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

Doctoral Thesis

Preharvest Strategies in Green Pepper Fruit: Quality Enhancement, Extended Shelf-Life and Integral Valorisation of By-products

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Codirectora: Dra. María Emma García Pastor

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Preharvest Strategies in Green Pepper Fruit: Quality Enhancement, Extended Shelf-Life and Integral Valorisation of By-products

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Degree in Food Science and Technology

Master's Degree in Agri-Food Technology and Quality

Supervisors:

Director: Dr. Pedro Javier Zapata Coll

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La presente Tesis Doctoral, titulada "**Preharvest Strategies in Green Pepper Fruit: Quality Enhancement, Extended Shelf-Life and Integral Valorization of By-products**", se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones**:

- Dobón-Suárez, A., Giménez, M.J., Castillo, S., García-Pastor, M.E., & Zapata, P.J. (2021). Influence of the Phenological Stage and Harvest Date on the Bioactive Compounds Content of Green Pepper Fruit. *Molecules*, 26(11), 3099. https://doi.org/10.3390/molecules26113099

- Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. (2021). Salicylic Acid Foliar Application Increases Crop Yield and Quality Parameters of Green Pepper Fruit during Postharvest Storage. *Agronomy*, 11(11), 2263. https://doi.org/10.3390/agronomy11112263

- Dobón-Suárez, A., Gutiérrez-Pozo, M., Serna-Escolano, V., Giménez, M.J., Valero, D., Serrano, M., García-Pastor, M.E., & Zapata, P.J. (2025). Antioxidant metabolism insights into ripening and senescence delay of green pepper fruit through the salicylic acid preharvest treatment. *Frontiers in Plant Science*, 16, 1475068. https://doi.org/10.3389/fpls.2025.1475068

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- Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. (2025). Methyl jasmonate fumigation enhances crop yield and delays physiochemical quality changes by modulating the secondary metabolism in green bell pepper. *Journal of the Science of Food and Agriculture*. *Under Review.*

- Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. (2025). Foliar or irrigation application of salicylic acid and methyl jasmonate regulate postharvest ripening and chilling tolerance of green pepper fruit by modulating both antioxidant and lipid metabolism. *Postharvest Biology and Technology*. *Under Review.*

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El **Dr. D. Pedro Javier Zapata Coll**, director, y la **Dra. Dña. María Emma García Pastor**, codirectora de la tesis doctoral titulada **"Preharvest Strategies in Green Pepper Fruit: Quality Enhancement, Extended Shelf-Life and Integral Valorization of By-products"**,

INFORMA/N:

Que **Dña. Alicia Dobón Suárez** ha realizado bajo nuestra supervisión el trabajo titulado **"Preharvest Strategies in Green Pepper Fruit: Quality Enhancement, Extended Shelf-Life and Integral Valorization of By-products"** conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

Lo que firmo/firmamos para los efectos oportunos, en Orihuela a 29 de abril de 2025

Director de la tesis
Dr. D. ***Pedro Javier Zapata Coll***

Codirectora de la tesis
Dra. Dña. ***María Emma García Pastor***

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

INFORMA:

Que **Dña. Alicia Dobón Suárez** ha realizado bajo la supervisión de nuestro Programa de Doctorado el trabajo titulado **"Preharvest Strategies in Green Pepper Fruit: Quality Enhancement, Extended Shelf-Life and Integral Valorsation of By-products"** conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

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Profa. Dra. Dña. Juana Fernández López

Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias,

Agroambientales y Alimentarias (ReTos-AAA)

'Nada en este mundo debe ser temido, solo entendido.

Ahora es el momento de entender más y temer menos'

Marie Curie (1867-1934)

Agradecimientos

Estar escribiendo estas líneas implica el cierre de una etapa que ha sido inolvidable. Por ello no quiero terminar sin agradecer a todos aquellos que han estado presentes durante estos años.

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Alicia ♥

Publications Category

This doctoral thesis is classified in a **compendium of publications category** to qualify for Doctor Degree from Miguel Hernandez University of Elche. Therefore, the 3 selected articles and their quality, in accordance with the 2023 edition of Journal Citation Reports® (JCR®), are shown:

Publication 1 — Research article

Dobón-Suárez, A., Giménez, M.J., Castillo, S., García-Pastor, M.E., & Zapata, P.J. (2021). Influence of the phenological stage and harvest date on the bioactive compounds content of green pepper fruit. ***Molecules***, 26(11), 3099. doi:10.3390/molecules26113099

Editors: Prof. Dr. Thomas J. Schmidt (among others of the editorial board).
ISSN: N/A
JCR® Category: *Chemistry, Multidisciplinary - SCIE*
Quartile: Q2
Rank: 86/231
Impact Factor (2023): 4.20 — Impact Factor (5 years): 4.60

Publication 2 — Research article

Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. (2021). Salicylic acid foliar application increases crop yield and quality parameters of green pepper fruit during postharvest storage. ***Agronomy***, 11(11), 2263. doi:10.3390/agronomy11112263

Editors: Prof. Dr. Leslie A. Weston (among others of the editorial board).
ISSN: N/A
JCR® Category: *Agronomy*
Quartile: Q1
Rank: 19/126
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Publication 3 — Research article

Dobón-Suárez, A., Gutiérrez-Pozo, M., Serna-Escolano, V., Giménez, M.J., Valero, D., Serrano, M., García-Pastor, M.E., & Zapata, P.J. (2025). Antioxidant metabolism insights into ripening and senescence delay of green pepper fruit through the salicylic acid preharvest treatment. ***Frontiers in Plant Science***, 16, 1475068. doi:10.3389/fpls.2025.1475068

Editors: Chun-Ming Liu (field chief editor).
ISSN: 1664-462X
JCR® Category: *Plant Sciences*
Quartile: Q1
Rank: 44/265
Impact Factor (2023): 4.10 — Impact Factor (5 years): 5.30

Other publications forming part of the doctoral thesis:

Publication 4 — Research article

Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. (2025). Methyl jasmonate fumigation enhances crop yield and delays physiochemical quality changes by modulating the secondary metabolism in green bell pepper. ***Journal of the Science of Food and Agriculture***. *Under Review.*

Editors: Prof. Dr. Andrew Waterhouse and Prof. Dr. Sandra Schmöckel (editors-in-chief).
ISSN: 0022-5142
JCR® Category: *Agriculture, Multidisciplinary*
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Impact Factor (2023): 3.30 — Impact Factor (5 years): 4.00

Publication 5 — Research article

Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. (2025). Foliar or irrigation application of salicylic acid and methyl jasmonate regulate postharvest ripening and chilling tolerance of green pepper fruit by modulating both antioxidant and lipid metabolism. ***Postharvest Biology and Technology***. *Under Review.*

Editors: Prof. Dr. Shi-Ping Tian and Prof. Dr. Chris Watkins (editors-in-chief).
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JCR® Category: *Agronomy*
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Publication 6 — Review article

Dobón-Suárez, A., García-Pastor, M.E., & Zapata, P.J. (2025). A comprehensive review on characterization of pepper seeds: Unveiling potential value and sustainable agrifood applications. ***Foods.*** *Under Review.*

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DOCTORAL THESIS STRUCTURE

This Doctoral Thesis has been structured in accordance with Miguel Hernández University internal regulations for the presentation of Doctoral Thesis as 'Compendium of Publications', this is:

- ***Abstract / Resumen*:** A brief description of the main results and conclusions obtained in this PhD thesis has been presented.

- ***Introduction:*** This PhD thesis has briefly addressed the scientific background and objective of the study, relating it to postharvest problems and market requirements on Lamuyo-type green pepper fruit. In addition, a comprehensive study of the production facts and importance of the crop has been undertaken to provide a robust rationale for the use of this crop. Furthermore, both the post- and preharvest elicitation strategies studied were thoroughly reviewed. Finally, it is vital to emphasise the significance of bell pepper by-products as a potential sustainable source in the context of the prevailing importance of the circular economy.

- ***Aim and Objectives:*** The main aim and specific objectives have been established in this section.

- ***Materials and Methods:*** The plant material, experimental design about the studied treatments and the analytical methods used to carry out the experiments included in this PhD thesis have been briefly explained and referenced.

- ***Publications:*** The 6 publications used for this PhD thesis are presented in the following order:

  i. ***Publication 1 — Research article.*** Dobón-Suárez, A., Giménez, M.J., Castillo, S., García-Pastor, M.E., & Zapata, P.J. (2021). Influence of the Phenological Stage and Harvest Date on the Bioactive Compounds Content of Green Pepper Fruit. *Molecules*, 26(11), 3099. doi:10.3390/molecules26113099

  ii. **Publication 2 — *Research article.*** Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. (2021). Salicylic Acid Foliar Application Increases Crop Yield and Quality Parameters of Green Pepper Fruit during Postharvest Storage. *Agronomy*, 11(11), 2263. doi:10.3390/agronomy11112263

  iii. ***Publication 3 — Research article.*** Dobón-Suárez, A., Gutiérrez-Pozo, M., Serna-Escolano, V., Giménez, M.J., Valero, D., Serrano, M., García-Pastor, M.E., & Zapata, P.J. (2025). Antioxidant metabolism insights into ripening and senescence delay of green pepper fruit through the salicylic acid preharvest treatment. *Frontiers in Plant Science*, 16, 1475068. doi:10.3389/fpls.2025.1475068

iv. ***Publication 4 — Research article.*** Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. (2025). Methyl jasmonate fumigation enhances crop yield and delays physiochemical quality changes by modulating the secondary metabolism in green bell pepper. *Journal of the Science of Food and Agriculture*. *Under Review.*

v. ***Publication 5 — Research article.*** Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., & Zapata, P.J. Foliar or irrigation application of salicylic acid and methyl jasmonate regulate postharvest ripening and chilling tolerance of green pepper fruit by modulating both antioxidant and lipid metabolism. *Postharvest Biology and Technology*. *Under Review.*

vi. ***Publication 6 — Review article.*** Dobón-Suárez, A., Zapata, P.J., & García-Pastor, M.E. (2025). A comprehensive review on characterization of pepper seeds: Unveiling potential value and sustainable agrifood applications. *Foods*. *Under Review.*

- ***Results and Discussion:*** In this section, the main results obtained in this PhD thesis are explained, discussed and summarized.

- ***Conclusions / Conclusiones:*** The main conclusions obtained in this PhD thesis have been listed.

- **Future Research Lines:** A brief description of future research lines that are already being developed as well as those that can be approached in an early and a long-distance future, both at a scientific and technology transfer levels from the results obtained in the present PhD thesis, is addressed.

- **References:** Literature used for writing and justifying this PhD thesis in the complementary sections to 'Publications' has been referenced.

– Abstract –

– Resumen –

ABSTRACT

The cultivation of peppers (*Capsicum annuum* L.) is of significant economic importance worldwide, due to their high nutritional value and culinary applications. Notwithstanding, considerable economic losses were incurred due to spoilage and deterioration during storage. Moreover, there is an increasing consumer demand for high-quality produce with enhanced nutritional value and treated throughout eco-friendly preharvest strategies. This is due to consumers' concerns and legal restrictions regarding the use of postharvest chemical treatments. The present PhD thesis explores the postharvest losses in Lamuyo-type green peppers and the increasing consumer demand for high-quality produce through preharvest strategies. The research focuses on the application of salicylic acid (SA) and methyl jasmonate (MeJA) as plant elicitors to enhance the quality and shelf-life of peppers, as well as on the integral valorisation of pepper seeds.

The PhD thesis, which comprises six publications, was the first to identify the optimal phenological stage S12 and harvest time (April) for maximum bioactive compound accumulation, thereby establishing a basis for subsequent studies. The findings from the second and third studies demonstrated that 0.5 mM SA application, via foliar and irrigation, improved crop yield, maintained quality up to 28 days at 7 °C, and upregulated the relative response of antioxidant-related genes, with irrigation being more practical. The fourth publication revealed that foliar application of 0.1 mM MeJA increased crop yield, delayed senescence, maintained quality attributes, and enhanced the total phenolic content, total antioxidant activity and the activity of antioxidant enzymes after 28 days at 7 °C. The fifth study showed that both 0.5 mM SA and 0.1 mM MeJA, applied via foliar or irrigation, induced chilling tolerance and maintained the postharvest quality of green bell peppers stored at 2 °C for 28 days plus 2 days at 20 °C, with SA irrigation being the most effective. The final publication took the form of a review article, the purpose of which was to emphasise the nutritional and functional value of pepper seeds as a sustainable alternative in agrifood, with the objective of promoting a circular bioeconomy.

In conclusion, recent advancements in understanding green bell pepper have encompassed two primary domains. Firstly, significant progress has been made in the optimisation of the physiological development stage. Secondly, there has been considerable progress in the application of SA and MeJA as preharvest strategies in green pepper fruit. These developments have the potential to assist horticultural companies in enhancing crop yield, ensuring the provision of green pepper fruits that meet higher quality standards at harvest, and extending shelf-life. Furthermore, a comprehensive review of bell pepper seeds as a by-product within the industry underscores their potential value for diverse agrifood applications. Subsequent research would likely concentrate on investigating: *1)* the effects of elicitors on resistance to rotting, as this is one of the major problems facing the marketing of peppers, *2)* the development of pre- and postharvest strategies to extend the shelf-life of fresh-cut pepper fruits, which are a popular choice for salads and various dishes due to the exponential growth of the processing industry, and *3)* the potential use of seeds as a food ingredient and plant biostimulants.

RESUMEN

El cultivo del pimiento (*Capsicum annuum* L.) tiene una gran importancia económica en todo el mundo, debido a su alto valor nutritivo y a sus aplicaciones culinarias. A pesar de ello, se producen pérdidas económicas considerables debido al deterioro durante el almacenamiento. Además, existe una creciente demanda por parte de los consumidores de productos de alta calidad con un valor nutricional mejorado y tratados mediante estrategias precosecha respetuosas con el medio ambiente. Esto se debe a la preocupación de los consumidores y a las restricciones legales en cuanto al uso de tratamientos químicos postcosecha. La presente tesis doctoral explora las pérdidas postcosecha en pimiento verde tipo Lamuyo y la creciente demanda de los consumidores de productos de alta calidad a través de estrategias precosecha. La investigación se centra en la aplicación de ácido salicílico (AS) y jasmonato de metilo (JaMe) como elicitores vegetales para mejorar la calidad y vida útil de los pimientos, así como en la valorización integral de las semillas de pimiento.

La tesis doctoral, compuesta por seis publicaciones, fue la primera en identificar el estado fenológico S12 y el momento de la cosecha (Abril) óptimos para la máxima acumulación de compuestos bioactivos, sentando así las bases para estudios posteriores. Los resultados del segundo y tercer estudio demostraron que la aplicación de AS a la concentración de 0,5 mM, vía foliar y por riego, mejoró el rendimiento del cultivo, mantuvo la calidad hasta 28 días a 7 °C y reguló al alza la respuesta relativa de los genes relacionados con antioxidantes, siendo más práctico el riego. La cuarta publicación reveló que la aplicación foliar de 0,1 mM de JaMe aumentó el rendimiento del cultivo, retrasó la senescencia, mantuvo los atributos de calidad, y mejoró el contenido fenólico total, la actividad antioxidante total y la actividad de los enzimas antioxidantes después de 28 días a 7 °C. El quinto estudio mostró que tanto AS 0,5 mM como JaMe 0,1 mM, aplicados vía foliar o por riego, indujeron la tolerancia al frío y mantuvieron la calidad postcosecha de pimientos verdes almacenados a 2 °C durante 28 días más 2 días a 20 °C, siendo el riego con AS el más efectivo. La publicación final adoptó la forma de un artículo de revisión, cuyo propósito era destacar el valor nutricional y funcional de las semillas de pimiento como alternativa sostenible en agroalimentación, con el objetivo de promover una bioeconomía circular.

En conclusión, los recientes avances en el conocimiento del pimiento verde han abarcado dos ámbitos principales. En primer lugar, se ha avanzado significativamente en la optimización de la fase de desarrollo fisiológico. En segundo lugar, se ha progresado considerablemente en la aplicación de AS y JaMe como estrategias precosecha en pimiento verde. Estos avances tienen el potencial de ayudar a las empresas hortícolas a mejorar el rendimiento de los cultivos, garantizar el suministro de pimientos verdes que cumplan con estándares de calidad más altos en la recolección y prolongar la vida útil. Además, una revisión exhaustiva de las semillas de pimiento como subproducto dentro de la industria subraya su valor potencial para diversas aplicaciones agroalimentarias. Las investigaciones posteriores se centrarían probablemente en investigar: *1)* los efectos de los elicitores sobre la resistencia a la incidencia de podredumbres, ya que es uno de los

principales problemas a los que se enfrenta la comercialización de los pimientos, *2)* el desarrollo de estrategias pre- y postcosecha para prolongar la vida útil de pimiento IV Gama, que es una opción popular para ensaladas y diversos platos debido al crecimiento exponencial de la industria de procesado, y *3)* el uso potencial de las semillas como ingrediente alimentario y bioestimulante vegetal.

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Introduction

1. INTRODUCTON

1.1. Production facts and crop and socio-economic importance

Pepper (*Capsicum annuum* L.) is among the most widely cultivated and produced vegetable crops in irrigated agriculture. It is widely regarded as one of the most significant horticultural crops on a global scale, with regard to both production and exportation. In recent years, there has been a significant increase in interest and demand for peppers as an economically significant horticultural crop on the global market (Das et al., 2015). Pepper fruits are a seasonal vegetable that have a high level of consumer acceptance, which results in an increase in the production of these crops. The cultivation of this crop is practised in a variety of geographic regions, where it can adapt to a range of climates and production systems. It is a pivotal component in the agricultural economies of numerous countries. Peppers, which encompass a wide range of fruit types, including green bell peppers, are among the most widely consumed vegetables globally (Food and Agriculture Organization (FAO, 2021).

As can be observed in **Figure 1**, according to the database of FAOSTAT (2024), the leading producer countries were China, Indonesia, Turkey, Mexico and Spain, which collectively accounted for over 40 % of global production. In accordance with the most recent statistics, the global production of peppers attained a total volume of 36.97 billion kilograms in 2022. China is the leading producer, with a total output of 17,158,086 tons (t), representing 45.47 % of global production. Turkey and Indonesia are the second-largest producers, with an output of 33,020,262 and 3,018,775 t, respectively. These are followed by Mexico (2,577,010 t), and Spain (1,533,280 t), which also play a crucial role in the global supply chain.

