, U. E., & Olapade, A. A. (2024). Quality Attributes, Physiology, and Postharvest Technologies of Tomatoes (Lycopersicum Esculentum)–A Review. *American Journal of Food Science and Technology*, 12(2), 42-64. doi: 10.12691/ajfst-12-2-1

-V-

Vaidya, S. N., Telrandhe, U. B., & Agrawal, S. (2022). Nutritional and health benefits of kiwifruit: An overview. *Annals of Phytomedicine*, 11(2), 176-185. doi: 0.54085/ap.2022.11.2.19

Valenzuela, J. L., Manzano, S., Palma, F., Carvajal, F., Garrido, D., & Jamilena, M. (2017). Oxidative stress associated with chilling injury in immature fruit: postharvest technological and biotechnological solutions. *International Journal of Molecular Sciences*, 18(7), 1467. doi: 10.3390/ijms18071467

Valero, D. & Serrano, M. (2010). Postharvest Biology and Technology for Preserving Fruit Quality. CRC Press/Taylor & Francis: Boca Raton, FL, USA, p. 138. doi: ç10.1201/9781439802670

Valero, D., Guillén, F., Valverde, J. M., Castillo, S., & Serrano, M. (2016). Recent developments of 1-methylcyclopropene (1-MCP) treatments on fruit quality attributes. *Eco-Friendly Technology for Postharvest Produce Quality*, 185-201. doi: 10.1016/B978-0-12-804313-4.00005-0

Van Ieperen, W., Van Meeteren, U., & Nijsse, J. (2002). Embolism repair in cut flower stems: a physical approach. *Postharvest Biology and Technology*, 25(1), 1-14. doi: 10.1016/S0925-5214(01)00161-2

Vázquez-López, Y., Iribe-Salazar, R., Carrazco-Escalante, M., Gaxiola-Camacho, S., & Corrales, J. C. (2022). Quality variables of “Hass” avocado stored in modified atmosphere packaging. *Agrociencia*. doi: 10.47163/agrociencia.v56i4.2460

Vignati, E., Lipska, M., Dunwell, J. M., Caccamo, M., & Simkin, A. J. (2022). Fruit development in sweet cherry. *Plants*, 11(12), 1531. doi: 10.3390/plants11121531

Vilhena, N. Q., Gil, R., Vendrell, M., & Salvador, A. (2022). Effect of preharvest 1-mcp treatment on the flesh firmness of ‘Rojo Brillante’ persimmon. *Horticulturae*, 8(5), 350. doi: 10.3390/horticulturae8050350

Vu, H. T., Scarlett, C. J., & Vuong, Q. V. (2019). Changes of phytochemicals and antioxidant capacity of banana peel during the ripening process; with and without ethylene treatment. *Scientia Horticulturae*, 253, 255-262. doi: 10.1016/j.scienta.2019.04.043

-W-

Wang, C., Zhang, J., Xie, J., Yu, J., Li, J., Lv, J., Gao, Y., Niu, T., & Patience, B. E. (2022). Effects of preharvest methyl jasmonate and salicylic acid treatments on growth, quality, volatile components, and antioxidant systems of Chinese chives. *Frontiers in Plant Science*, 12, 767335. doi: 10.3389/fpls.2021.767335

Wang, G., & Zhang, X. (2020). Evaluation and optimization of air-based precooling for higher postharvest quality: literature review and interdisciplinary perspective. *Food Quality and Safety*, 4(2), 59-68. doi: 10.1093/fqsafe/fyaa012

Wang, H., Wang, J., Mujumdar, A. S., Jin, X., Liu, Z. L., Zhang, Y., & Xiao, H. W. (2021). Effects of postharvest ripening on physicochemical properties, microstructure, cell wall polysaccharides contents (pectin, hemicellulose, cellulose) and nanostructure of kiwifruit (*Actinidia deliciosa*). *Food Hydrocolloids*, 118, 106808. doi: 10.1016/j.foodhyd.2021.106808