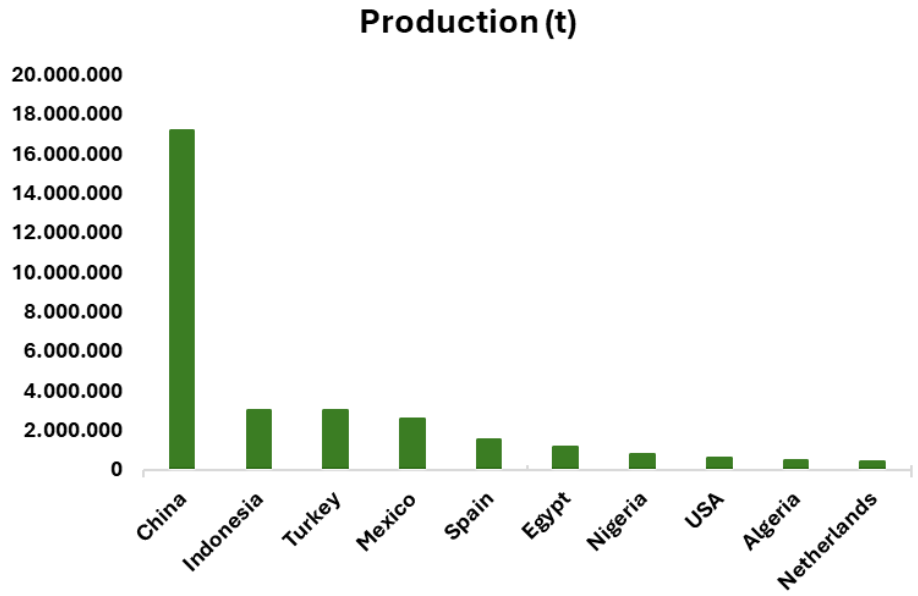

Figure 1. *The top ten countries in terms of production of pepper fruits in 2022. Source: FAOSTAT. (https://www.fao.org/faostat/es/#rankings/countries_by_commodity). Accessed: December 2024.*

Spain is the primary producer of fresh peppers in Europe and is the main supplier of this vegetable to the European Union (EU) market. In the 2023-2024 season, Spanish pepper exports attained a record value of €1.61 billion, amounting to 815.86 million kilograms. This represented a 5.52 % increase on the previous season (2022-2023). Germany, France and the United Kingdom constituted the primary markets (Hortoinfo, 2024).

The predominant region of pepper horticultural production in Spain is the south-eastern peninsular area, particularly the Andalucía and Murcia regions (**Figures 2A and 2B**). Andalucía is the most significant producing area, accounting for 64 % of the total pepper production in Spain and 59 % of the national area dedicated to this crop (MAPA, 2024). The pepper was cultivated on 14,565 hectares (ha) (**Figure 2A**) and reached 1,077,592 t (**Figure 2B**) in 2022 (MAPA, 2024). The preeminence of this geographical area is chiefly ascribable to the pervasive presence of greenhouse agriculture, a phenomenon that is especially pronounced in the province of Almería. Conversely, the region of Murcia had a total area dedicated to pepper production of 1,745 ha and produces 197,436 t in 2022 (**Figures 2A and 2B**, respectively). The cultivation of pepper crop in Spain has increased in recent years, driven by the growing demand for fresh peppers in European markets, which has been experiencing significant annual growth. This trend has led to the development of greenhouse cultivation along the Spanish Mediterranean coast.

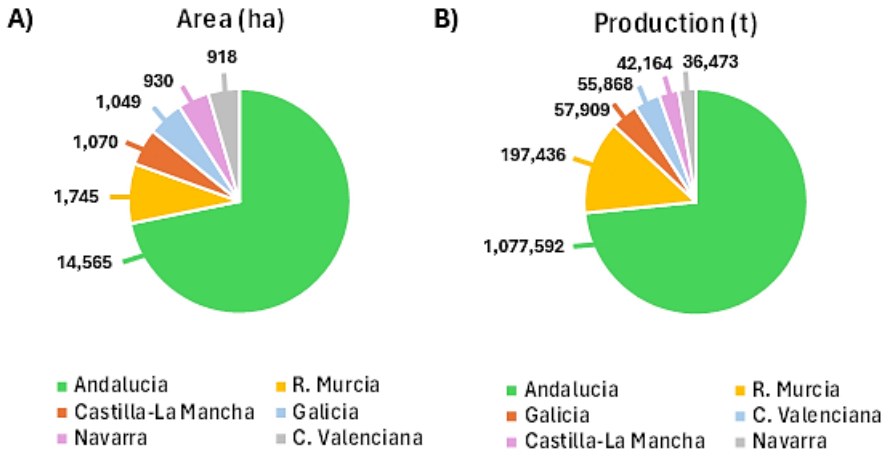

Figure 2. A) *Area dedicated to the cultivation of pepper fruits in hectares (ha) in Spain in 2022. Source: MAPA (https://www.mapa.gob.es/es/). Accessed December 2024.* ***B)*** *Total production of pepper fruit in terms of tons (t) in the primary production areas of Spain in 2022. Source: MAPA (https://www.mapa.gob.es/es/). Accessed: December 2024.*

The harvesting of peppers (*Capsicum spp.*) is conducted at two distinct stages of ripening, contingent on the specific cultivar of pepper and the market for which it is intended. This encompasses both ripe (red) and unripe (green) pepper fruits. However, it is important to acknowledge the absence of information on the extant documentary evidence concerning the discrepancy on crop yield between red and green peppers. Furthermore, it is crucial to emphasise that a notable proportion of total production is harvested at unripe stage, *i.e.* green pepper fruits.

Green pepper fruits exhibit a heightened degree of sensitivity when harvested at an early stage of ripening in comparison to red peppers. The reduced thickness of the walls of green peppers renders them more vulnerable to mechanical damage during postharvest handling and transport. This, in turn, has a detrimental effect on the shelf-life and market quality of the produce (Wills et al., 1998). Furthermore, it has been observed that green pepper fruits are more susceptible to certain diseases and pests affecting the pepper crop, which can result in significant financial losses for farmers (Wills et al., 1998). In addition, the reduced sugar content of the green pepper fruits means that they are less sweet (Wills et al., 1998). The diminished levels of antioxidants, including carotenoids, capsaicinoids, capsanthin and capsorubin (Topuz & Ozdemir, 2007), contribute to their reduced resistance to environmental stress and oxidation. Finally, ripe pepper fruits, *i.e.* those which have already reached their characteristic red colour, are less susceptible to some physiological disorders, such as chilling injury (CI), than green pepper fruits (Cantwell, 2023). These findings emphasise the necessity for further research focused on the search for tools or strategies to improve the quality of green peppers, with a view to enhancing their agronomic and nutritional traits and, thus, promoting their cultivation and consumption, which are of great importance in the south-eastern region of Spain.

1.2. Market requirements for the quality of green pepper fruit

Fresh peppers are a globally consumed vegetable (Jones, 2019), renowned for their bright colours, distinctive flavours and nutritional benefits (Smith et al., 2021). Different cultivars of *Capsicum spp.* are cultivated on a wide scale for their fruits, which can be consumed in a variety of ways, including fresh, dried or cooked. Peppers are a highly sought-after commodity in the marketplace, owing to their organoleptic qualities and sensory characteristics, including size, colour and flavour. The external appearance of the pepper fruit is of critical importance to consumers in the decision-making process regarding its purchase. The physical attributes of the pepper fruit, including size, firmness, colour and overall freshness, are pivotal quality traits. In the context of fruit intended for fresh consumption, deviations in visual appearance from established standards can readily result in rejection.

Green pepper fruits are considered a valuable nutritional source due to their high concentrations of phytochemical constituents that have been demonstrated to be beneficial to human health. These include antioxidants, flavonoids, phenolic acids, vitamins and carotenoids. These bioactive components in question have been demonstrated to confer functional properties to green pepper fruits, encompassing antioxidant, antifungal, and antibacterial activity (Abdalla et al., 2019). However, the quality of the pepper fruit is subject to deterioration due to physiological and biochemical changes that are induced by both abiotic and biotic stresses. Furthermore, it has been demonstrated that inadequate or inappropriate postharvest management, including dehydration, colour loss, skin shrivelling and texture softening, can result in severe alteration (Charoenphun et al., 2024).

It is evident that there has been a surge of interest in the bioactive compounds present in pepper fruits, owing to the myriad benefits they offer to human health. These include antioxidant, analgesic and anti-inflammatory properties (Chanda et al., 2012; Deng et al., 2013). Furthermore, it is hypothesised that known antioxidants play a pivotal role in the protection against a wide range of diseases, most notably cardiovascular disease and cancer (Mayne, 1996). In addition, these compounds can reduce cholesterol and blood sugar levels. Nevertheless, the compositional quality and content of phytochemicals in different cultivars or genotypes of the same fruits and vegetables, including peppers, are found to be influenced by numerous factors. These include biotic and abiotic stresses, agronomics, genotypes, ripening stages, cultivation techniques, harvesting time, and different processing conditions (Amakura et al., 2002; Menichini et al., 2009).

Notwithstanding the beneficial properties of fresh green peppers, it is a highly perishable vegetable and, thus, it is consumed in the freshest possible stage. Consequently, factors such as fruit quality and shelf-life are of great importance in determining the commercial value of the product. The most effective method to maintain the quality and prolong the shelf-life of fresh peppers is the application of low temperatures (Xu et al., 2023; Zhao et al., 2024). Nevertheless, storing produce at low temperatures significantly increases the likelihood of CI when the fruit is stored at temperatures below its optimal storage conditions, *i.e.* below 5 °C in the case of pepper fruits. Furthermore, CI has been demonstrated to diminish the nutritional benefits attributed to functional compounds (Wang et al., 2016). The most prevalent visible symptoms of CI affecting quality deterioration during postharvest storage of green pepper fruits are mainly attributed to surface pitting, watery spots, shrinkage, seed browning, calyx discolouration and accelerated senescence (Liu et al., 2015; Ge et al., 2020). In contrast, when peppers are stored at an optimal temperature range of 7-10 °C, the quality of the fruit is subject to deterioration, which is characterised by significant weight loss, dehydration, and accelerated softening of the fruit. In addition, a high incidence of rot is observed, which is predominantly caused by *Alternaria alternata* (Rao et al., 2011).

Research on green pepper is of great importance, due to its increasing global demand and its nutritional value, as it is rich in bioactive compounds that benefit health. The visual appearance of the pepper is an important factor in the consumer's purchasing decision, which underlines the importance of preserving and optimising the quality of the product. However, the fruit of the *Capsicum* genus is susceptible to deterioration due to its fragile structure, which renders it vulnerable to mechanical damage during handling and transportation. This poses significant challenges in terms of shelf-life and market quality. In view of the challenges identified, there is an emerging requirement to develop effective strategies, particularly for protection against CI incidence at low temperatures and prevention of both quality losses and fungal decay incidence at optimal temperatures to satisfy the market demand.

1.3. Salicylic acid (SA) and methyl jasmonate (MeJA) elicitation strategies

Nowadays, there is an imperative to enhance the yield of high-quality foodstuffs, given the ongoing expansion of the human population and the deleterious consequences of climate change. The escalating consumption of energy and resources in the agricultural sector has rendered it

unsustainable, thereby posing a threat to global food security (FAO, 2021; IPCC, 2022). In the agricultural sector, the utilisation of chemical fertilisers endowed with fungicidal properties has become a prevalent strategy in the production of fruits and vegetables. The objective of this approach is to regulate the activity of pathogens, thereby enhancing the quality and consumer acceptance of produce, while addressing global food demands. Nevertheless, the increasing concern regarding the utilisation of artificial products in food production, in conjunction with the excessive application of these chemicals, has given rise to numerous issues, including the escalating legislated restrictions on their application, as well as the eutrophication, root deterioration and soil acidification. Consequently, a global trend has emerged in which the use of synthetic fungicides is being reduced, and eco-friendly strategies are being promoted. The aforementioned strategies advocate for the implementation of more sustainable agricultural practices, with the objective of reducing environmental impact. Furthermore, these strategies seek to control the occurrence of pests and diseases, whilst simultaneously improving the quality of nutraceuticals and extending the shelf-life of food (Wang et al., 2014; Saavedra et al., 2016; Konsue et al., 2020; Sewal et al., 2025).

Elicitors are compounds naturally synthesised in plants, and their roles in regulating physiological and biochemical processes, and activating the plant defence system to respond to abiotic and biotic stresses have been well documented (Martínez-Esplá et al., 2018; Ramírez-Godoy et al., 2018; Klimek-Szczykutowicz et al., 2020). It has been demonstrated that elicitors can induce the biosynthesis of a wide variety of metabolites and inducing the expression of enzyme coding genes related to plant defense responses, as well as due to the accumulation of secondary metabolites (Ruiz-García & Gómez-Plaza, 2013; Baenas et al., 2014; Gorni & Pacheco, 2016). Moreover, these metabolites have been demonstrated to exert a substantial influence on plant adaptation, and mounting evidence suggests that they may also confer health benefits to humans if these fruits and vegetables are consumed (Klimek-Szczykutowicz et al., 2020). Recent studies have demonstrated the efficacy of chemical elicitors in regulating disease control during the postharvest phase. The induction of local resistance instigates a direct response at the site of stress, whilst systemic resistance induces a response from an uninfected part to the whole plant (Valero & Serrano, 2010). The classification of systemic resistance is dependent on the signal molecule that initiates the expression pathway, with systemic acquired resistance (SAR) and induction of systemic response (ISR) being the two main categories (Thakur & Sohal, 2013; Nazar et al., 2017).

Elicitors can be classified as abiotic or biotic compounds, although some plant hormones should be also considered as plant elicitors. Biotic elicitors are defined as substances of biological origin, which can be categorised into two main types: exogenous and endogenous. Exogenous biotic elicitors are derived from external sources, such as pathogens, including microorganisms, or substances produced during the process of plant enzymes breaking down microbial cell walls (Gadzovska-Simic et al., 2015; Wani et al., 2024). Conversely, endogenous biotic elicitors are produced within the plant in response to pathogen attacks or stress. These include polysaccharides resulting from pathogens inducing degradation of the plant cell wall, as well as intracellular proteins and small molecules, such as plant growth regulators, including and salicylic acid (SA) and methyl jasmonate (MeJA) (Singh & Dwivedi, 2018; Bhaskar et al., 2022; Alcalde et al., 2022).

In this sense, SA, also known as 2-hydroxybenzoic acid, is a phenolic compound that possesses an aromatic ring with a hydroxyl group or other functional derivative and its naturally occurring plant growth regulator. As demonstrated in the relevant literature, SA has been shown to play a pivotal role in a variety of developmental and physiological processes (Ali, 2021). These include, but are not limited to, ethylene production, stomatal movements, photosynthesis, membrane functions, pigment accumulation, and enzyme activities, as can be observed in **Figure 3**. In addition, SA has been found to be associated with plant growth and development. The most salient effect of SA is the induction of SAR, which is achieved by intervening in the resistance to local and endemic plant diseases by activating the plant's defences. Thus, SA is synthesised and transported by the phloem throughout the plant, thus protecting the entire plant from future infections (Lefevere et al., 2020). Moreover, the physiological effects of the substance under investigation include the stimulation of seed germination, fruit growth and flowering.

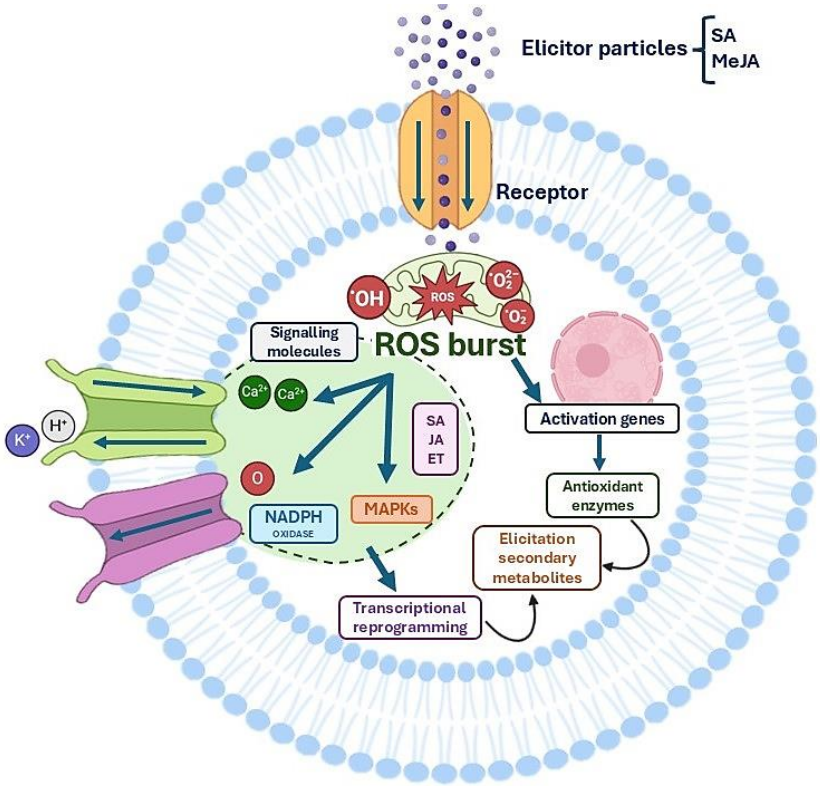

Figure 3. *The general mechanism that follows the perception of elicitors influences the levels of secondary metabolites by altering reactive oxygen species, modulating gene expression, and impacting signalling pathways. Abbreviations: MeJA, methyl jasmonate; SA, salicylic acid; JA, jasmonic acid; ET, ethylene; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; MAPKs, protein kinases and mitogen-activated protein kinases (Adapted from Baenas et al., 2014; Sewal et al., 2025). Source: The information has been compiled by the authors and the figure was created with BioRender (Toronto, ON, Canada).*

On the other hand, MeJA is a naturally occurring variant of jasmonic acid (JA), from which the methyl ester has been eliminated during bioconversion. MeJA is frequently utilised as an elicitor in both *in vitro* and *in vivo* research models due to its capacity to stimulate enzymes responsible for the synthesis of phenolic compounds and flavonoids in plants. This stimulation of

enzymes instigates a defence response against injury and pathogenic invasion (Castro et al., 2021). MeJA has been identified as a volatile methyl ester that functions as a major signalling molecule in abiotic and biotic stresses (Wang et al., 2021). Furthermore, it plays an essential role in plants, impacting both morphological and molecular functions. Due to its volatile nature and ability to permeate biological membranes, it is regarded as a significant phytohormone that facilitates both intra- and intercommunications in plants. The **Figure 3** highlights the general mechanism of this plant hormone after the perception through membrane receptors on the regulation of defence responses, particularly throughout the modulation of antioxidant systems (Jeyasri et al., 2023).

To date, several significant results have been published by different authors about the effect of elicitors on certain vegetables of the *Solanaceae* family. The efficacy of the elicitors in mitigating the occurrence of CI, whilst concurrently preserving the quality of the fruits and vegetables, has been demonstrated by these studies. Moreover, the findings of the studies have elucidated the capacity of the elicitors to enhance the health benefits of the vegetable produce associated with their consumption by augmenting the antioxidant capacity. A literature review of the effects of these plant elicitors on the following parameters is presented in **Table 1**:

- The increase of crop yield.
- The maintenance of fruit quality and the delay of postharvest ripening or senescence.
- The enhancement of antioxidant systems: both non-enzymatic (bioactive compound content and total antioxidant activity) and the enzymatic one (antioxidant enzymes activity).
- The alleviation of CI symptoms, improving chilling tolerance during cold storage.
- The induction of resistance against pathogen and decay incidence.