Wang, H., Wu, Y., Yu, R., Wu, C., Fan, G., & Li, T. (2019a). Effects of postharvest application of methyl jasmonate on physicochemical characteristics and antioxidant system of the blueberry fruit. *Scientia Horticulturae*, 258, 108785. doi: 10.1016/j.scienta.2019.108785

Wang, L. S., & Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters*, 269(2), 281-290. doi: 10.1016/j.canlet.2008.05.020

Wang, L., Jin, P., Wang, J., Jiang, L., Shan, T., & Zheng, Y. (2015). Methyl jasmonate primed defense responses against *Penicillium expansum* in sweet cherry fruit. Plant Molecular Biology Reporter, 33, 1464-1471. doi: 10.1007/s11105-014-0844-8

Wang, M., Cao, J., Lin, L., Sun, J., & Jiang, W. (2010). Effect of 1-methylcyclopropene on nutritional quality and antioxidant activity of tomato fruit (*Solanum lycopersicon* L.) during storage. *Journal of Food Quality*, 33(2), 150-164. doi: 10.1111/j.1745-4557.2010.00308.x

Wang, Q., Wei, Y., Chen, X., Xu, W., Wang, N., Xu, F., Wang, H., & Shao, X. (2020). Postharvest strategy combining maturity and storage temperature for 1-MCP-treated peach fruit. *Journal of Food Processing and Preservation*, 44(4), e14388. doi: 10.1111/jfpp.14388

Wang, X., Liang, D., Xie, Y., Lv, X., Wang, J., & Xia, H. (2019b). Melatonin application increases accumulation of phenol substances in kiwifruit during storage. *Emirates Journal of Food and Agriculture*, 31(5), 361-367. doi: 10.9755/ejfa.2019.v31.i5.1954

Wang, Y. T. (2007). Temperature, duration in simulated shipping, and thermal acclimatization on the development of chilling injury and subsequent flowering of Phalaenopsis. *Journal of the American Society for Horticultural Science*, 132(2), 202-207. doi: 10.21273/JASHS.132.2.202

Wang, Y., Luo, Z., Huang, X., Yang, K., Gao, S., & Du, R. (2014). Effect of exogenous γ-aminobutyric acid (GABA) treatment on chilling injury and antioxidant capacity in banana peel. *Scientia Horticulturae*, 168, 132-137. doi: 10.1016/j.scienta.2014.01.022

Wang, Y., Niu, Y., Ye, L., Shi, Y., & Luo, A. (2024). Transcriptomic analysis reveals ozone treatment delays kiwifruit postharvest softening by modulating cell wall metabolism. *Journal of Food Science*, 89(4), 2001-2016. doi: 10.1111/1750-3841.16979

Watkins, C. B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances*, 24(4), 389-409. doi: 10.1016/j.biotechadv.2006.01.005

Wei, X., Xie, D., Mao, L., Xu, C., Luo, Z., Xia, M., Zhao, X., Han, X., & Lu, W. (2019). Excess water loss induced by simulated transport vibration in postharvest kiwifruit. *Scientia Horticulturae*, 250, 113-120. doi: 10.1016/j.scienta.2019.02.009

Wenden, B., Campoy, J. A., Lecourt, J., López Ortega, G., Blanke, M., Radičević, S., Schüller, E., Spornberger, A., Christen, D., Magein, H., Giovannini, D., Campillo, C., Malchev, S., Peris, J. M., Meland, M., Stehr, R., Charlot, G., & Quero-García, J. (2016). A collection of European sweet cherry phenology data for assessing climate change. *Scientific Data*, 3(1), 1-10. doi: 10.1038/sdata.2016.108

Winkler, A., Blumenberg, I., Schürmann, L., & Knoche, M. (2020). Rain cracking in sweet cherries is caused by surface wetness, not by water uptake. *Scientia Horticulturae*, 269, 109400. doi: 10.1016/j.scienta.2020.109400