As demonstrated in **Table 1**, most postharvest treatments appear to be focused on mitigating CI symptoms and enhancing cold tolerance during storage. A substantial body of research has demonstrated the efficacy of compounds such as methyl salicylate (MeSA) and MeJA on cherry tomatoes (Zhang et al., 2011; Zhang et al., 2012). In tomato, studies have shown that the application of SA and MeJA has yielded favourable outcomes (Aghdam et al., 2014; Zhang et al., 2016). In green and red bell peppers, glycine betaine (GB) and MeJA have been shown to be effective, respectively (Wang et al., 2016; Wang et al., 2019). For eggplants, eugenol and MeJA have been found to be effective (Huang et al., 2019; Shi et al., 2019). In addition, the combination of MeJA and MeSA, as well as SA and trisodium phosphate (TSP), have demonstrated favourable outcomes in hot and red bell peppers, respectively (Seo et al., 2020; Ge et al., 2020). Furthermore, melatonin (MT) and oxalic acid (OA) have been demonstrated to be efficacious in the context of both horn and green bell peppers, respectively (Wang et al., 2022a; Wang et al., 2022b).

On the other hand, the **Table 1** also comprises several postharvest treatments that have been investigated for the purpose of maintaining quality and delaying senescence. The use of compounds such as 1-methylcyclopropene (1-MCP), MeJA, SA and calcium chloride ($CaCl_2$) has been a common feature in studies of eggplant and tomato (Massolo et al., 2011; Fan et al., 2016; Nurettin & Seyda, 2020; Rivero Meza et al., 2021; Kumar et al., 2021; Ban et al., 2021). In a similar manner, the use of postharvest treatments based on 1-MCP, SA, MeJA, and calcium (Ca), among other

compounds, has been the subject of study in order to maintain the functional quality and increase the content of bioactive compounds and antioxidant capacity (**Table 1**) (Massolo et al., 2011; Aghdam et al., 2014; Zhang et al., 2016; Fan et al., 2016; Shi et al., 2019; Nurettin and Seyda, 2020; Ban et al., 2021). Finally, the induction of resistance against pathogens by treatments with arginine (Arg), MeSA, *Origanum dictamnus* essential oil and combinations of propolis and chitosan has been investigated in tomatoes, peppers and eggplants (**Table 1**) (Zhang et al., 2017; Stavropoulou et al., 2021).

Regarding preharvest treatments (**Table 1**), a range of alternatives have been investigated to increase crop yield, maintain postharvest quality, enhance the content of functional compounds, induce cold tolerance and reduce fruit decay. Several preharvest treatments have been trialled, including spirulina, SA, MeJA, chitosan, pyraclostrobin + boscalide, thyme essential oil, nitrogen (N), naphthalene acetic acid (NAA), Kelpak®, $CaCl_2$, chitosan oligosaccharide (COS), humic acid (HA), and fulvic acid (FA). Calcium thiosulphate (CaTS) and potassium thiosulphate (KTS) have been demonstrated to be efficacious in increasing crop yield, maintaining postharvest quality and enhancing the functional quality of tomatoes, both yellow and green bell peppers, chilli peppers and eggplant (Domínguez et al., 2012; Dias et al., 2016; Migliori et al., 2017; Mbandlwa et al., 2020; Frías-Moreno et al., 2020; Baek et al., 2021; Mazumder et al., 2021; Ghahremani et al., 2021; Aires et al., 2022; Kramchote & Suwor, 2022; Zheng et al., 2023; Al-Saif et al., 2024; Moosavi-Nezhad et al., 2024). Furthermore, studies have demonstrated that treatments incorporating MT and iodine (KIO_3) in 'California Wonder' red pepper and cherry and mini-plum tomatoes have been shown to enhance functional quality, resulting in increased bioactive compound content (Somma et al., 2024; Nasiri et al., 2024). Under cold storage conditions, sodium nitroprusside (SNP) and SA treatments have been investigated for the enhancement of cold tolerance in tomato (Ahmadi-Soleimanie et al., 2020; Baninaiem & Dastjerdi, 2023).

Finally, the utilisation of preharvest treatments to induce resistance to pathogens emerges as a promising strategy for crop protection. In this context, the use of various compounds to strengthen natural plant defences has been investigated. For instance, in tomatoes, the efficacy of pyraclostrobin + boscalide (Domínguez et al., 2012), propolis and chitosan (Migliori et al., 2017), and $CaCl_2$ (Mazumder et al., 2021) has been reported, suggesting their potential to induce resistance against pathogens. In eggplants, SA has also demonstrated favourable outcomes in this regard (Ghahremani et al., 2021). In a similar manner, a combination of SA and MT has been demonstrated to enhance resistance to pathogens in 'California Wonder' red pepper (Nasiri et al., 2024). The findings indicate that the strategic application of these compounds during the preharvest phase has the potential to enhance plant immunity, thereby reducing disease incidence and improving crop yield and fruit quality (**Table 1**).

Table 1. *Literature review of different elicitors applied as post- or preharvest treatments in some fruits and vegetables of the Solanaceae family.*

Effect	Fruit	Treatment[ǂ]	References
The maintenance of fruit quality and the delay of postharvest ripening or senescence	Eggplant (*Solanum melongena* L., cv. 'Lucía')	1-MCP	Massolo et al. (2011)
	Eggplant (*Solanum melongena* L., cv. 'Brigitte')	MeJA	Fan et al. (2016)
	Eggplant (*Solanum melongena* L., cv. 'Anamur Karasi')	MeJA	Nurettin & Seyda (2020)
	Tomato (*Solanum lycopersicum* L., cv. 'Grape')	MeJA	Rivero Meza et al. (2021)
	Tomato (*Solanum lycopersicum* L., cv. 'BSS-488' and cv. 'Hisar Aru')	SA	Kumar et al. (2021)
	Eggplant (*Solanum melongena* L., cv. 'Heilong')	$CaCl_2$	Ban et al. (2021)
The enhancement of antioxidant systems: both non-enzymatic (bioactive compound content and total antioxidant activity) and the enzymatic one (antioxidant enzymes activity)	Eggplant (*Solanum melongena* L., cv. 'Lucía')	1-MCP	Massolo et al. (2011)
	Tomato (*Lycopersicon esculentum* L., cv. 'Newton')	SA	Aghdam et al. (2014)
	Tomato (*Solanum lycopersicum* L., cv. 'UC82B')	MeJA	Zhang et al. (2016)
	Eggplant (*Solanum melongena* L., cv. 'Brigitte')	MeJA	Fan et al. (2016)
	Eggplant (*Solanum melongena* L., cv. 'Brigitte')	MeJA	Shi et al. (2019)
	Eggplant (*Solanum melongena* L., cv. 'Anamur Karasi')	MeJA	Nurettin & Seyda (2020)
	Eggplant (*Solanum melongena* L., cv. 'Heilong')	Ca	Ban et al. (2021)
The alleviation of CI symptoms, improving chilling tolerance during cold storage	Cherry tomato (*Solanum lycopersicum* L., cv. 'Messina')	MeSA	Zhang et al. (2011)
	Cherry tomato (*Solanum lycopersicum* L., cv. 'Messina')	MeJA	Zhang et al. (2012)
	Tomato (*Lycopersicon esculentum* L., cv. 'Newton')	SA	Aghdam et al. (2014)
	Tomato (*Solanum lycopersicum* L., cv. 'UC82B')	MeJA	Zhang et al. (2016)

PREHARVEST		Green bell pepper (*Capsicum annuum* L., cv. 'Mutianqiushuo')	GB	Wang et al. (2016)
		Red bell pepper (*Capsicum annuum* L., cv. 'Champion')	MeJA	Wang et al. (2019)
		Eggplant (*Solanum melongena* L., cv. 'Chongming')	Eugenol	Huang et al. (2019)
		Eggplant (*Solanum melongena* L., cv. 'Brigitte')	MeJA	Shi et al. (2019)
		Hot pepper (*Capsicum annuum* L., cv 'Cheongyang')	MeJA; MeSA; MeJA + MeSA	Seo et al. (2020)
		Green bell pepper (*Capsicum annuum* L., cv. 'Jinli')	MeJA	Ma et al. (2020)
		Red Bell pepper (*Capsicum annuum* L., cv '606')	SA; TSP; SA + TPS	Ge et al. (2020)
		Horn pepper fruit (*Capsicum annuum* L., cv. 'Ciban')	MT	Wang et al. (2022a)
		Green bell pepper (*Capsicum annuum* L., cv. 'Jin 3')	OA	Wang et al. (2022b)
	The induction of resistance against pathogen and decay incidence	Tomato (*Solanum lycopersicum* L., cv. 'Badun')	Arg; MeSA	Zhang et al. (2017)
		Tomato (*Solanum lycopersicum* L.; Pepper (*Capsicum annuum* L.; Eggplant (*Solanum melongena* L.)	*O. dictamnus* oil	Stavropoulou et al. (2021)
	The increase of crop yield	Eggplant (*Solanum melongena* L., cv. 'Embu')	Spirulina	Dias et al. (2016)
		Tomato (*Solanum lycopersicum* L., cv. 'Kumato')	SA; MeJA	Baek et al. (2021)
		Eggplant (*Solanum melongena* L., cv. 'IR3121')	SA	Ghahremani et al. (2021)
		Chili pepper (*Capsicum annuum* L., cv. 'Super-Hot' and cv. 'Num Khao')	Chitosan	Kramchote & Suwor (2022)
		Tomato (*Solanum lycopersicum* L., cv. 'Colossal')	SA	Aires et al. (2022)
	The maintenance of fruit quality and the delay of	Tomato (*Solanum lycopersicum* L., cv. 'Raf' and cv. 'Amadeo')	PYR + Bos	Domínguez et al. (2012)
		Eggplant (*Solanum melongena* L., cv. 'Embu')	Spirulina	Dias et al. (2016)

postharvest ripening or senescence	Tomato (*Solanum lycopersicum* L., cv. 'Vesuviano')	Thyme oil; CHS	Migliori et al. (2017)
	Tomato (*Lycopersicon esculentum* L., cv. 'Newton')	SNP	Ahmadi-Soleimanie et al. (2020)
	Cherry tomato (*Solanum lycopersicum* L., cv. 'Caballero' and 'Victoria')	N	Frías-Moreno et al. (2020)
	Yellow sweet pepper (*Capsicum annuum* L., cv. 'Citrine')	NAA; Kelpak®	Mbandlwa et al. (2020)
	Tomato (*Solanum lycopersicum* L., cv. 'MT1' and cv. 'MT3')	$CaCl_2$	Mazumder et al. (2021)
	Tomato (*Solanum lycopersicum* L., cv. 'Kumato')	SA; MeJA	Baek et al. (2021)
	Eggplant (*Solanum melongena* L., cv. 'IR3121')	SA	Ghahremani et al. (2021)
	Chili Pepper (*Capsicum annuum* L., cv. 'Super Hot' and cv. 'Num Khao')	CHS	Kramchote & Suwor (2022)
	Tomato (*Solanum lycopersicum* L., cv. 'Kumato')	SA; MeJA	Baek et al. (2023)
	Tomato (*Solanum lycopersicum* L., cv. 'Baraka')	SA	Baninaiem & Dastjerdi (2023)
	Tomato (*Solanum lycopersicum* L., cv. 'Ruixinghongniu')	CHS; COS	Zheng et al. (2023)
	Tomato (*Solanum lycopersicum* L., cv. 'Prensisa')	HA; FA; SA	Al-Saif et al. (2024)
	Green Bell Pepper (*Capsicum annuum* L., cv. 'Karisma')	CaTS; KTS	Moosavi-Nezhad et al. (2024)
The enhancement of antioxidant systems: both non-enzymatic (bioactive compound content and total antioxidant activity) and the enzymatic one (antioxidant enzymes activity)	Tomato (*Solanum lycopersicum* L., cv. 'Raf' and cv. 'Amadeo')	PYR + Bos	Domínguez et al. (2012)
	Tomato (*Solanum lycopersicum* L., cv. 'Vesuviano')	Thyme oil; CHS	Migliori et al. (2017)
	Tomato (*Solanum lycopersicum* L., 'Micro-Tom' and '*slmyc2*' mutants)	MeJA	Shu et al. (2020)
	Tomato (*Solanum lycopersicum* L., cv. 'Caballero' and cv. 'Victoria')	N	Frías-Moreno et al. (2020)
	Yellow sweet pepper (*Capsicum annuum* L., cv. 'Citrine')	NAA; Kelpak®	Mbandlwa et al. (2020)
	Tomato (*Solanum lycopersicum* L., cv. 'MT1' and cv. 'MT3')	$CaCl_2$	Mazumder et al. (2021)
	Tomato (*Solanum lycopersicum* L., cv. 'Colossal')	SA	Aires et al. (2022)

	Tomato (*Solanum lycopersicum* L., cv. 'Baraka')	SA	Baninaiem & Dastjerdi (2023)
	Tomato (*Solanum lycopersicum* L., cv. 'Prensisa')	HA; FA; SA	Al-Saif et al. (2024)
	Cherry tomato (*Solanum lycopersicum* L., cv. 'Reddery RZ F1') Mini-plum tomato (*Solanum lycopersicum* L., cv. 'Delisher')	KIO_3	Somma et al. (2024)
	Red bell pepper (*Capsicum annuum* L., cv 'California Wonder')	SA; MT	Nasiri et al. (2024)
	Green bell pepper (*Capsicum annuum* L., cv. 'Karisma')	CaTS; KTS	Moosavi-Nezhad et al. (2024)
The alleviation of CI symptoms, improving chilling tolerance during cold storage	Tomato (*Lycopersicon esculentum* L., cv. 'Newton')	SNP	Ahmadi-Soleimanie et al. (2020)
	Tomato (*Solanum lycopersicum* L., cv. 'Baraka')	SA	Baninaiem & Dastjerdi (2023)
The induction of resistance against pathogen and decay incidence	Tomato (*Solanum lycopersicum* L., cv. 'Raf' and cv. 'Amadeo')	PYR + Bos	Domínguez et al. (2012)
	Tomato (*Solanum lycopersicum* L., cv. 'Vesuviano')	Propolis; CHS	Migliori et al. (2017)
	Eggplant (*Solanum melongena* L., cv. 'IR3121')	SA	Ghahremani et al. (2021)
	Tomato (*Solanum lycopersicum* L., cv. 'MT1' and cv. 'MT3')	$CaCl_2$	Mazumder et al. (2021)
	Red Bell Pepper (*Capsicum annuum* L., cv. 'California Wonder')	SA; MT	Nasiri et al. (2024)

† Abbreviations: CI, chilling injury; 1-MCP, 1-methylcyclopropene; MeJA, methyl jasmonate; SA, salicylic acid; $CaCl_2$, calcium chloride; Ca, calcium; MeSA, methyl salicylate; GB, glycine betaine; TSP, trisodium phosphate; MT, melatonin; OA, oxalic acid; Arg, arginine; *O. dictamnus* oil, *Origanum dictamnus* oil; PYR, pyraclostrobin; Bos, boscalid; CHS, chitosan; SNP, sodium nitroprusside; N, nitrogen; NAA, naphthalene acetic acid; COS, chitosan oligosaccharide; HA, humic acid; FA, fulvic acid; CaTS, calcium thiosulfate; KTS, potassium thiosulfate; KIO_3, iodine.

1.4. Green pepper fruit by-products: Seeds

The food industry is responsible for generating significant amounts of waste, which often raises environmental concerns due to its high biodegradability. According to the United Nations Environment Programme (UNEP) and the Food and Agriculture Organization of the United Nations (FAO), approximately 1.05 billion tonnes of food waste will be generated globally in 2022, representing about one third of the edible parts of food produced for human consumption (FAO, 2024). The issue of food waste from fruit and vegetable consumption is a significant global concern. It is estimated that approximately 45 % of the global production of fruit and vegetables is wasted during the supply chain, from the initial stages of production to the final consumption stage. Fruit and vegetable processing generates significant amounts of waste and by-products, accounting for 20-60 % of fruit and vegetable inputs. This by-product fraction accounts for 10 % of total food waste (Banerjee et al., 2017). In addition, agricultural by-products, such as those from fruit and vegetable processing, have increased significantly over the last 25 years, largely because of studies linking fruit and vegetable consumption to health benefits, including reduced mortality from cancer and cardiovascular disease (Pimentel-Moral et al., 2020).

Losses and wastage of fruit and vegetables are significant economic and environmental sources of bioactive compounds that need to be valorised rather than disposed of in landfills. From a socio-economic perspective, a few options have been identified for the potential use of fruit and vegetable by-products in the food, cosmetic and pharmaceutical industries due to their bioactive compounds. From an environmental perspective, the impact of fruit and vegetable wastage and loss on the environment and human health is of great concern (Coman et al., 2020; Anaya-Esparza et al., 2021). Consequently, there is a need to establish an approach aimed at reducing food waste.

The valorisation of post-production agrifood by-products, to produce value-added food ingredients, has become a major focus of research to improve the sustainability of the food chain. The use of agrifood by-products for the extraction of bioactive compounds and nutrients represents a valuable opportunity, offering significant possibilities for waste reduction and indirect income generation (Iriondo-DeHond et al., 2018; Pimentel-Moral., et al. 2020). Therefore, the biotransformation of fruit and vegetable processing wastes into value-added bioproducts could be a viable approach from an environmental and techno-economic perspective. The recovery of these high value-added antioxidant compounds, including phenolic compounds, flavonoids, chlorogenic and gallic acids, anthocyanins, carotenoids, lycopene, proteins, polysaccharides and tannins, is further supported by growing scientific evidence from studies on co-products such as eggplant peel (Boulekbache-Makhlouf et al., 2013), apricot peel and marc (Han et al, 2013; Cheaib et al., 2018), mango peel, seeds and pulp (Vega-Vega et al., 2013), careway seeds (Yara-Varón et al., 2016), grape seeds (Da Porto & Natolino, 2017; Boger et al., 2018), potato, tomato, avocado and pomegranate peels (Singh & Saldaña, 2011; Ho et al., 2015; Figueroa et al., 2018; Dimitrov et al., 2019), and broccoli by-products (Ferreira et al., 2018).

Peppers are a vegetable with a high nutritional value (Smith et al., 2021). However, the processing of peppers typically produces a by-product in the form of stalks, seeds and leaves.

However, this by-product can be used as a raw material for the extraction of phytochemical compounds, offering a wide range of possibilities for reuse and valorisation, thus contributing to the principles of the circular economy. Several bioactive compounds have been identified in the waste edible parts, seeds and leaves of pepper, including phenolics, flavonoids, carotenoids, tocopherol and pectic polysaccharides (Anaya-Esparza et al., 2021; Cvetković et al., 2022). These bioactive compounds have been shown to have a variety of biological activities, including antioxidant, antimicrobial, antifungal, immunosuppressive and immunostimulant properties, as well as anti-diabetic, anti-tumour and neuroprotective activities (Anaya-Esparza et al., 2021). In addition, the use of these by-products can serve as a strategy to generate new functional or nutraceutical products, including functional food additives for human consumption.

The widespread use of pre-prepared convenience products, such as fresh-cut pepper fruit, frozen sliced of pepper fruit and minimally processed pepper fruits, has increased significantly in society. However, the increased consumption of these fresh, chilled or frozen products, while convenient, results in increased waste from the washing, cutting and packaging processes. Consequently, this waste needs to be re-evaluated to minimise the environmental impact and maximise the use of resources, and to this end it is essential to develop sustainable practices in the food industry. As stated in the relevant literature on fresh-cut and frozen fruits and vegetables (Sharma et al., 2016; Berkenkamp et al., 2012; James, 2021), the following section outlines the various applications and their effect on the waste of fresh-cut, frozen-sliced and minimally processed pepper fruits.