Winkler, A., Ossenbrink, M., & Knoche, M. (2015). Malic acid promotes cracking of sweet cherry fruit. *Journal of the American Society for Horticultural Science*, 140(3), 280-287. doi: 10.21273/JASHS.140.3.280

Woisky, R. G., & Salatino, A. (1998). Analysis of propolis: some parameters and procedures for chemical quality control. *Journal of Apicultural Research*, 37(2), 99-105. doi: 10.1080/00218839.1998.11100961

Woolf, A. B., Requejo-Tapia, C., Cox, K. A., Jackman, R. C., Gunson, A., Arpaia, M. L., & White, A. (2005). 1-MCP reduces physiological storage disorders of 'Hass' avocados. *Postharvest Biology and Technology*, 35(1), 43-60. doi: 10.1016/j.postharvbio.2004.07.009

Wu, C., Hao, W., Yan, L., Zhang, H., Zhang, J., Liu, C., & Zheng, L. (2023b). Postharvest melatonin treatment enhanced antioxidant activity and promoted GABA biosynthesis in yellow-flesh peach. *Food Chemistry*, 419, 136088. doi: 10.1016/j.foodchem.2023.136088

Wu, X., Chen, Y., Zhu, J., Zhang, N., Wei, Y., Jiang, S., Ye, J., & Shao, X. (2023a). 1-Methylcyclopropene reduces postharvest water loss by modulating cuticle formation in tomato fruit. *Postharvest Biology and Technology*, 206, 112564. doi: 10.1016/j.postharvbio.2023.112564

Wu, X., Jia, Q., Ji, S., Gong, B., Li, J., Lü, G., & Gao, H. (2020). Gamma-aminobutyric acid (GABA) alleviates salt damage in tomato by modulating Na+ uptake, the GAD gene, amino acid synthesis and reactive oxygen species metabolism. *BMC Plant Biology*, 20, 1-21. doi: 10.1186/s12870-020-02669-w

Xia, Y., Wu, D. T., Ali, M., Liu, Y., Zhuang, Q. G., Wadood, S. A., Liao, Q. H., Liu, H. Y., & Gan, R. Y. (2024). Innovative postharvest strategies for maintaining the quality of kiwifruit during storage: An updated review. *Food Frontiers*, doi: 10.1002/fft2.442

Xia, Y., Zhuo, R., Li, B., & Tian, S. (2021). Effects of 1-methylcyclopropene on disease resistance of red-fleshed kiwifruit during long-term cold storage and the possible mechanisms. *New Zealand Journal of Crop and Horticultural Science*, 49(2-3), 182-195. doi: 10.1080/01140671.2020.1856888

Xiong, S., Sun, X., Tian, M., Xu, D., & Jiang, A. (2023). 1-Methylcyclopropene treatment delays the softening of Actinidia arguta fruit by reducing cell wall degradation and modulating carbohydrate metabolism. *Food Chemistry*, 411, 135485. doi: 10.1016/j.foodchem.2023.135485

Xu, F., Dong, S., Xu, Q., & Liu, S. (2020). Control of brown heart in Huangguan pears with 1-methylcyclopropene microbubbles treatment. *Scientia Horticulturae*, 259, 108820. doi: 10.1016/j.scienta.2019.108820

Xu, H., Ediger, D., & Sharifi, M. (2023). Horticultural practices in early spring to mitigate the adverse effect of low temperature on fruit set in 'Lapins' sweet cherry. *Plants*, 12(3), 468. doi: 10.3390/plants12030468

-Y-

Yan, R., Li, S., Cheng, Y., Kebbeh, M., Huan, C., & Zheng, X. (2022). Melatonin treatment maintains the quality of cherry tomato by regulating endogenous melatonin and ascorbate-glutathione cycle during room temperature. *Journal of Food Biochemistry*, 46(10), e14285. doi: 10.1111/jfbc.14285