- ***Fresh-cut pepper fruit:*** Its use in culinary contexts such as salads, stir-fries and pizzas, as well as its use as a garnish, underlines its versatility. During processing, a significant number of stalks and seeds are discarded. Finally, the packaging also contributes to the generation of plastic waste.
- ***Frozen-sliced pepper fruit:*** They are used in stews, sauces, stir-fries and as an ingredient in ready meals. The waste generated during the initial processing stage is like that of fresh slices. Plastic materials are also used in the freezing process.
- ***Minimally processed pepper fruits:*** This category includes peppers that have been washed, cut and packaged for immediate consumption. This results in the generation of stalk and seed waste in addition to packaging waste. This type of product is closely linked to the catering industry, where a significant amount of organic waste is generated.

In this sense, the valorisation of food waste is an area of technological and innovative research that brings benefits to the population, the economy and the environment. The implementation of sustainable economic practices in the food industry, including the valorisation of pepper by-products, has been shown to reduce waste and create economic opportunities through the production of value-added products. This approach is in line with the principles of the circular bioeconomy, where waste is transformed into resources, contributing to an efficient and environmentally eco-friendly food system. As the consumption of fresh-cut products continues to grow, it is essential to implement by-product management and valorisation strategies that balance consumer convenience with environmental responsibility in the near future.

- 2 -

Aim and Objectives

2. AIM AND OBJECTIVES

The Lamuyo-type green pepper fruit displays an earlier and shorter vegetative cycle in comparison to other types of pepper fruits. Consequently, the influence of the harvesting date and the phenological stage at which the green pepper is consumed as a possible factor influencing its content in bioactive compounds and its antioxidant capacity remains to be elucidated. In view of the quality issues experienced with green pepper fruit and the strategies and previous research that have been previously commented in different horticultural products of the *Solanaceous* family, it is imperative to address the study of preharvest treatments with elicitors in the Lamuyo-type green pepper fruit. The purpose of this study is to propose solutions to a significant problem in the marketing of green pepper fruits, due to this cultivar being characterised by a short shelf-life during postharvest, as mentioned above.

Conversely, based on the previous knowledge, the capacity that these elicitors could have to induce antioxidant defence systems in pepper fruits, as well as the possible mitigation that could cause to reduce CI symptoms, would be a great preharvest strategy to minimise quality losses and some physiological disorders of green pepper fruit during postharvest storage and marketing. Moreover, these compounds have been demonstrated to preserve or augment the antioxidant properties of the different fruit and vegetable of *Solanaceous* family, thereby could ensuring the well-being of consumers. The present study aims to address this knowledge gap by investigating the effects of the preharvest application of these elicitors on green pepper fruits of 'Lamuyo' type, cv. 'Herminio'.

The main aim of the present PhD thesis was to extend the shelf-life of green pepper fruit by applying preharvest treatments with methyl jasmonate (MeJA) and salicylic acid (SA) during the developmental cycle of the crop (**Figure 4A**). Furthermore, novel strategies for the revalorisation of pepper seeds as by-products generated from agrifood industries have been considered as the last objective of the present PhD thesis (**Figure 4B**).

In accordance with this aim, the following specific objectives were pursued (**Figure 4**):

I. Determine the optimal phenological stage for harvesting in relation to phytochemical quality and storability.

II. Evaluate the impact of preharvest application of MeJA and SA on crop yield, green pepper fruit quality and shelf-life.

III. Investigate the effect of preharvest application of MeJA and SA on the antioxidant systems, lipid metabolism and its relationship with CI tolerance.

IV. Review the possible potential for the revalorization of pepper seeds as by-products.

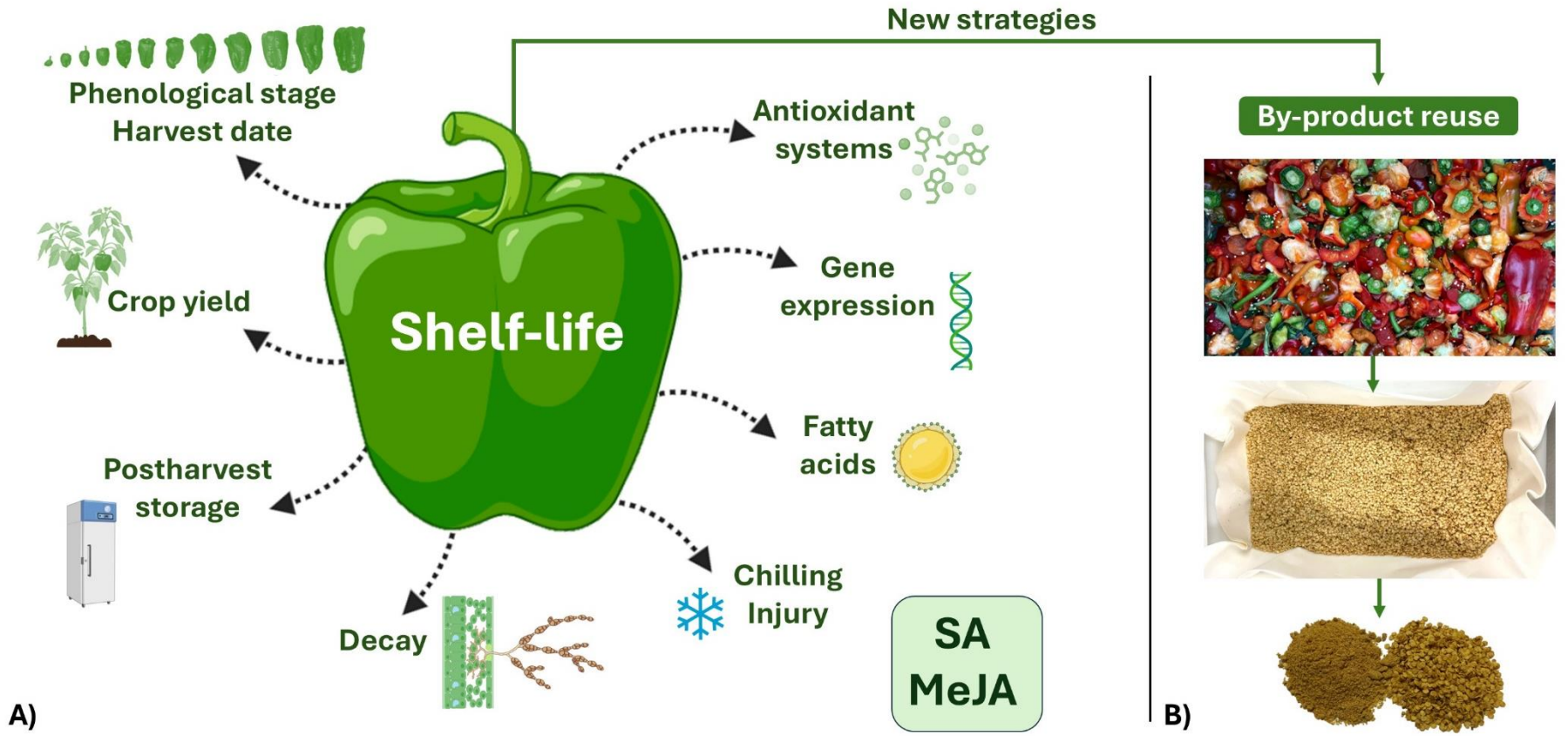

Figure 4. A) *Diagram illustrating the aim and objectives of this PhD Thesis based on the elicitation strategies with SA (salicylic acid) and MeJA (methyl jasmonate). Some targeted parameters have been represented based on the analyses carried out in the publications 1, 2, 3, 4 and 5: research articles. Source: The information has been compiled by the authors and the figure was created with BioRender (Toronto, ON, Canada).* ***B)*** *New studies and strategies are needed to reuse and valorise the green pepper seeds as by-product (Review of existing characterization studies and the identification of their potential use as a source for agrifood applications: publication 6). Source: The photographs have been compiled by the authors.*

- 3 -

Materials and Methods

3. MATERIALS AND METHODS

In this section, the chief characteristics of the plant material, the experimental conditions, the preharvest treatments, the analytical determinations and the statistical design employed in this PhD thesis are included. For a more detailed exposition of these aspects, the publications that constitute the result section can be consulted.

3.1. Plant material and experimental design

This PhD thesis comprises experimental studies that were performed during three growing seasons (2020, 2021 and 2022) on *Capsicum annuum* L. Thus, 'Lamuyo'-type pepper plants, 'Herminio' cultivar, were cultivated on an annual basis in January in a commercial plot that was being cultivated in a plastic-roofed greenhouse located in El Raal (Murcia, Spain). The crop management programme that was followed for the short growing cycle of 'Lamuyo' pepper was the same one that the company usually employs for this purpose. The programme involved the use of rockwool as the soil substrate, drip irrigation, and nutrient levels that were optimal. Pepper plants were established in a randomised block design, with varying numbers of replicates assigned to each treatment and year. The harvesting of pepper fruits was primarily conducted in accordance with the stipulated commercial harvesting stage for consumption, as well as the commercial criteria for harvest that had been established by the company.

The elicitor treatments utilised in the experimental studies presented in this PhD thesis were prepared using a uniform methodology and under the same conditions. The compounds utilised in this study were methyl jasmonate (MeJA) and salicylic acid (SA), obtained from Sigma (Sigma-Aldrich, Madrid, Spain). The administration of treatments was conducted through foliar spraying or irrigation, performed at the early hours of the morning and depending on the publication. It was observed that solutions for all treatments were supplemented with 0.5 % Tween®-20 as a surfactant. The initial treatment was administered prior to the onset of the flowering stage. The equidistance among the application dates of the treatments was approximately 21 days, due to the staggered flowering cycle, except for the last application, which was performed near to the final commercial harvest. The selection of the specific pepper cultivar was based on its crop cycle. A total of seven exogenous applications and ten harvest dates were made throughout the development and growth cycle of the 2020 and 2021 seasons, although only four consecutive times at 21-day intervals until the date of the only harvest performed were carried out in the 2022 season. The treatments and concentrations that were the focus of this study are outlined in **Figure 5** and were as follows:

- ***Control:*** Control pepper plants were sprayed with 0.5 % Tween®-20 aqueous solution.
- ***Methyl jasmonate (MeJA):*** In the 2020 season, treatments were administered at concentrations of 0.1 and 1 mM for pepper plants by means of foliar spraying. In the subsequent years, namely 2021 and 2022, the optimal concentration of MeJA, as determined by the findings of the preceding season, was employed for the cultivation of green peppers. This concentration of MeJA, at 0.1 mM, was administered through two distinct methods: foliar spraying and irrigation.

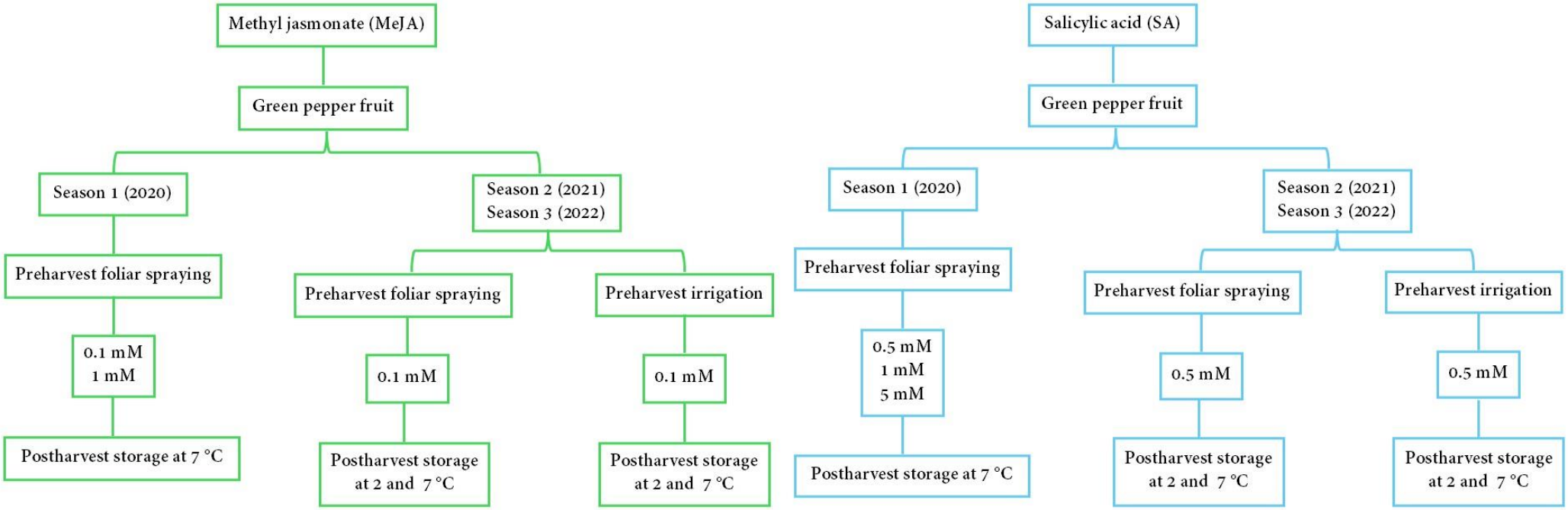

Figure 5. *The experimental design scheme of the different elicitors (MeJA and SA) preharvest treatments included in this PhD thesis, which was conducted over three growing seasons (2020, 2021 and 2022). The temperatures assayed were 2 and 7 ºC ± 1 ºC. Source: The information has been compiled by the authors.*

- ***Salicylic acid (SA):*** In the 2020 season, treatments were administered at concentrations of 0.5, 1 and 5 mM for pepper plants by means of foliar spraying. In the subsequent years, namely 2021 and 2022, the optimal concentration that had been determined as effective in the preceding season (i.e. SA at 0.5 mM) was employed once more for the cultivation of green peppers. This was achieved through two distinct methods: foliar spraying and irrigation.

The postharvest storage conditions that were examined were contingent upon the objective of each scientific publication presented. The present PhD thesis focuses on the conservation of control and treated peppers at optimum temperatures (7 ± 1 ºC) and chilling-temperature conditions (2 ± 1 ºC) for a long-stored period (up to 28 days).

3.2. Crop yield

In consideration of the staggered harvesting characteristic of this crop, a total of ten harvests were conducted on an annual basis, in accordance with the commercial quality criteria established by the company. On each occasion, the total crop yield was determined, with the quantity in kilograms of fruit harvested per plant and the number of commercial fruits produced per plant being recorded. The production of the plants was expressed as accumulative yield (kilograms per plant) from the first to the last harvest date in each season studied. Furthermore, the average fruit weight (g) at the various harvesting dates was ascertained.

3.3. Physiological, physiochemical and functional determinations

The weight of the green peppers was measured at harvest and after each storage period. The ***weight loss*** of each pepper fruit was determined individually, with the fruit's weight recorded on the day of harvest (day 0) and on the different analysis dates during postharvest storage. The aggregation of these losses was expressed as a percentage (%) of the initial weight. The ***respiration rate*** of the green pepper fruits was measured by subjecting them to an experiment in which they were hermetically sealed in a 2-litre glass jar for a period of 60 min. Subsequently, 1-mL samples were extracted from the airtight glass jar using a syringe of atmosphere and injected into the Shimadzu 14B gas chromatograph (Shimadzu Europe GmbH, Duisburg, Germany). This instrument was equipped with a thermal conductivity detector. As previously outlined in the publication of Dobón-Suárez et al. (2021b), the chromatography conditions have been thoroughly delineated. The results of respiration rate were expressed as mg CO_2 kg^{-1} h^{-1}.

The ***firmness*** of the green pepper fruits was measured individually using a TX-XT2i texture analyser (Stable Micro Systems, Godalming, UK). The TX-XT2i texture analyser was equipped with a flat probe, the purpose of which was to produce a deformation force on the fruit equivalent to 5 % of the fruit diameter. The results obtained were expressed as the ratio of the applied force to the distance travelled (N mm^{-1}). The ***external colour*** of pepper fruits was measured individually at three points around the outer equatorial zone of the fruit using a Minolta colourimeter (CRC200, Minolta Camera Co., Kanto, Tokyo, Japan). The results of the green colour measurement were

expressed using the CIE Lab system. Following the recording of the L*, a*, and b* parameters, the colour was defined as the b* parameter or hue angle (h^o = arctg b*/a*), depending on the publication.

The ***total soluble solids (TSS)*** were determined in duplicate in the juice obtained from green pepper samples ground per replicate of each treatment, using an Atago PR-101 digital refractometer (Atago Co. Ltd., Tokyo, Japan) at 20 °C. Furthermore, the ***total acidity (TA)*** of each replicate was determined by automatic titration with 0.1 N NaOH to pH 8.10, utilising 1 mL of juice diluted in 25 mL of distilled H_2O. The ***ripening index (RI)*** was determined through the calculation of the quotient of TSS and TA.

The quantification of ***individual sugars and organic acids*** was conducted through the extraction of 5 g of green pepper fruit sample from each replicate, according to the protocol described by García-Pastor et al. (2020a). The extraction process was conducted using a 5-mL aqueous solution of 0.5 % phosphoric acid. Subsequently, the samples were subjected to a centrifugation process at 10,000 *g* for 10 min at a temperature of 4 °C. After this, the resultant supernates were filtered through 0.45 µm Millipore filters and injected in duplicate into a high-performance liquid chromatography (HPLC) system (Hewlett Packard HPLC series 1100). The elution system consisted of 0.1 % phosphoric acid, which was run isocratically at a rate of 0.5 mL min^{-1}, through a Supelco column (Supelco Gel C-610H, 30 cm x 7.8 mm, Supelco DBAK, Bellefonte, United States). The detection of organic acids was accomplished through the utilisation of 210 nm light absorption, while the detection of sugars was achieved by means of a refractive index detector. A standard curve of pure sugars and organic acids purchased from Sigma (Poole, UK) was utilised for quantification purposes.

The quantification of the ***antioxidant capacity*** and the ***bioactive compounds content*** was conducted on the frozen samples, which were stored under liquid nitrogen at -80 °C. Analytical determinations of functional traits were conducted on the edible portion of the green pepper (skin and flesh). The analysis of the ***total antioxidant activity (TAA)*** and the ***total phenolic content (TPC)*** was performed in accordance with previously reported methods (Sayyari et al., 2011; García-Pastor et al., 2020a). Accordingly, 5 g of frozen green pepper fruit samples were homogenised in 10 mL of 50 mM phosphate buffer (pH = 7.80) and 5 mL of ethyl acetate. The homogenate was then subjected to a centrifugal process at 10,000 *g* for 15 min at a temperature of 4 °C. The upper and lower fractions were utilised to quantify the ***lipophilic (L-TAA)*** and ***hydrophilic (H-TAA) total antioxidant activity***, respectively. The TPC was quantitatively measured in duplicate on the lower fraction for each extract using the Folin-Ciocalteu reagent, as previously described by García-Pastor et al. (2020a). The determination of TAA, in both H-TAA and L-TAA, was achieved through the utilisation of an enzymatic system comprising the chromophore 2,20-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), in conjunction with the horseradish peroxidase enzyme and its oxidant substrate (hydrogen peroxide). In this system, the generation and monitoring of $ABTS^+$ radicals occurs at a wavelength of 730 nm. The decrease in the degree of absorption following the addition of green pepper extract was proportional to the total antioxidant activity (TAA) of the sample. A calibration curve was performed with Trolox (R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0-20

nmol) from Sigma-Aldrich (Madrid, Spain), and the results were expressed as g of Trolox Equivalent (TE) kg^{-1}.