Yang, S., He, M., Li, D., Shi, J., Peng, L., & Jinjing, L. (2023). Antifungal activity of 40 plant essential oil components against *Diaporthe fusicola* from postharvest kiwifruits and their possible action mode. Industrial Crops and Products, 194, 116102. doi: 10.1016/j.indcrop.2022.116102

Yang, W., Wu, Y., Hu, Q., Pei, F., & Mariga, A. M. (2019). Preharvest treatment of *Agaricus bisporus* with methyl jasmonate inhibits postharvest deterioration. LWT, 106, 158-163. doi: 10.1016/j.lwt.2019.02.069

Yao, H., & Tian, S. (2005). Effects of pre-and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. *Postharvest Biology and Technology*, 35(3), 253-262. doi: 10.1016/j.postharvbio.2004.09.001

Yong, K. T., Yong, P. H., & Ng, Z. X. (2023). Tomato and human health: A perspective from post-harvest processing, nutrient bio-accessibility, and pharmacological interaction. *Food Frontiers*, 4(4), 1702-1719. doi: 10.1002/fft2.299

Yu, C., Zeng, L., Sheng, K., Chen, F., Zhou, T., Zheng, X., & Yu, T. (2014). γ-Aminobutyric acid induces resistance against *Penicillium expansum* by priming of defence responses in pear fruit. *Food Chemistry*, 159, 29-37. doi: 10.1016/j.foodchem.2014.03.011

Yu, S., Zhen, C., Zhao, P., Li, J., Qin, Z., & Gao, H. (2023). Antifungal mechanisms of γ-aminobutyric acid against the postharvest pathogen *Alternaria alternata*. *LWT*, 173, 114314. doi: 10.1016/j.lwt.2022.114314

Yuan, X., Zheng, H., Fan, J., Liu, F., Li, J., Zhong, C., & Zhang, Q. (2022). Comparative study on physicochemical and nutritional qualities of kiwifruit varieties. *Foods*, 12(1), 108. doi: 10.3390/foods12010108

-Z-

Zalewski, K., Lahuta, L. B., Martysiak-Żurowska, D., Okorski, A., Nitkiewicz, B., & Zielonka, Ł. (2019). Effect of exogenous application of methyl jasmonate on the lipid and carbohydrate content and composition of winter triticale (*Triticosecale* Wittm.) grain and the severity of fungal infections in triticale plants and grain. *Journal of Agricultural and Food Chemistry*, 67(21), 5932-5939. doi: 10.1021/acs.jafc.9b00799

Zarei, L., Koushesh Saba, M., & Vafaee, Y. (2020). Effect of gamma-amino-butyric acid (GABA) foliar application on chilling and postharvest quality of tomato (cv. Newton). *Plant Productions*, 43(2), 199-212. doi: 10.22055/ppd.2020.27796.1681

Ze, Y., Gao, H., Li, T., Yang, B., & Jiang, Y. (2021). Insights into the roles of melatonin in maintaining quality and extending shelf life of postharvest fruits. *Trends in Food Science & Technology*, 109, 569-578. doi: 10.1016/j.tifs.2021.01.051

Zeng, L., Shi, L., Lin, H., Lin, Y., Lin, Y., & Wang, H. (2021). Containing 1-methylcyclopropene treatment suppresses fruit decay of fresh Anxi persimmons by enhancing disease resistance. *Food Quality and Safety*, 5, fyab007. doi: 10.1093/fqsafe/fyab007

Zewdie, T., Desaiegn, A., Olijira, G., Amare, H., Fetene, K., & Gebrie, L. (2021). Review on effects of pre and post-harvest factors affecting the quality and shelf life of tomato (*Lycopersicon esculentum* Mill.). *South Asian Journal of Agricultural Sciences*, 1 (1), 51-56.