The content of ***ascorbic acid (AA)*** and ***dehydrorascorbic acid (DHA)*** of a frozen sample of green pepper fruit was measured in accordance with the method established by Peña-Estévez et al. (2016). In order to extract AA and DHA, 5 g of the sample was homogenised with 5 mL of a methanol: water (5:95, *v/v*) solution containing 0.1 mM citric acid, 0.05 mM ethylenediamine tetraacetic acid disodium salt, and 4 mM NaF. The mixture was then subjected to processing using an Ultraturrax T18 basic (IKA, Berlin, Germany) for a period of 30 s. Subsequently, the extract was filtered through a four-layer cheesecloth, the pH was adjusted to 2.35-2.40 with 2 N ClH, and the sample was subjected to centrifugation at 10,000 *g* for 15 min at 4 °C. The resulting filtrate was purified through a methanol-activated C18 cartridge (Sep-Pak cartridges C18, Waters, Dublin, Ireland) and filtered through a 0.45 µm PFTE filter. In the context of the DHA derivatisation process, a volume of 750 µL of the extract was amalgamated with 250 µL of 7.7 M 1,2-phenylenediamine within an HPLC amber vial. The mixture was left to react for a period of 37 min, after which 20 µL of the sample was injected into the column. The column employed was a Luna (250 mm x 4.6 mm, 5 µm particle size) C18 column (Phenomenex, Macclesfield, United Kingdom) with a C18 security guard (4.0 mm x 3.0 mm) cartridge system (Phenomenex, Macclesfield, United Kingdom) using a HPLC system (Agilent HPLC 1200 Infinity series). The mobile phase was composed of 50 mM KH_2PO_4, 5 mM hexadecyl trimethyl ammonium bromide and 5 % methanol, with a pH of 4.59. The isocratic flow rate was set at 1 mL min^{-1}. Spectrophotometric analysis was conducted at 261 nm for AA [R_T (retention time) = 9.4 min] and at 348 nm for DHA (R_T = 4.5 min). The concentrations of both compounds were determined by comparison with AA and DHA standards (Sigma-Aldrich, Germany), employing a method that has been previously described by García-Pastor et al. (2020a).

The extraction of ***total chlorophylls*** *(the sum of* ***chlorophyll a and b****)* and ***total carotenoids*** was conducted in accordance with the methodologies previously outlined by Knee (1972) and Xie et al. (2023), with minor adjustments. Approximately 0.20 g of freeze-dried powder for each of the replicates were manually ground in a mortar and pestle and mixed with 5 mL of acetone extract solution containing 0.1 % butylated hydroxytoluene (BHT) to prevent the pigment from oxidising. In the subsequent stage of the process, the mixed extraction was subjected to ultrasonic extraction for a period of 15 min. Subsequently, the mixture was subjected to a centrifugation process at a speed of 10,000 *g* for a duration of 10 min at a temperature of 4 °C. This procedure was repeated multiple times until the residue was found to be colourless. To estimate the chlorophyll and total carotenoid contents of the collected sample, an acetone solution containing 0.1 % BHT was utilised. The estimation was conducted for a constant volume of the solution (25 mL). The methods reported by Lichtenthaler and Wellburn (1983) were utilised to detect the extracts' extinction coefficient at 470, 645, and 662 nm, employing a spectrophotometric absorption (UV-1900i-UV-VIS Spectrophotometer, Shimadzu Corporation, Germany) to quantify the content of both total chlorophylls and total carotenoids. The following equations were used in the calculation: Ca = 11.75662 – 2.35645; Cb = 18.6l645 – 3.96662; and TCC = (1000470 – 2.27*Ca – 81.4*Cb)/227.

The variables Ca and Cb are indicative of the content of chlorophyll a and chlorophyll b, respectively. Furthermore, the abbreviature of TCC signifies the total carotenoid content.

3.4. Antioxidant enzymes activities

The ***antioxidant enzymes*** were measured in freeze-dried powder (flesh and skin tissues) that were maintained at -80 °C, in order to determine ***ascorbate peroxidase (APX)***, ***catalase (CAT)*** and ***peroxidase (POD)*** enzymes. To analyse the APX, CAT and POD activities, 0.20 g of green pepper powder was homogenised with 5 mL of a phosphate buffer (50 mmol L^{-1}, pH 6.80) solution that contained 1 % (*w/v*) polyvinylpyrrolidone (PVP) and 1.0 mmol L^{-1} ethylenediamine-tetraacetic acid. Subsequently, the extracts were subjected to a centrifugal process at 10,000 *g* for 15 min at 4 °C. The resulting transparent upper layer was then utilised for the quantification process, which was conducted in duplicate as previously outlined by García-Pastor et al. (2021). To quantify the amount of APX, the following reaction mixture was prepared: 200 µL of extract in 3 mL of 50 mmol L^{-1} potassium phosphate (pH 7.00), 0.5 mmol L^{-1} ascorbic acid and 1.0 mmol L^{-1} H_2O_2. Subsequently, the decrease of the absorbance at 290 nm from time 0 to 60 s was measured. For the CAT activity, 100 µL of extract were added to 3 mL of reaction mixture containing 15 mmol L^{-1} H_2O_2 and 50 mmol L^{-1} phosphate buffer (pH 7.00). The decrease of extinction coefficient at 240 nm over a period of one minute was measured. In conclusion, the reaction mixture for the POD activity comprised 200 µL of extract, with a final volume of 3 mL of 50 mmol L^{-1} phosphate buffer (pH 7.00), 12 mmol L^{-1} H_2O_2 and 7 mmol L^{-1} guaiacol. The increase in the absorption at 470 nm over a period of one minute was measured. The activity of APX, CAT or POD was expressed in terms of units of enzymatic activity, with one enzymatic unit (U) being defined as a decrease or increase, depending on the enzyme, of 0.01 ascorbate min^{-1}.

3.5. Relative *Ca*APX, *Ca*CAT, *Ca*POD, *Ca*PAL and *Ca*DHAR2 genes expression

The RNA was extracted from 0.03 g of freeze-dried flesh and skin tissues of green pepper fruit, in accordance with the protocol described by García-Pastor et al. (2021), with slight modifications. The ***relative expression levels of targeted genes*** at harvest (day 0) and after 28 days of storage were analysed. Total RNA extraction was conducted using the RNeasy Plant Mini Kit (Qiagen, Düsseldorf, Germany), following the manufacturer's instructions. The protocol stipulated a DNase treatment for the eluted RNA, which was facilitated by Baseline-ZERO DNase (Epicentre/Lucigen USA). The quantification of RNA was conducted by spectrophotometric absorption using an Implen Nanophotometer® (IMPLEN, Munich, Germany). The RNA extracts were stored at -80 °C. The synthesis of single-strand cDNA from 500 ng of total RNA was performed using the PrimeScript RT Master Mix (Perfect Real Time) kit (Takara Bio, Japan) in a Mastercycler Nexus X2 (Eppendorf, Germany) PCR machine, in accordance with the instructions provided by the manufacturer. The synthesis and subsequent quantitative real-time polymerase chain reaction (qPCR) for the expression of the targeted genes was carried out by the Genomic Centre of the Complutense University of Madrid (Madrid, Spain). Total RNA (15-40 ng per reaction) from the different biological replicates and treatments was utilised as the template for the OneStep qPCR reactions.

In the third publication of the present PhD thesis, two housekeeping genes, **ubiquitin (*Ca*UBI)** and ***actin (CaACT)***, were selected as reference genes in *Capsicum annuum* L. (Li et al., 2020; Seo et al., 2020; Ge et al., 2020). The relative expression of five genes was evaluated as targeted genes: ***L-ascorbate peroxidase gene (CaAPX)***, ***catalase gene (CaCAT)***, ***peroxidase gene (CaPOD)***, ***phenylalanine ammonia-lyase gene (CaPAL)*** and ***dehydroascorbate reductase 2 gene (CaDHAR2)***. The gene-specific primers utilised are listed in **Table 2**. Gene sequences were obtained from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). The amplicon length for each primer is outlined below: The base pairs for *Ca*UBI are 123, for *Ca*ACT 130, for *Ca*APX 238, for *Ca*CAT 104, for *Ca*POD 131, for *Ca*PAL 676 and for *Ca*DHAR2 136. The primers for qRT-PCR (**Table 2**), purchased from Merck (Sigma-Aldrich, Darmstadt, Germany), were utilised. The reactions were prepared in duplicate on 384-well plates using PowerUp SYBR® Green Master Mix (Applied Biosystems, California) with the primers at a concentration of 300 nM in a reaction volume of 10 µL. Subsequently, the qPCR analysis was conducted in a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, California) utilising an initial step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Furthermore, the quality of the amplicons was assessed through a melt curve analysis step, which revealed the absence of any side products. The data obtained from the qPCR were then processed using the QuantStudio Real-Time PCR Software (Applied Biosystems, California). Subsequently, relative targeted gene expression in treated green pepper fruit was normalised using the expression levels of the *Ca*UBI and *Ca*ACT genes. This calculation was conducted with reference to control fruit, utilising each biological replicate.

Table 2. *Transcriptomic details of primers for the reference and targeted genes analysed.*

Gene[†]	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')	NCBI Reference
*Ca*UBI	GGCATGTCTGGGACTTTTGC	AGACCCGTTCCTTGACAACC	AY486137.1
*Ca*ACT	ACCCTGTGCTTCTCACTGAAG	GCATAAAGAGACAACACCGCC	AY572427.1
*Ca*APX	ACTGGTGGACCGAATGGTTC	GTAACCGCCCTTCCTTTGGA	NM_001324587.1
*Ca*CAT	TATCCGATCCCCGAGCAACT	CACAGTGAGACGAGAAGCG	AF227952.1
*Ca*POD	AACAGGGAAACCCGAATGGG	TTTGGTGCAGCCCTCTTCTC	FJ596178
*Ca*PAL	ATGCTCTTAGAACGTCGCCC	AAGACGTATTCCCTGTCCACG	NM_001325423
*Ca*DHAR2	GTTGATTTGAGCTTGGCCCC	TCTGGAAAGACTCACGCTCG	KJ950368.1

[†] Abbreviations for the different reference genes analysed in *Capsicum annuum* L. (*Ca*): *Ca*UBI, ubiquitin gene; *Ca*ACT, actin gene; *Ca*APX, L-ascorbate peroxidase gene; *Ca*CAT, catalase gene; *Ca*POD, peroxidase gene; *Ca*PAL, phenylalanine ammonia-lyase gene; *Ca*DHAR2, dehydroascorbate reductase 2 gene.

3.6. Incidence of fruit decay and chilling injury

In the first publication, the ***incidence of fruit decay*** was analysed. On a weekly basis, the number of decay peppers per treatment stored at 7 °C was recorded for each sampling date. The results were expressed as the percentage (%) of accumulated decay at 21 days of storage with

respect to the total number of pepper fruits utilised in that experiment. Furthermore, ***photographic documentation*** was conducted to illustrate the visual characteristics of green pepper fruits treated with SA and those that were not (control), over a 21-day storage period. Photographs of pepper fruits were captured using a digital camera (Nikon D3400) in a light box with a white background. The camera setup was configured in accordance with the methodology outlined by García-Pastor et al. (2020b), with the following parameters: two LEDs emitting light at a colour temperature of 5600 K, a flash speed of 1/5 s, an ISO setting of 200, a focal opening of f/20, and a lens length of 35 mm. The substandard pieces of pepper fruit were evaluated, documented through photography, and subsequently eliminated from the experiment.

The ***external CI index*** was evaluated in each fruit according to a 5-point hedonic scale (**Figure 6**). This scale was based on the percentage (%) of surface affected by CI symptoms (*i.e.* dehydration, browning and pitting). The scale ranges from 0 (no symptoms), 1 (1–20 %), 2 (21–40 %), 3 (41–60 %), 4 (61–80 %) and 5 (> 81%). Scale photographs of green pepper fruits were obtained from previous studies and utilised as a visual reference for the evaluation of CI symptoms (**Figure 6**). The severity of CI symptoms was subsequently determined based on these photographs. The calculation of the CI index was conducted as outlined below: the total value from the hedonic scale was multiplied by the number of fruits with the corresponding score, and this result was divided by the total number of fruits in the sample.

Figure 6. *Reference hedonic scale to evaluate chilling injury (CI) index in Lamuyo-type green pepper fruit during 28 storage days at 2 ºC. Source: Photographs were compiled by the authors.*

3.7. Fatty acids profile and content

The procedure for the methylation of the fat of flesh and skin of green peppers was conducted in accordance with the methodology outlined by Trigueros & Sendra (2015). In employing a trans-esterification reaction, 20 mg of the sample was reacted with 2 mL of 0.5 M sodium methoxide. The fatty acid profile was determined and quantified by high-resolution gas chromatography, which analysed the fatty acid methyl esters obtained. The separation of methyl esters was accomplished through the utilisation of a Shimadzu GC17A gas chromatography unit, which was integrated with a mass spectrometry detector GC–MS QP5050 (Shimadzu, Kyoto, Japan). The separation process was conducted using a SupraWax-280 column, which was filled with 100 % polyethylene glycol (Teknokroma S. Co. Ltd., 165 Barcelona, Spain; 30 m length, 0.25 mm internal diameter, 0.25 µm

film thickness) and were identified by comparison of their retention times with the Supelco 37-component FAME Mix reference standard (Sigma-Aldrich Co., St. Louis, MO, USA). The chromatographic conditions were applied in accordance with the methodology detailed by García-Pastor (2020c), and quantification was performed for each sample using nonadecanoic acid (19:0, C19) as the internal standard.

3.8. Statistical analyses

The results of this PhD thesis were expressed as the mean ± standard error (SE) of three (n = 3) or five (n = 5) replicates, depending on the experimental design of each publication. The data were then analysed using a variety of statistical methods, including analyses of variance (ANOVA), HSD Tukey's test or HSD Duncan's test to examine mean comparisons, and Student's *t*-test. The sources of variation were found to vary according to each experimental design of each study. Statistically significant differences were $p < 0.05$, and these were indicated using different lowercase or capital letter designations in each publication included. In certain instances, these disparities were denoted by an asterisk (*) positioned in the corresponding bars or points for each parameter. In some publications, resulting differences were represented as *, **, and *** symbols when $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. No statistically significant differences were identified when $p \geq 0.05$ and, thus, represented as NS. All analyses were executed using the SPSS software package version 17.0 for Windows. The PCA model was also constructed with normalized data using the version 17.0 of SPSS software package. The heatmap analysis was conducted with Microsoft Excel® for Windows (Excel, 2016, Microsoft Corporation, Redmond, Washington, USA).

- 4 -

Publications

4. PUBLICATIONS

4.1. Publication 1 — Research article

PUBLICATION 1 (Open access)

Influence of the phenological stage and harvest date on the bioactive compounds content of green pepper fruit

Dobón-Suárez, A., Giménez, M.J., Castillo, S., García-Pastor, M.E., Zapata, P.J.

Molecules, 2021, 26(11), 3099

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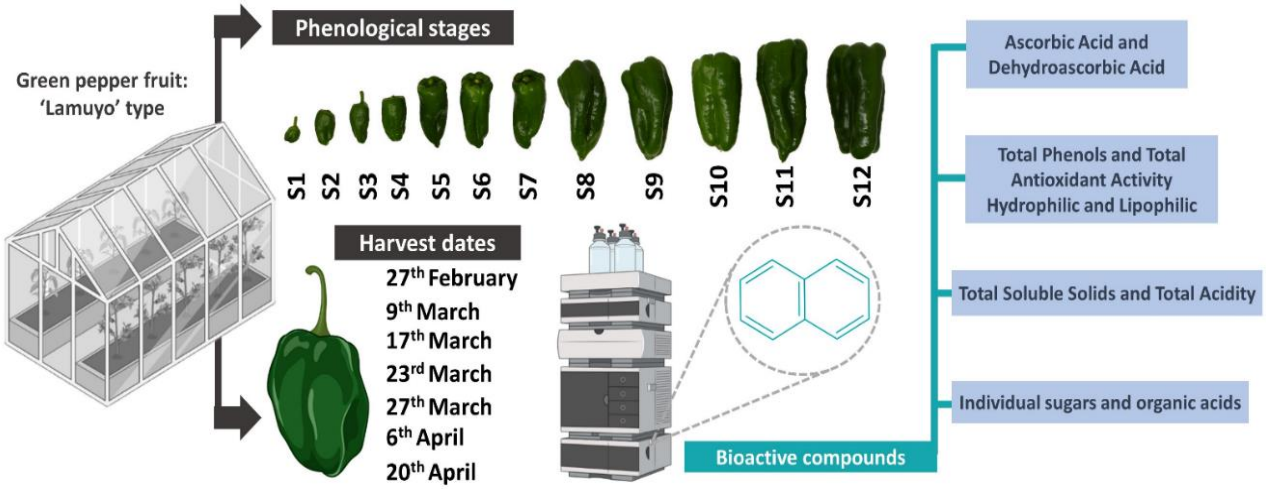
Graphical abstract:



PUBLICATIONS

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Article

Influence of the Phenological Stage and Harvest Date on the Bioactive Compounds Content of Green Pepper Fruit

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Abstract: Green pepper fruit is often consumed before it is completely ripe. However, the influence of the phenological stage in which the green pepper is consumed as a potential influencing factor in its bioactive compounds content and antioxidant capacity remains unknown. In addition, no literature is available concerning the bioactive compounds changes in 'Lamuyo' green peppers along its developmental and growth cycle. For this, two different approaches have been carried out, one using twelve different phenological stages (S1 to S12), and in the other, seven different harvest dates (from 27 February to 20 April). Moreover, bioactive compounds changes during 21 days of postharvest storage at 8 °C were investigated. In this study, bioactive compounds (ascorbic acid, dehydroascorbic acid, and total phenolic content) and the total hydrophilic and lipophilic (TAA-H and TAA-L) antioxidant activity were analysed. In addition, total soluble solids, total acidity, individual sugars, and organic acids were determined. Vitamin C levels increased along the phenological stages and harvest dates due to significant increases in ascorbic and dehydroascorbic acid levels. Our results show that the total phenol content decreases as vegetables develop and subsequently increases both as ripening begins and by the last harvest date. Furthermore, TAA-H was also greater by the phenological stage S12 and the 20 April harvest date. In conclusion, the phenological stage and harvest date are key factors that significantly influence the bioactive compounds of green peppers, and those that appear by S12 and 20 April could be more beneficial to health.

Keywords: antioxidant activity; *Capsicum annuum*; organic acids; phenolics; vitamin C

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1. Introduction

Currently, there is great interest in maintaining good health, and the intake of bioactive compounds in the diet has important health-protecting benefits related to antioxidant activities [1]. Pepper fruit (*Capsicum annuum* L.) is a vegetable of great economic importance worldwide, and it is greatly appreciated in the market for its organoleptic qualities [2], especially for its colour variability and its high levels of bioactive compounds [3]. This vegetable is high in bioactive compounds like phenols, mainly flavonoids which are a group of phenolic compounds, carotenoids, capsaicinoids, and vitamin C [3,4], and there is good correlation between its antioxidant activity and total phenolic content, and ascorbic acid content [5]. A broad number of cultivars are available in supermarkets, most of which range in colour from green to yellow, orange, red, or purple when they are completely ripe. Green peppers are often harvested before they ripen completely, and the stage of ripeness can partly account for the phytonutrient content [6]. Some authors have reported that bioactive compounds levels can vary by genotype and ripening stage in red pepper fruit [7,8]. Moreover, harvesting time is another important factor affecting the shelf-life and antioxidant capacity of peppers. Numerous biochemical and physiological changes occur at different stages of pepper development due to changes in the synthesis, transportation, and degradation of various metabolites [9], like changes in the concentration of organic acids and sugars, the synthesis and degradation of pigments and the accumulation of volatile

compounds. The phytochemical changes that occur during ripening and the resulting effect on antioxidant activity are important dietary considerations that may affect the consumption of different pepper types.

The characterisation of phytochemical changes in peppers that occur during ripening is essential since these changes could affect antioxidant activities, aroma, taste, postharvest storage, and, ultimately, consumer preference [10]. Chávez-Mendoza et al. [11] have characterised the bioactive compounds and antioxidant activity of different cultivars of grafted bell pepper harvested on three different sampling dates in the same crop cycle. This study revealed differences in the content of bioactive compounds and antioxidant activity among the grafted cultivars of bell pepper and concluded that September was the best sampling date to have the highest content in bioactive compounds and strongest antioxidant capacity in these bell peppers. As far as we know, only two studies have investigated the effect of ripening stage and harvest time on the bioactive compounds content of five different coloured *Capsicum* genotypes [12] using four sweet commercial peppers [13]. However, all of these studies have focused on the changes that occur in the bioactive compounds content during the ripening process from green to red pepper fruit and highlight the benefits of consuming these peppers in a ripe and coloured stage. In addition, the results published about the influence of harvesting time on bioactive compounds changes are limited to three sampling dates, in April, May, and June, along the developmental, growth, and ripening cycle of pepper fruit [13]. In green pepper fruit like, 'Lamuyo', whose consumption is increasingly in demand and has an earlier and shorter-term crop cycle than other peppers (from February to April) with a staggered flowering, the effect of the harvest date on antioxidant levels remains unclear. Furthermore, the influence of the phenological stage in which the green pepper is consumed as a potential influencing factor in its bioactive compounds content and antioxidant capacity remains also unknown. For this reason, two different approaches have been carried out in the present work. In one, we have studied twelve different phenological stages of green pepper fruit, and in the other, we have analysed green pepper fruit with the same phenological stage harvested at seven different sampling dates throughout the winter and spring periods of the same growth cycle. The aim of this work was therefore to elucidate the influence of the phenological stage and harvest date on the bioactive compounds content of green pepper fruit in order to fill this knowledge gap. In addition, we have also looked at the postharvest behaviour of bioactive compounds in order to study how the functional and nutritive parameters behave during 21 days of storage at 8 °C.

2. Results and Discussion

2.1. Effect of the Phenological Stage and Harvest Date on Ascorbic Acid (AA) and Dehydroascorbic Acid (DHA) Content

Fresh peppers present high levels of vitamin C reaching more than three times the recommended dietary allowance (RDA) [14]. Vitamin C is one of the most important water-soluble vitamins for human health, known for its high antioxidant activity. It has two active forms: ascorbic acid (AA), which is the main one, and the oxidized form dehydroascorbic acid (DHA), which also has biological activity and is easily transformed to ascorbic acid in the human body, making it interesting to measure [13]. The vitamin C content of foods is usually considered to be the sum of the AA and DHA [15]. Figure 1A,B show the AA and DHA content, respectively, found in the green pepper fruits harvested during the twelve phenological stages of this study. Both parameters showed an increasing trend defined by a quadratic model regression (AA: $y = 4.86 \times 10^{-3}x^2 - 0.012x + 0.32$, $r^2 = 0.888$; DHA: $y = 2.82 \times 10^{-3}x^2 - 0.016x + 0.139$, $r^2 = 0.961$). The statistical analysis revealed significant differences ($p < 0.05$) for both ascorbic acid forms among the studied phenological stages. Specifically, AA increased significantly (1.75-fold) from S1 to S9, and then a sudden 1.88-fold increase was observed between S9 and S12 (Figure 1A). On the other hand, DHA levels did not show significant differences ($p \geq 0.05$) until the green pepper fruit reached the S8 phenological stage. Between this stage and the last phenological stage, a significant 1.61-fold increase was observed (Figure 1B). The total vitamin C content, as the sum of both

forms, was therefore significantly influenced by the phenological stage of the green pepper fruit, and a 3-fold increase in total content was observed between the first and last stage.

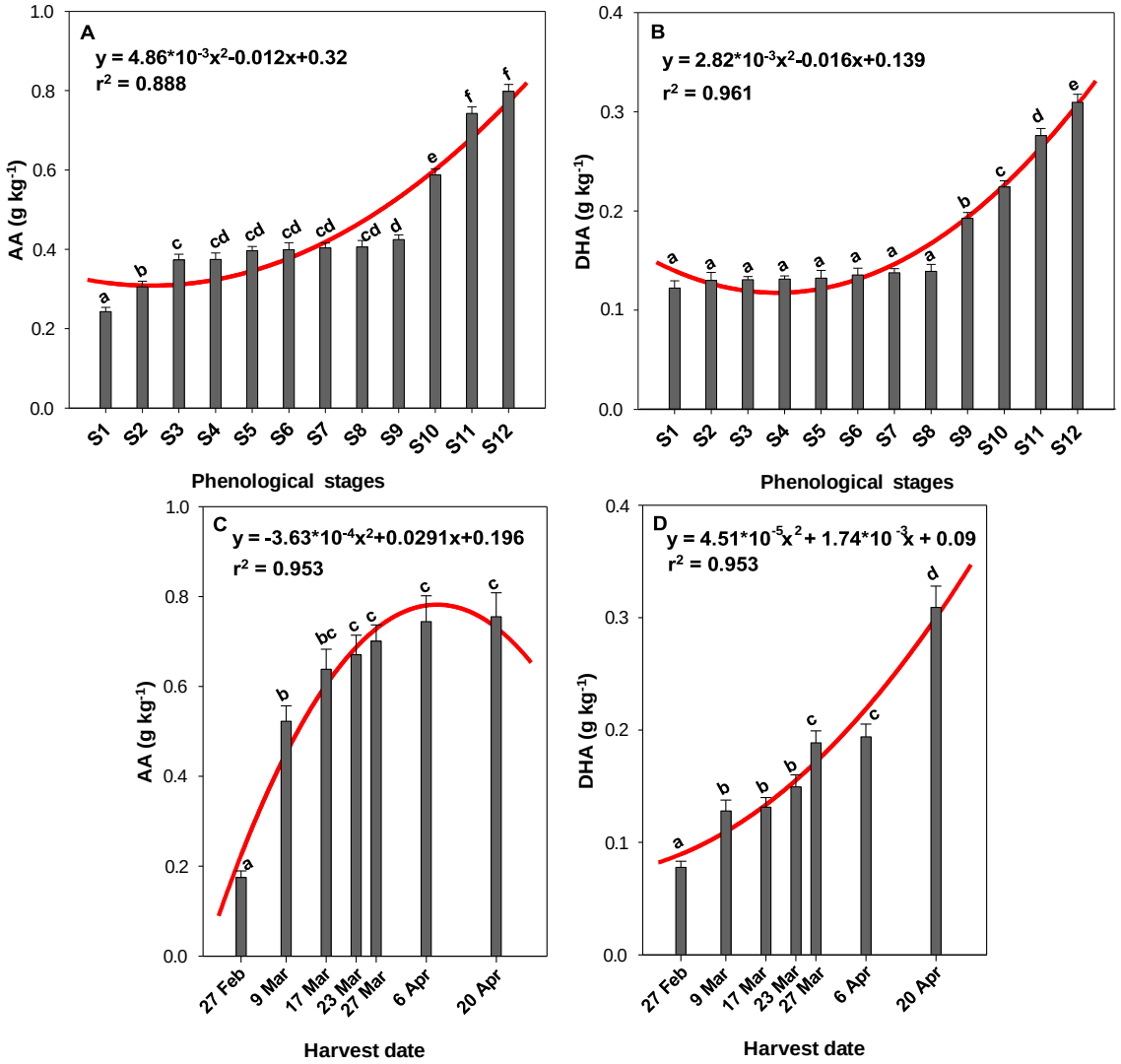

Figure 1. Influence of phenological stages (**A**,**B**) and harvest date (**C**,**D**) on ascorbic acid (AA) and dehydroascorbic acid (DHA) content (g kg^{-1}) in green pepper fruit. Lowercase letters show significant differences ($p < 0.05$) among phenological stages or harvest dates. A quadratic model regression and its coefficients are shown in each graph.

With respect to the influence of harvest date on AA and DHA content, the results are shown in Figure 1C,D, respectively. An increasing trend was observed again for both functional parameters with two new quadratic model regressions (AA: $y = -3.63 \times 10^{-4}x^2 + 0.029x - 0.196$, $r^2 = 0.953$; DHA: $y = 4.51 \times 10^{-5}x^2 + 1.74 \times 10^{-3}x + 0.09$, $r^2 = 0.953$). The green peppers harvested on the first harvest date, 27 February, showed significantly lower average levels of AA (0.18 ± 0.02 g kg^{-1}) and DHA (0.08 ± 0.01 g kg^{-1}) than those harvested on the later harvest dates. By the second harvest date, 9 March, the average AA and DHA content had significantly increased, by 2.99- and 1.65-fold, respectively (Figure 1C,D, respectively). From this point, the AA content increased 1.28-fold in the green peppers until 23 March, when the levels remained constant without significant differences until the last harvest date (Figure 1C). The DHA content, on the other hand, did not significantly increase between 9 March and 23 March. This oxidant form of ascorbic acid started to increase again by 27 March, when it showed a 1.26-fold increase with respect to the 23 March harvest date. In addition, DHA significantly increased by the last harvest date of the growth cycle, when it reached average levels of 0.31 ± 0.02 g kg^{-1} (Figure 1D).

Some authors have found differences of 30% in ascorbic acid levels between red and green peppers [7]. Specifically, Zhuang et al. [16] have reported that the vitamin C content in nine fresh peppers ranged from 0.93 g kg^{-1} FW for Creasing Pepper (green pepper) to 3.93 g kg^{-1} FW for Long-Point Pepper (red pepper), depicting a 4-fold variation between cultivars. Our total vitamin C content results (~0.25–1.11 g kg^{-1} FW) are within the ranges found in other studies for green peppers (0.12–1.80 g kg^{-1} FW) [17]. Peppers are a good source of vitamin C. Per 100 g, fresh peppers exceed the recommended daily allowance (RDA) of 60 mg [18], especially peppers harvested in S9–12 (Figure 1A,B) or those harvested from 9 March to 20 April (Figure 1C,D). These results highlight the importance of the phenological stage and harvest date on vitamin C content in green pepper fruit. Navarro et al. [19] showed that the ascorbic acid content in peppers also depends on the ripening stage. Accordingly, Ghasemnezhad et al. [12] have reported that fully developed bell pepper fruit just before the onset of colour change had more ascorbic acid than whole-coloured fruit. In a previous work, vitamin C tended to increase at the onset of ripening, but then decreased gradually with the advanced ripening most probably due to its antioxidant role, which increases with the increasing respiration rate in the climacteric fruit [20]. In green pepper fruit, which is harvested before full ripening and therefore does not undergo colour changes, the ascorbic acid content increases in the most advanced phenological stages, when the pepper fruit is fully developed (Figure 1A,B), confirming the last hypothesis. In addition, this content increased from the end of February until the end of April (Figure 1C,D). Martí et al. [13] have observed that ripe 'Almuden' and 'Cabezo' sweet pepper cultivars show an increase in vitamin C content in May. Accordingly, we found that 'Lamuyo' green pepper fruit, which is characterized by a shorter-term crop cycle than other cultivars, shows an increase in total vitamin C content along its developmental and growth cycle. A wide range of ascorbic acid levels has been reported in a number of pepper cultivars, indicating that the differences are related to cultivar, genetics, ripening stages, and agro-climatic conditions [14,21]. Mozafar [22] has suggested that the higher level of ascorbic acid in the ripening stage is due to the light intensity and glucose levels, which are the precursors of ascorbic acid. The higher ascorbic acid levels in 'Lamuyo' pepper fruit (Figure 1A–D) observed on 20 April could therefore be related to the significantly higher endogenous glucose levels found at this harvest date (Figure 5A).

Under oxidative conditions, AA is easily converted through a free radical intermediate to dehydroascorbic acid (DHA) in a reversible process which, in part, may explain the antioxidant effect attributed to AA. DHA is quite unstable and, under continued oxidative conditions, is further degraded to 2,3-diketogulonic acid, which is not biologically active as vitamin C. We measured both ascorbic acid forms, and the results show that the latest phenological stages and harvest dates presented significantly higher AA and DHA levels than the other stages and dates (Figure 1A–D). Martí et al. [13] have also measured the oxidised form of ascorbate (DHA) in sweet peppers and found that different factors are operating in the different cultivars during ripening, affecting the ascorbate oxidation. A decrease in ascorbic acid usually coincides with ripening and with an increase in ascorbate peroxidase activity [23]. This enzyme catalyses the oxidation of ascorbate [24], influencing the redox state of ascorbate. Despite this effect, the ascorbic acid content was not found to decrease during ripening in 'Lamuyo' green pepper fruit, while a significant increase in DHA content was observed by 20 April (Figure 1D), when higher temperatures and solar radiation influenced oxidation according to other studies [13]. Finally, variability in vitamin C could be influenced by different factors at harvest. Some authors have reported that the ripening stage, cultivar or growing conditions of a particular sample of a given plant could result in different levels of antioxidant compounds, which could affect the chemical stability of AA and DHA, and/or the enzyme activity [25]. Our results could contribute two additional key factors that can also impact antioxidant compounds levels: the phenological stage and the harvest date along the growth cycle of the green pepper fruit.

2.2. Effect of the Phenological Stage and Harvest Date on Total Phenol Content and the Total Hydrophilic (TAA-H) and Lipophilic (TAA-L) Antioxidant Activity

Compounds that contribute to the total antioxidant activity (TAA) in pepper fruits are numerous and include ascorbic acid and phenolic compounds, mainly flavonoids which are a group of phenolics. These two compounds are thus important in assessing their quality. Specifically, the main individual phenolic compounds in green pepper fruit are caffeic acid derivative and trans-*p*-coumaroyl-β-D-glucopyranoside such as hydroxycinnamic acids, and quercetin-3-*O*-rhamnoside and luteolin 7-*O*-(2-apiosyl-6-malonyl)-glucoside which are flavonoids [21]. All these bioactive compounds are determinant on the organoleptic characteristics (colour, appearance, flavour, and taste) and functional quality of fruits and vegetables, as well as a parameter involved in enzymatic browning and other reactions which could occur during fruit processing. The results obtained for total phenolic compounds in 'Lamuyo' pepper fruits at the different phenological stages showed a quadratic regression (Figure 2A; $y = 4.60 \times 10^{-3}x^2 - 0.05x + 0.77$, $r^2 = 0.83$). Peppers from earlier (S1 and S2) and later (S10–12) phenological stages showed significantly higher total phenol levels (between 1.15 and 1.11-fold higher, respectively) than the intermediate stages (S3 to S9). Harvest date also affected the total phenol content, which reached values of 0.83 ± 0.02 and 0.99 ± 0.03 g kg^{-1} by the two last harvest dates (6 and 20 April), respectively, but no significant differences were observed before these sampling dates. This increasing trend seen in the effect of harvest time on total phenol content is represented by a quadratic regression equation ($y = 1.51 \times 10^{-4}x^2 - 1.04 \times 10^{-3}x + 0.636$, $r^2 = 0.988$) in Figure 2B.

On the other hand, a significant increasing trend of TAA-Hydrophilic was defined for the phenological stage in a linear model (Figure 2C; $y = 0.097x + 0.425$, $r^2 = 0.976$) and for harvest date in exponential form (Figure 2D; $y = -4.28 \times 10^{-4}x^2 + 0.04x + 0.68$, $r^2 = 0.996$), respectively. Specifically, the green peppers harvested at S12 and on the last two harvest dates (6 and 20 April) showed the highest TAA-Hydrophilic values (~1.59 g kg^{-1} ; Figure 2C,D). Nevertheless, TAA-Lipophilic values showed a drastic decrease as the phenological stages advanced (Figure 2E), resulting in 2.40-fold variations. This descent was drawn with a quadratic regression; $y = -0.05x + 1.029$ with values of $r^2 = 0.947$. Contradictorily, the harvest date factor showed a 1.24-fold increase in TAA-Lipophilic values along the developmental and growth cycle in 'Lamuyo' peppers (Figure 2F; $y = -3.42 \times 10^{-5}x^2 + 3.59 \times 10^{-3}x + 0.52$, $r^2 = 0.774$).

Phenolic compounds are secondary plant metabolites that play an essential role in antioxidant activity. The total phenol content of 'Lamuyo' pepper (~0.60–1.00 g kg^{-1}; Figure 2A,B) was lower than that reported in other green-stage pepper cultivars (~2.10 to 5.58 g kg^{-1}) [10]. However, our total phenol content was accordance with the results observed in the Sweet/Robusto cultivar (green bell pepper fruit), averaging 0.70 g kg^{-1}, by Chávez-Mendoza et al. [11]. This is the first report in which the effect of the phenological stage on total phenol content has been analysed, and the results show that this content decreases as the vegetable develops and subsequently increases as ripening begins but prior to full ripening (Figure 2A). The highest total phenol content values were thus observed at the S1 and S12 phenological stages. A similar effect or downward trend was observed in lemon fruit during growth and ripening on trees from 11 September through 13 December [26].