Zhang, B., Zhou, J., Meng, Y., Zhang, N., Gu, B., Yan, Z., & Idris, S. I. (2018). Comparative study of mechanical damage caused by a two-finger tomato gripper with different robotic grasping patterns for harvesting robots. *Biosystems Engineering*, 171, 245-257. doi: 10.1016/j.biosystemseng.2018.05.003

Zhang, C., & Whiting, M. (2013). Plant growth regulators improve sweet cherry fruit quality without reducing endocarp growth. *Scientia Horticulturae*, 150, 73-79. doi: 10.1016/j.scienta.2012.10.007

Zhang, M., Liu, W., Li, C., Shao, T., Jiang, X., Zhao, H., & Ai, W. (2019). Postharvest hot water dipping and hot water forced convection treatments alleviate chilling injury for zucchini fruit during cold storage. *Scientia Horticulturae*, 249, 219-227. doi: 10.1016/j.scienta.2019.01.058

Zhang, W., Jiang, H., Cao, J., & Jiang, W. (2021a). Advances in biochemical mechanisms and control technologies to treat chilling injury in postharvest fruits and vegetables. *Trends in Food Science and Technology*, 113, 355-365. doi: 10.1016/j.tifs.2021.05.009

Zhang, W., Zhao, H., Jiang, H., Xu, Y., Cao, J., & Jiang, W. (2020). Multiple 1-MCP treatment more effectively alleviated postharvest nectarine chilling injury than conventional one-time 1-MCP treatment by regulating ROS and energy metabolism. *Food Chemistry*, 330, 127256. doi: 10.1016/j.foodchem.2020.127256

Zhang, X., Sheng, J., Li, F., Meng, D., & Shen, L. (2012a). Methyl jasmonate alters arginine catabolism and improves postharvest chilling tolerance in cherry tomato fruit. *Postharvest Biology and Technology*, 64(1), 160-167. doi: 10.1016/j.postharvbio.2011.07.006

Zhang, Y., Lin, B., Tang, G., Chen, Y., Deng, M., Lin, Y., Li, M., He, W., Wang, Y., Zhang, Y., Luo, Y., Chen, Q., Wang, X. & Tanget, H. (2024). Application of γ-aminobutyric acid improves the postharvest marketability of strawberry by maintaining fruit quality and enhancing antioxidant system. *Food Chemistry: X*, 21, 101252. doi: 10.1016/j.fochx.2024.101252

Zhang, Y., Zhang, W., Wu, W., Farag, M. A., Wang, L., Xiao, S., Gao, H., & Jiang, W. (2023). Critical assessment of the delivery methods of chemical and natural postharvest preservatives for fruits and vegetables: a review. *Critical Reviews in Food Science and Nutrition*, 1-23. doi: 10.1080/10408398.2023.2289071

Zhang, Z., Huber, D. J., & Rao, J. (2011). Ripening delay of mid-climacteric avocado fruit in response to elevated doses of 1-methylcyclopropene and hypoxia-mediated reduction in internal ethylene concentration. *Postharvest Biology and Technology*, 60(2), 83-91. doi: 10.1016/j.postharvbio.2011.01.001

Zhang, Z., Huber, D. J., Hurr, B. M., & Rao, J. (2009). Delay of tomato fruit ripening in response to 1-methylcyclopropene is influenced by internal ethylene levels. *Postharvest Biology and Technology*, 54(1), 1-8. doi: 10.1016/j.postharvbio.2009.06.003

Zhang, Z., Tian, S., Zhu, Z., Xu, Y., & Qin, G. (2012b). Effects of 1-methylcyclopropene (1-MCP) on ripening and resistance of jujube (*Zizyphus jujuba* cv. Huping) fruit against postharvest disease. LWT-Food science and technology, 45(1), 13-19. doi: 10.1016/j.lwt.2011.07.030

Zhang, Z., Wang, T., Liu, G., Hu, M., Yun, Z., Duan, X., Cai, K., & Jiang, G. (2021b). Inhibition of downy blight and enhancement of resistance in litchi fruit by postharvest application of melatonin. *Food Chemistry*, 347, 129009. doi: 10.1016/j.foodchem.2021.129009