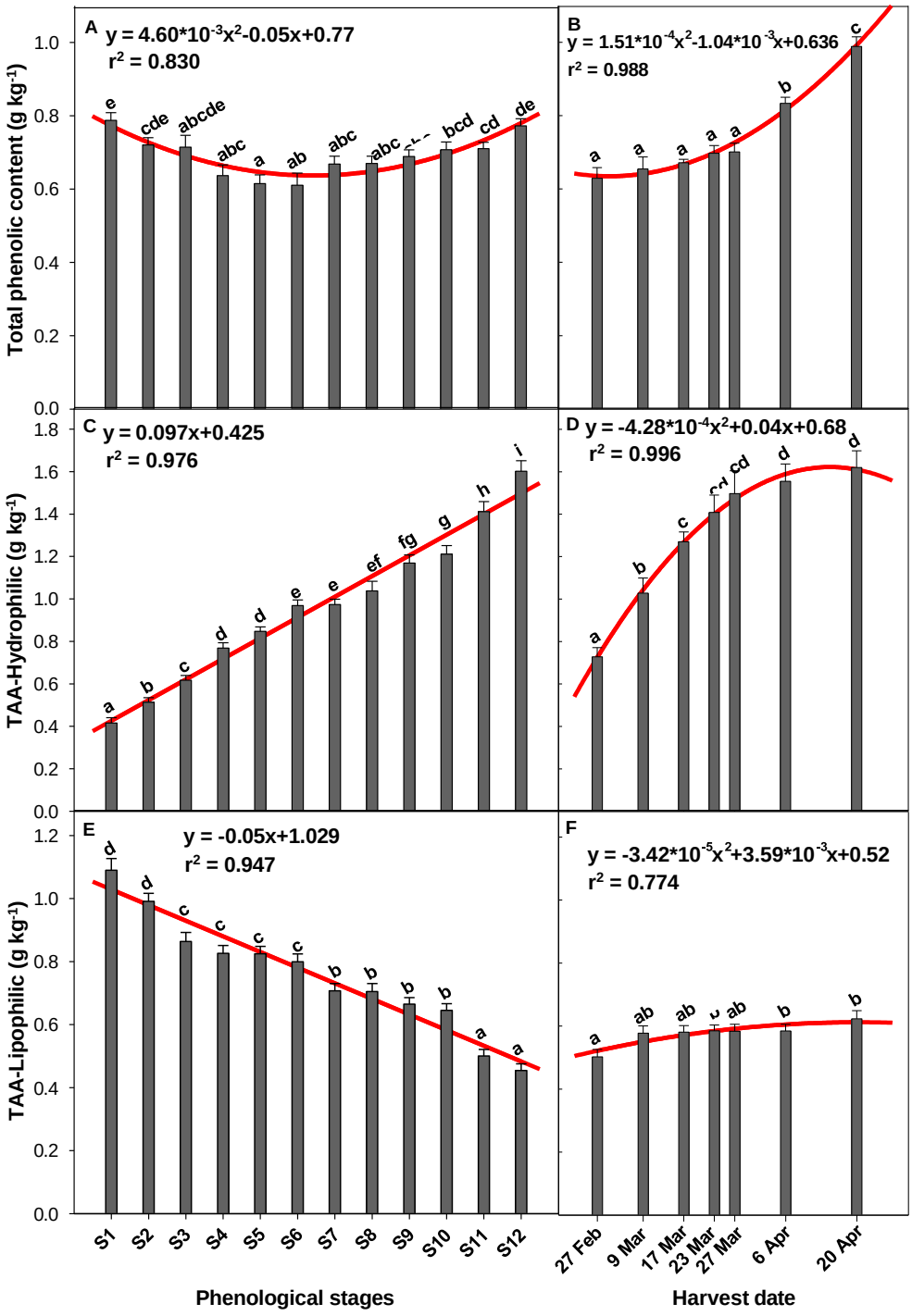

Figure 2. Influence of phenological stages (**A**,**C**,**D**) and harvest date (**B**,**D**,**F**) on total phenolic content (**A**,**B**), and the TAA-Hydrophilic (**C**,**D**) and Lipophilic (**E**,**F**) (g kg^{-1}) levels of green pepper fruit. Lowercase letters show significant differences ($p < 0.05$) among phenological stages or harvest dates. A quadratic model regression and its coefficients are shown in each graph.

The increase in total phenolic content during the last phenological stages could be related with earlier reports suggesting that fruit and vegetable ripening is associated with a significant accumulation of total phenolic content [27]. Phenolic compounds are produced by the phenylpropanoid pathway during ripening and contribute to fruit pigmentation and the disease resistance response found in many fleshy fruits [28]. In general, the mature red stage displays a higher content of total phenolics than the green stage in pepper

fruits [16]. The type of green pepper fruit in this study, however, is harvested and consumed before reaching the colour changes associated with the ripening process. According to Ghasemnezhad et al. [12], a high concentration of quercetin in green pepper fruit may be connected with the function of protecting the photosynthetic apparatus. It was shown that flavonoids, which strongly absorb radiation within the range 280–315 nm, that is UVB, could act as filters of UV radiation, in this way protecting photosynthesizing cells-that are situated deeper-against damage.

The significant increase noticed on the last two harvest dates (6 and 20 April; Figure 2B) was also observed in previous studies, which found that harvest date is an important factor that influences the total phenol content in pepper fruit [11–13]. However, the total phenol increase in the present work along the different harvest dates could be related to the increase in temperatures during the month of April. Accordingly, it has been reported that the season in which pepper fruits develop influences changes in phenolic compounds content. Nevertheless, the developmental conditions of the fruits, as well as the cultivar analysed, are decisive factors influencing antioxidants like phenolic compounds in fruits and vegetables [14].

Ultimately, the antioxidant capacity is given by compounds present on hydrophilic and lipophilic fractions (Figure 2C–F). Lipophilic compounds are mainly chlorophylls, carotenoids, and vitamin E, while the hydrophilic ones are vitamin C, glutathione (GSH), and phenols, mainly flavonoids. Both antioxidant activity fractions are related to potential health functionality against various chronic non-communicable diseases [1]. Some authors have reported that the TAA of pepper cultivars increases significantly with ripening [12,14,19]. Our TAA-Hydrophilic and TAA-Lipophilic results along the phenological stages show for the first time that the contribution of both fractions is inversely proportional as the pepper develops. Green pepper fruits harvested at S1 showed higher TAA-Lipophilic values than those harvested at S12, but the opposite occurred with the antioxidant activity provided by hydrophilic compounds (Figure 2C,E). The difference in the antioxidant activities reflects the nature and level of the antioxidant compounds found in green pepper fruit. The increase in TAA-Hydrophilic levels along the phenological stage could be related to increases in both ascorbic acid content, in AA and DHA forms (Figure 1A,B), and phenolic compounds (Figure 2A). However, the increasing TAA-Hydrophilic levels seem to be more related to the observed increase in vitamin C than the total phenolic content in the last phenological stages (Figure 2C). On the other hand, the drastic decrease in TAA-Lipophilic levels seem to be mediated by the loss of some lipophilic compounds along the phenological stages studied (Figure 2E). Chlorophylls are the main compounds that change during pepper development on the plant, and lipophilic-nature pigments are responsible for the characteristic green colour of each cultivar. As can be seen in Figure S1, the green colour of 'Lamuyo' pepper fruit ranged from light green in S1 to a deep green in S12. Currently, a new and promising set of assays showing the health-promoting activities of chlorophylls has promoted the development of studies dealing with their in vivo antioxidant actions [29]. Enhanced TAA at the fully developed stage of green pepper fruit, mainly provided by the TAA-Hydrophilic fraction, reflects the nutritional and functional importance of consuming the pepper fruits at this stage.

Finally, both TAA-Hydrophilic and TAA-Lipophilic levels increased as the harvest date progressed (Figure 2D,F). These results agree with those reported by Chávez-Mendoza et al. [11] and Martí et al. [13]. Again, results point to the importance of environmental factors like temperature, radiation, and humidity in fruits development. Higher temperatures and solar radiation seem to be good for fruits in order to increase the levels of both TAA fractions.

2.3. Evolution of Functional Parameters during Postharvest Storage

The bioactive compounds behaviour of pepper fruit at 8 °C (non-chilling temperature) during postharvest storage is shown in Figure 3. The AA and DHA content significantly decreased (by 31 and 55%, respectively) by 21 days of storage. Nevertheless, the total phenolic content and TAA-Hydrophilic and TAA-Lipophilic levels were significantly higher

(1.12, 1.21, and 2.03-fold, respectively) at the end of postharvest storage. It has been described that vitamin C levels in fruits depend on storage conditions among other factors, including the cultivar, production practices or ripening stage [14,21,25,30]. Vitamin C loss in the green peppers studied is in accordance with that observed by Barzegar et al. [31] and could be due to chemical (non-enzymatic oxidation), and/or enzymatic processes. It is worth mentioning the effect of the enzymes on L-AA stability, especially in fresh produce, in which enzymatic activity may be an important contributor to L-AA degradation. During weight loss, ascorbic acid can be rapidly lost as a result of oxidation [25]. In addition, substantial vitamin C degradation could occur due to the storage time, temperature or exposure to light [25,32,33].

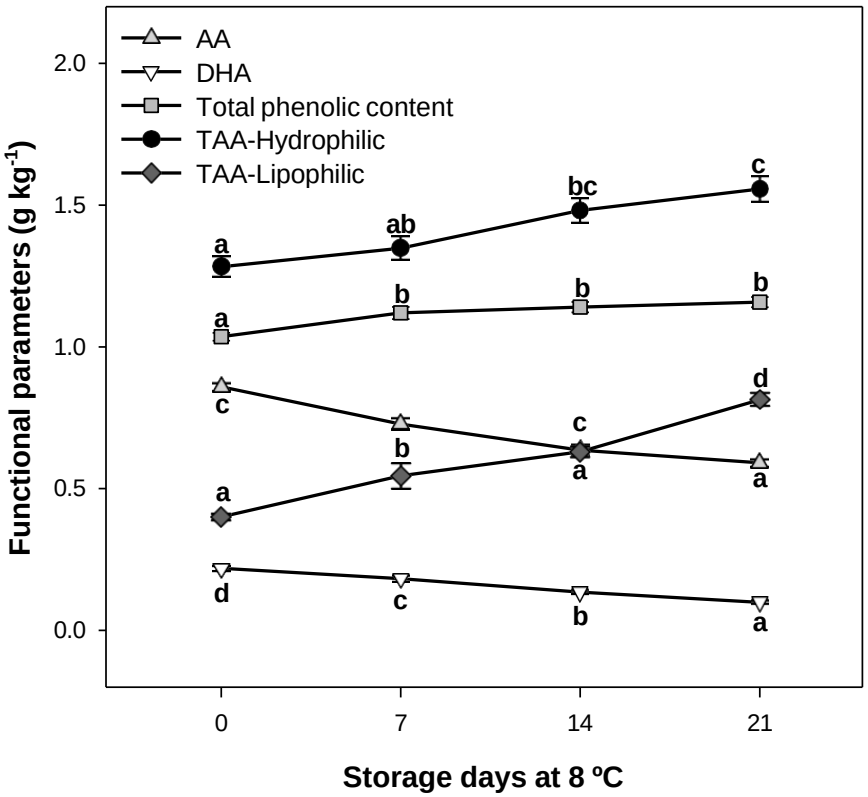

Figure 3. Evolution of functional parameters (g kg^{-1}); ascorbic acid (AA), dehydroascorbic acid (DHA), total phenolic content, and TAA-Hydrophilic and TAA-Lipophilic levels during 21 days of postharvest storage at 8 ˚C. Lowercase letters show significant differences ($p < 0.05$) among storage days at 8 ˚C.

Our results show that total phenolic content and TAA-Hydrophilic and TAA-Lipophilic levels increased in 'Lamuyo' green pepper fruit up to 20 days of storage at 8 ˚C (Figure 3). Similar results were reported by Barzegar et al. [31] and Barbagallo et al. [34], who found that that the total phenolic content and DPPH scavenging activity in control green peppers increased up to day 20 and the 3rd week of storage, respectively, and then decreased. Phenolic compounds accumulated in pepper fruit are affected by storage condition. Raffo et al. [35] demonstrated that sweet peppers stored at 8 ˚C accumulated hydroxycinnamic acid derivatives, whereas at 4 ˚C phenolics accumulation appeared to be partially inhibited. Our results about the increase of total phenolic content and total antioxidant activity during 21 days of storage at 8 ˚C could be related with an increase of hydroxycinnamic acid derivatives.

2.4. The Effect of the Phenological Stage, Harvest Date, and Postharvest Storage on Total Soluble Solids (TSS) and Total Acidity (TA)

The phenological stage influenced the TSS and TA content (Figure 4A,B). Both parameters significantly increased at S12, reaching values of 48.30 ± 0.41 and 1.99 ± 0.04 g kg^{-1}, respectively. The trend was described by a quadratic model correlation for TSS ($y = 0.122x^2 + 0.5x + 29.55$, $r^2 = 0.985$) and TA ($y = 0.012x^2 - 0.027x + 0.784$, $r^2 = 0.982$). Furthermore, harvest date was also highly correlated with the TA content ($y = 0.023x + 0.904$, $r^2 = 0.954$; Figure 4D), but no significant differences were observed in TSS among the studied harvest dates (Figure 4C). Therefore, green pepper fruits harvested on 20 April showed 2.46-fold higher total acidity levels than peppers harvested on 27 February (Figure 4D). TSS and TA were also affected by postharvest storage, showing an opposite trend (Figure 4E,F). The TSS content in green peppers had significantly increased, by 15%, by 21 days of storage at 8 °C (Figure 4E), while the TA had significantly decreased, by 51%, by the end of the storage period (Figure 4F).

It has been reported that TSS content increases with fruit ripening as a result of greater degradation of the polysaccharides and accumulation of sugars [36], confirming that our TSS values at S12 were the highest (Figure 4A). However, no significant differences were observed for TSS along the harvest dates (Figure 4C). This is because the 'Lamuyo' pepper fruits were harvested on different dates according to commercial criteria for TSS (~45.00 g kg^{-1}).

The main organic acids contributing to pepper acidity were citric and malic acid (Figure 5B), and TA increased with fruit ripening (Figure 4B), as previously reported by Serrano et al. [37]. The increases in TA along the different harvest dates (Figure 4D) could be related to an increase in the average temperature in April, as has been reported by Hernández-López et al. [38]. This is because metabolic processes occur more slowly in low temperature conditions, while an increase in temperature causes an opposite effect. Finally, the increase in TSS content could potentially be due to pepper fruit weight loss and the conversion of organic acids to sugars [39]. Organic acid losses lead to significant decreases in TA values from harvest up until 21 days of storage (Figure 4F), according to Barzegar et al. [31].

2.5. The Effect of Harvest Date and Postharvest Storage on Individual Sugars and Organic Acid Content

Changes in TSS an TA are based on changes in individual sugars and organic acid content (Figure 5A,B) along the harvest dates (27 February to 20 April) and during postharvest storage (from 20 April to 11 May). No significant differences were observed for the individual sugars, glucose and fructose, between the first harvest date, 27 February, and the last one, 20 April (Figure 5A). The 'Lamuyo' pepper showed 55% glucose and 45% fructose at the first harvest date, and no changes in the sugar profile were found by the last harvest date. Nevertheless, a significant increase in citric, malic, and ascorbic acid (51%, 53%, and 69%, respectively) was observed by 20 April (Figure 5B).

The organic acid profile of 'Lamuyo' green peppers was as follows: fumaric acid (0.67%), oxalic acid (1.47%), succinic acid (8.09%), ascorbic acid (13.98%), malic acid (37.53%), and citric acid (38.26%). This is similar to the profile reported by Serrano et al. [37]. However, these researchers reported that citric acid was the main organic acid contributing to pepper acidity in the 'Herminio' red cultivar. Our results showed that the proportion of malic and citric acid was similar in the green peppers of this cultivar (Figure 5B). Finally, the glucose and fructose levels were significantly higher (1.22 and 1.15-fold, respectively) after 21 days of storage at 8 °C (Figure 5A). By the end of the storage period, the levels of citric, malic, ascorbic, and succinic acids had significantly decreased, by 51%, 44%, 60%, and 82%, respectively; fumaric acid, on the other hand, increased by 83% (Figure 5B). The organic acid profile thus changed during 21 days of postharvest storage at 8 °C.

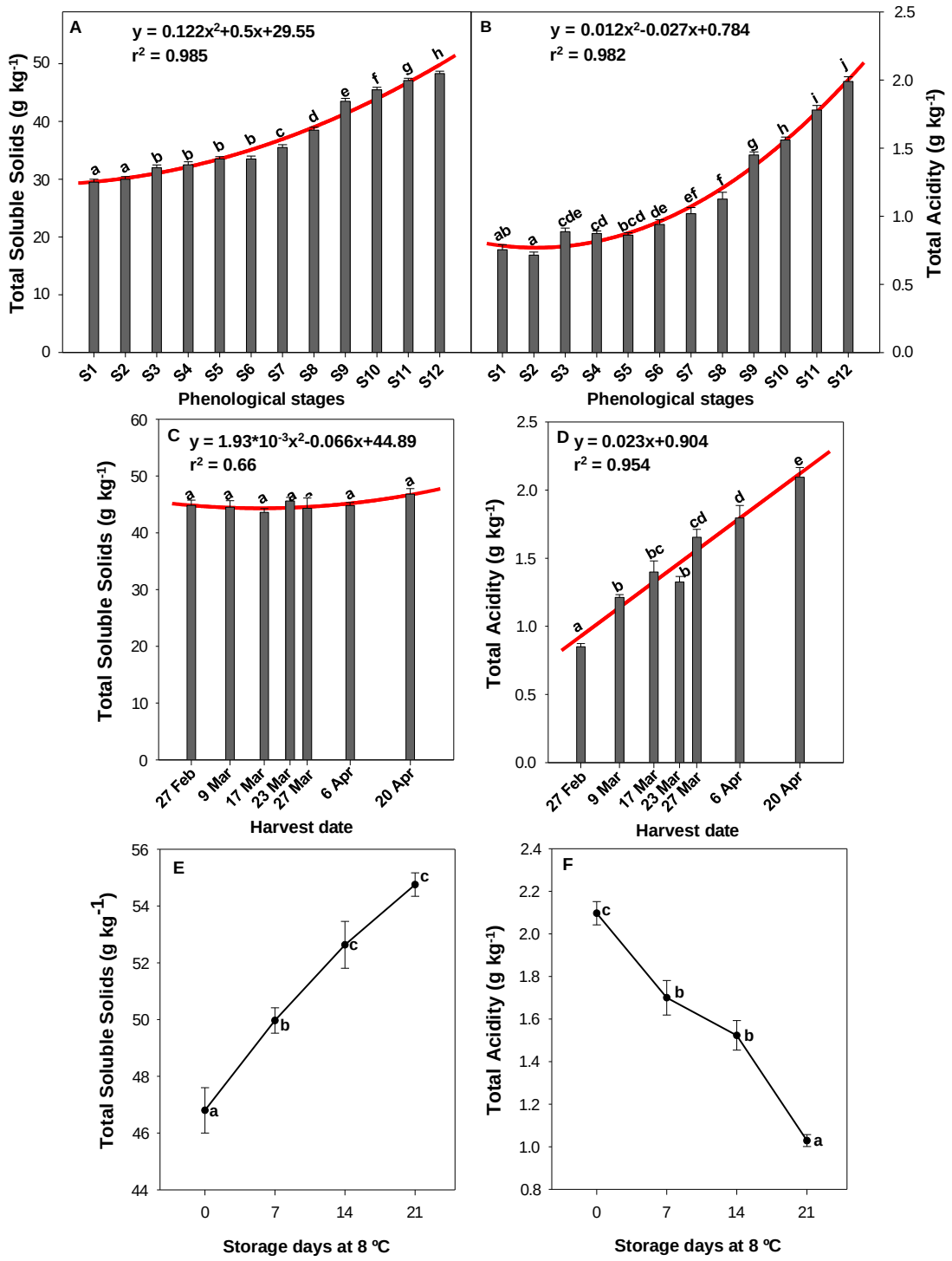

Figure 4. Influence of phenological stages (**A**,**B**), harvest date (**C**,**D**), and postharvest storage (**E**,**F**) on total soluble solids (TSS) (**A**,**C**,**E**) and total acidity (TA) (**B**,**D**,**F**) (g kg^{-1}) in green pepper fruit. Lowercase letters show significant differences ($p < 0.05$) among phenological stages, harvest dates, or storage days at 8 °C. A quadratic model regression and its coefficients are shown in some graphs.