Zhao, D. Y., Shen, L., Fan, B., Liu, K. L., Yu, M. M., Zheng, Y., Ding, Y., & Sheng, J. P. (2009). Physiological and genetic properties of tomato fruits from 2 cultivars differing in chilling tolerance at cold storage. *Journal of Food Science*, 74, C348-C352. doi: 10.1111/j.1750-3841.2009.01156.x

Zhao, D., Wang, R., Meng, J., Li, Z., Wu, Y., & Tao, J. (2017). Ameliorative effects of melatonin on dark-induced leaf senescence in gardenia (Gardenia jasminoides Ellis): leaf morphology, anatomy, physiology and transcriptome. *Scientific Reports*, 7(1), 10423. doi: 10.1038/s41598-017-10799-9

Zhao, H., Ye, L., Wang, Y., Zhou, X., Yang, J., Wang, J., Cao, K., & Zou, Z. (2016). Melatonin increases the chilling tolerance of chloroplast in cucumber seedlings by regulating photosynthetic electron flux and the ascorbate-glutathione cycle. *Frontiers in Plant Science*, 7, 1814. doi: 10.3389/fpls.2016.01814

Zhao, J., Wang, X., Pan, X., Jiang, Q., & Xi, Z. (2021). Exogenous putrescine alleviates drought stress by altering reactive oxygen species scavenging and biosynthesis of polyamines in the seedlings of Cabernet Sauvignon. *Frontiers in Plant Science*, 12, 767992. doi: 10.3389/fpls.2021.767992

Zhao, Q., Shi, Y., Xu, C., Jiang, Z., Liu, J., Sui, Y., & Zhang, H. (2023). Control of postharvest blue and gray mold in kiwifruit by *Wickerhamomyces anomalus* and its mechanism of antifungal activity. *Postharvest Biology and Technology*, 201, 112345. doi: 10.1016/j.postharvbio.2023.112345

Zheng, H., Deng, W., Yu, L., Shi, Y., Deng, Y., Wang, D., & Zhong, Y. (2024). Chitosan coatings with different degrees of deacetylation regulate the postharvest quality of sweet cherry through internal metabolism. *International Journal of Biological Macromolecules*, 254, 127419. doi: 10.1016/j.ijbiomac.2023.127419

Zheng, X., Du, X., Wang, Y., Wang, L., Dai, B., Zhou, L., Mao, j., & Huan, C. (2023b). 1-methylcyclopropene treatment improves chilling tolerance by regulating IAA biosynthesis, auxin signaling transduction and cell wall degradation in peach fruit. *Scientia Horticulturae*, 321, 112265. doi: 10.1016/j.scienta.2023.112265

Zheng, Y., Wang, X., Cui, X., Wang, K., Wang, Y., & He, Y. (2023a). Phytohormones regulate the abiotic stress: An overview of physiological, biochemical, and molecular responses in horticultural crops. *Frontiers in Plant Science*, 13, 1095363. doi: 10.3389/fpls.2022.1095363

Zhou, C., Dong, W., Jin, S., Liu, Q., Shi, L., Cao, S., Li, S., Chen, W., & Yang, Z. (2022). γ-Aminobutyric acid treatment induced chilling tolerance in postharvest peach fruit by upregulating ascorbic acid and glutathione contents at the molecular level. *Frontiers in Plant Science*, 13, 1059979. doi: 10.3389/fpls.2022.1059979

Zulfiqar, F., Moosa, A., Darras, A., Nafees, M., Ferrante, A., & Siddique, K. H. (2023). Preharvest melatonin foliar treatments enhance postharvest longevity of cut tuberose via altering physio-biochemical traits. *Frontiers in Plant Science*, 14, 1151722. doi: 10.3389/fpls.2023.1151722