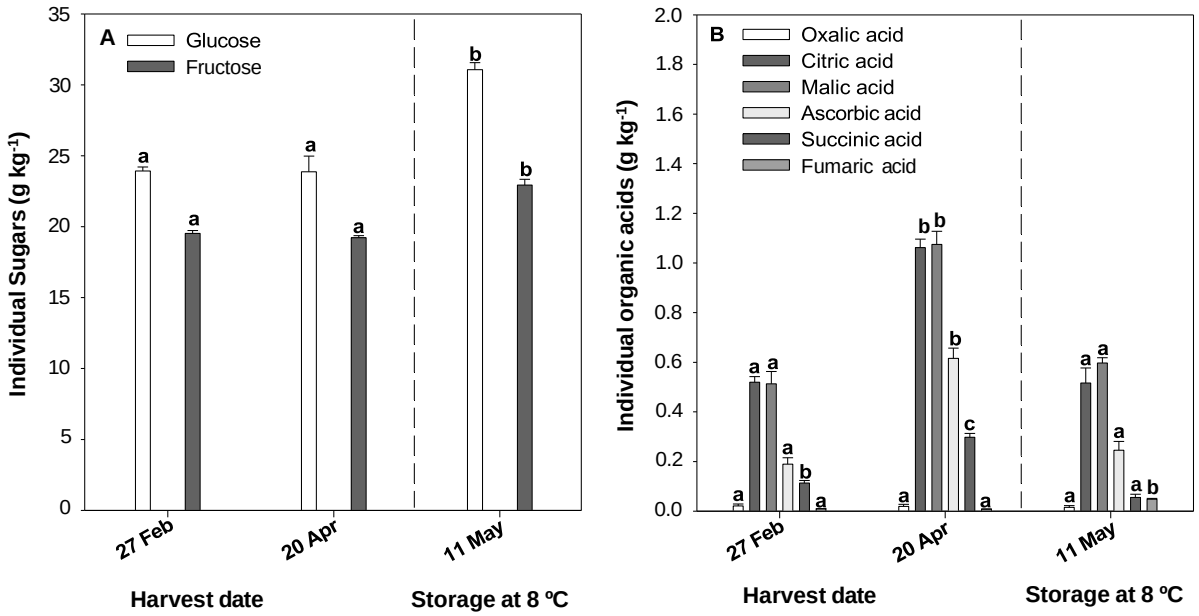

Figure 5. Influence of harvest date (27 February and 20 April) and postharvest storage (at harvest; 20 April, and at 21 storage days; 11 May) on individual sugar (**A**) and organic acid (**B**) levels (g kg^{-1}) in green pepper fruit. Lowercase letters show significant differences ($p < 0.05$) among harvest dates and storage days at 8 °C.

No differences in glucose and fructose content were observed along the harvest dates, because all pepper fruits were harvested with the same commercial criteria (Figure 5A). Nevertheless, the increase in both individual sugars during postharvest storage could be related to the ripening process that continuously occurs under storage at 8 °C (Figure 5A), according to Mashabela et al. [36]. With respect to organic acid profile (Figure 5B), Barzegar et al. [31] have also reported changes in the citric, malic, and ascorbic acid content of sweet pepper fruits. Concentrations of these acids are known to diminish during ripening. Our organic acid results are in accordance with previous reports showing that overall acidity increases after harvest and then decreases in storage [40]. Medlicott and Thompson [41] also reported reduced acidity levels with prolonged storage due to the fact that the predominant malic acid diminishes in the fruit as the ripening process advances. Carbohydrate and acid metabolism are therefore closely connected during the postharvest ripening period [42]. Finally, these compositional differences on TSS, TA, individual sugars, and organic acid content could determine sensory differences of perceived sweetness as well as could be related with the consumers' preferences. Sweet pepper taste is largely determined by the sugar-to-acid ratio and, in general, these two components can vary independently altering taste attributes of fruit and vegetables [35].

3. Materials and Methods

3.1. Green Pepper Fruit Cultivar and Growing Conditions

'Lamuyo' pepper plants (*C. annuum* L.), of the 'Herminio' cultivar, were grown under plastic-roofed greenhouses (Hortalizas Sanper S.L., El Raal, Murcia, Spain). The experiment was carried out during the winter-spring season (February–April 2020). According to the usual crop programme designed by the company for the early cycle of this type of pepper, automatic drip irrigation and optimal nutrient levels were applied and rockwood was used as the soil substrate. The soil texture was sandy loam with a pH of 7.50. Meteorological data were collected from a station close to the experimental greenhouses (38°2′2.64″ North, 1°1′18.9″ West). The mean long-term climate data during the growing season (2020) is shown in Table 1. The green pepper fruits were harvested on 10 April at different phenological stages in the developmental and growth cycle; we also harvested fruits

on seven different harvest dates, ranging from the end of February to the end of April. Pepper fruit harvested on different harvest dates were from the same phenological stage (S12). For this reason, the importance to study, on the one hand, the influence of different phenological stages on the bioactive compounds content and, on the other hand, the influence of different harvest dates on this content in peppers with the same phenological traits. We used three replicates of 30 plants (n = 90 plants) for these two approaches. The pepper fruits were harvested and classified at 12 different phenological stages, from S1 until S12, along the crop cycle. The biometrical characteristics of the stages studied are described in Figure S1. Specifically, ten green pepper fruits, one fruit per plant (n = 10 plants), were analysed for each replicate and phenological stage (n = 30 green fruit per stage). Previous to the analytical determinations, the green peppers harvested from each replicate were weighed (g) and measured in length and diameter (mm), and results were expressed as the mean ± SE (Figure S1). For the harvest date study, green-pepper fruits were harvested from 10 different plants per replicate (n = 30 pepper plants) along the short-term crop cycle of 'Lamuyo' type. According to a staggered production, 10 green peppers, one fruit per plant from each replicate (n = 30 green peppers), were analysed in each harvest date (n = 210 pepper fruit per crop cycle) and phenological stage (n = 30 green pepper fruit per stage). The harvest dates studied were 27 February, 9 March, 17 March, 23 March, 27 March, 6 April, and 20 April. The equidistance among harvest dates was stablished according to commercial criteria of harvesting in green pepper fruit established by the company. All green pepper fruits were cut to remove the peduncle and the seeds and then frozen in liquid N_2, and maintained at −80 °C until analysis.

Table 1. Meteorological data provided by a station close to the experimental greenhouses in El Raal, Murcia, Spain.

		27 Feb	9 Mar	17 Mar	23 Mar	27 Mar	6 Apr	20 Apr
Temperature (°C)	Average	12.90	16.37	15.06	12.25	11.15	12.09	14.24
	Maximum	17.20	23.63	22.42	16.86	14.85	18.09	20.29
	Minimum	8.70	9.44	8.36	7.64	8.23	6.89	8.88
Relative Humidity (%)	Average	75.47	50.65	69.01	84.53	85.91	82.01	81.37
Hours of Sunshine/Day	Average	8.44	9.50	8.67	7.14	5.80	9.09	9.47

3.2. Experimental Postharvest Storage Design

At the end of the harvest period, 20 April, 40 green pepper fruits that were homogeneous in colour and size were selected from another 10 different pepper plants for each replicate (n = 120 green peppers). The pepper fruits were immediately transferred to the laboratory and randomly divided into four batches of 30 green peppers each. One batch was analysed after harvest and the other ones stored at 8 ± 1 °C and 85% Relative Humidity (RH) for 21 days (until 11 May). At 7-day intervals, one group was taken at random and subjected to the following analyses.

3.3. Ascorbic Acid (AA) and Dehydroascorbic Acid (DHA)

Ascorbic (AA) and dehydroascorbic (DHA) acids were measured according to the method of Peña-Estévez et al. [43]. Briefly, 5 g of frozen green pepper fruit was homogenised with 5 mL of a methanol: water (5:95) solution containing 0.1 mM citric acid, 0.05 mM ethylenediamine tetraacetic acid disodium salt, and 4 mM NaF for 30 s on an Ultraturrax (T18 basic, IKA, Berlin, Germany). Then, the extract was filtered through a four-layer cheesecloth and the pH was adjusted to 2.35–2.40 with 2 N HCl; it was then centrifuged at 10,000 *g* for 15 min at 4 °C. The supernatant was purified through a methanol-activated C_{18} cartridge (Sep-Pak cartridges C18, Waters, Dublin, Ireland) and filtered through a 0.45 µm PFTE filter. For DHA derivatization, 750 µL of extract were mixed with 250 µL of 7.7 M 1,2-phenylenediamine in an HPLC amber vial. The mixture was allowed to react for 37 min and then 20 µL were injected onto a Luna (250 mm × 4.6 mm, 5 µm

particle size) C18 column (Phenomenex, Macclesfield, UK) with a C18 security guard (4.0 mm × 3.0 mm) cartridge system (Phenomenex) using an HPLC system (1200 Infinity series, Agilent Technologies, Waldbronn, Germany). The mobile phase was 50 mM KH_2PO_4 containing 5 mM hexadecyl trimethylammonium bromide and 5% methanol (pH 4.59) with an isocratic flow of 1 mL min^{-1}. Absorbance was recorded at 261 nm for AA (Rt = 9.4 min) and at 348 nm for DHA (Rt = 4.5 min), and both values were quantified by comparison with AA and DHA standard areas (Sigma-Aldrich, Darmstadt, Germany). Vitamin C was defined as the sum of both AA and DHA content. The results (mean ± SE) were expressed as g kg^{-1} fresh weight (FW).

3.4. Total Phenolic Content and Total Hydrophilic (H-TAA) and Lipophilic (L-TAA) Antioxidant Activity

To measure the total phenolic content and total antioxidant activity (TAA), 5 g of frozen green pepper fruit were homogenised in 10 mL of 50 mM phosphate buffer pH = 7.8 and 5 mL of ethyl acetate. The homogenate was centrifuged at 10,000 *g* for 15 min at 4 °C and the upper and lower fractions were used to quantify total lipophilic (L-TAA) and hydrophilic (H-TAA) antioxidant activity, respectively. In addition, the total phenol content was quantified in duplicate on the lower fraction for each extract using the Folin-Ciocalteu reagent as previously described [44]. The results were expressed as g gallic acid equivalent (GAE) kg^{-1} and are the mean ± SE of three replicates. H-TAA and L-TAA were determined in duplicate in each extract as previously described, also by Sayyari et al. [44]. A reaction mixture containing 2-2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horseradish peroxidase enzyme, and its oxidant substrate (hydrogen peroxide) was performed to monitor at 730 nm the $ABTS^+$ radicals generated. The decrease in absorbance after adding the green pepper extract was proportional to the TAA of the sample calculated using a calibration curve made with Trolox [(*R*)-(+)-6-hydroxy- 2, 5, 7, 8-tetramethyl-croman-2-carboxylic acid] (0–20 nmol) from Sigma Aldrich (Madrid, Spain). The results are expressed as g of Trolox Equivalent (TE) kg^{-1} FW and are the mean ± SE of three replicates.

3.5. Total Soluble Solids (TSS) and Total Acidity (TA)

Ten frozen pepper samples of each replicate were combined to obtain a homogeneous sample of juice for each replicate. Total soluble solids (TSS) were measured in duplicate in the same juice using a digital refractometer (Atago PR-101, Atago Co., Ltd., Tokyo, Japan) at 20 °C and expressed as g kg^{-1} FW. Titratable acidity (TA) was determined in duplicate in each sample using 1 mL of diluted juice (in 25 mL distilled H_2O) obtained from 50 g of pepper fruit, which was automatically titrated (785 DMP Titrino, Metrohm, Burladingen, Germany) with 0.1 N NaOH up to pH 8.10; the results were expressed as g malic acid equivalent kg^{-1} FW.

3.6. Individual Sugars and Organic Acids

For sugar and organic acid quantification, extraction was performed according to the protocol described by García-Pastor et al. [45]. The supernatant was filtered through a 0.45 µm Millipore filter and injected into a high-performance liquid chromatography (HPLC) system (Hewlett-Packard HPLC series 1100, Waldbronn, Germany). The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min^{-1} through a Supelco column (Supelcogel C-610H, 30 cm 7.8 mm, Supelco, Bellefonte, PA, USA). Sugars were detected by a refractive index detector and organic acids by absorbance at 210 nm. The results were expressed as g kg^{-1} FW and are the mean ± SE of three replicates. A standard curve of pure sugars and organic acids purchased from Sigma (Poole, UK) was used to quantify these compounds.

3.7. Statistical Analysis

The analysis was carried out in three replicates for all analytical determinations. Results are expressed as mean ± SE. Data were subjected to analysis of variance (ANOVA).

The sources of variation were the phenological stages, harvest dates or storage time. Mean comparisons were performed using Tukey's HSD test to determine whether the differences among the phenological stages, harvest dates or storage time were significant at $p < 0.05$. All analyses were performed using the SPSS software package v.17.0 for Windows (SPSS, 2001, IBM Corporation, Armonk, NY, USA).

4. Conclusions

In conclusion, phenological stages and harvest dates are two key factors that significantly influence the bioactive compounds content and the antioxidant activity of 'Lamuyo' green pepper fruit. Firstly, green peppers harvested in S12 and on 20 April showed the highest levels of antioxidant compounds, mainly ascorbic acid, dehydroascorbic acid, and total phenolic content. Secondly, these peppers showed the highest total acidity due to the significant increase in citric, malic, ascorbic, and succinic acids at these two points. Thirdly, some of these bioactive compounds and organic acids significantly decreased during 21 days of postharvest storage at 8 °C. Therefore, it is advisable to harvest the green pepper fruits at the most advanced phenological stage (S12) and on the latest harvest dates, in April, in order to achieve maximum health benefits in terms of functional traits.

Supplementary Materials: The following are available online. Figure S1: Biometrical characteristics of phenological stages in 'Lamuyo' green pepper fruit: weight (g), length (mm), and diameter (mm). Data are the mean ± SE.

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4.2. Publication 2 — Research article

PUBLICATION 2 (Open access)

Salicylic acid foliar application increases crop yield and quality parameters of green pepper fruit during postharvest storage

Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., Zapata, P.J.

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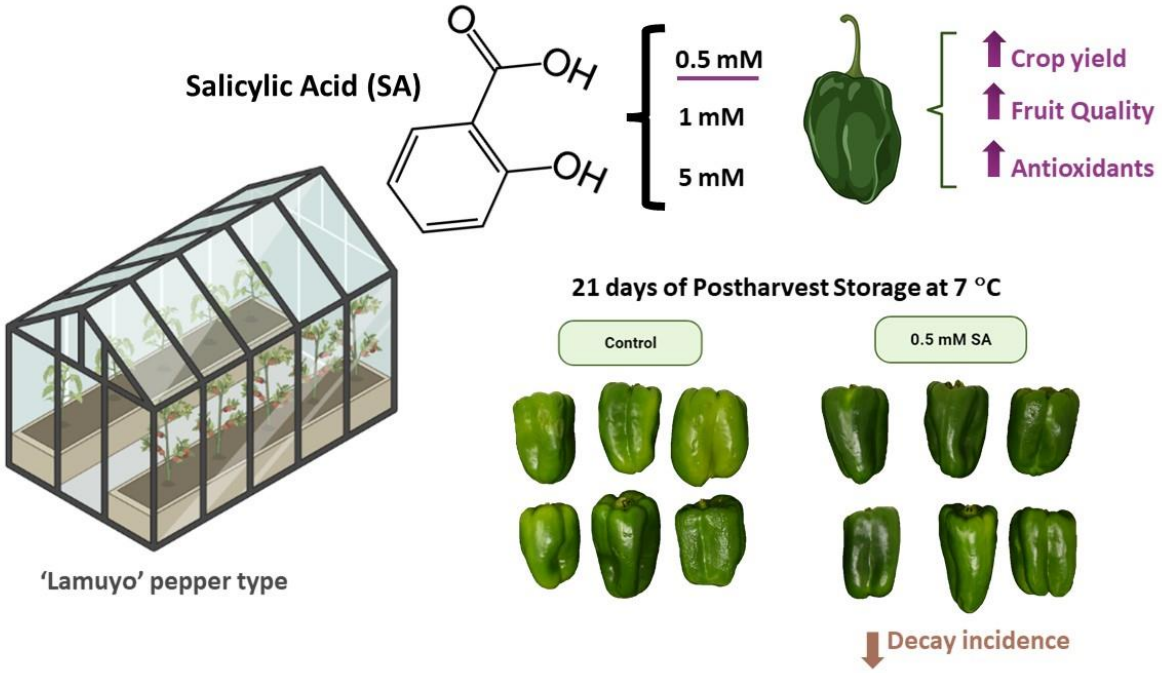
Graphical abstract:



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Salicylic Acid Foliar Application Increases Crop Yield and Quality Parameters of Green Pepper Fruit during Postharvest Storage

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Abstract: The main aim of this study was to evaluate the effect of salicylic acid (SA) as a preharvest treatment on crop yield throughout the crop cycle of green pepper fruit as well as on its quality parameters, including functional quality, at harvest and during 21 days of storage at 7 °C. Thus, 'Herminio' pepper plants were treated with SA at 0.5, 1 and 5 mM, and higher crop yield (kg per plant, number of fruits per plant and average fruit weight) and quality parameters (firmness, green color and total acidity) at harvest were obtained with the 0.5 mM dose, as well as greater phenolic compounds content and total antioxidant activity. These quality traits and functional quality were also maintained at higher levels for this treatment than in controls during postharvest storage, leading to a delay of fruit quality losses. In addition, the decay incidence for 0.5 mM SA-treated pepper fruits reached a ca. value of 2% at the end of the storage, which was lower than untreated fruits (16.6%). These results suggest that preharvest application of SA at low doses tested on pepper plants could be a useful tool to increase crop yield and fruit quality parameters at harvest and maintain them during storage, delaying quality losses and decay incidence.

Keywords: average weight; decay incidence; firmness; SA; total phenolics

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1. Introduction

Crops and their supplies require substantial increments for servicing the gap between production and demand [1]. In the last few years, the necessity of improving crop yield has been much more emergent due to the expanding population [2]. Research has been performed in recent years regarding the use of preharvest treatments using naturally occurring plant compounds to increase crop yield and fruit quality at harvest and to maintain it during storage, due to consumers' concerns and legal restrictions concerning postharvest chemical treatments [3]. The use of plant growth regulators [4] has been recently introduced to improve the quality of vegetable products. Salicylic acid (SA) is a water-soluble secondary metabolite and a phenolic compound produced by the plant organism [5]. This ubiquitous phytohormone is essential for plant growth, development and stress resistance [6]. SA acts as a signaling agent in plants which promotes tolerance against several biotic and abiotic stresses [7] and regulates many physiological and metabolic processes [8], such as proline metabolism, photosynthesis, transpiration, ion uptake and transportation [2,9]. Moreover, SA has been shown to intervene with the ethylene, abscisic acid and cytokinins roles in plants [10]. Therefore, SA has recently been proposed to be a new kind of plant growth regulator.

Pepper fruit (*Capsicum annuum* L.) is a vegetable of great economic importance worldwide, and it is highly appreciated in the market for its organoleptic qualities [11]. Further, consumers purchasing decisions commonly focused on the color, size and firmness, among other quality traits [12]. In particular, the freshness of the pepper is an important quality

attribute to consumers [10]. In addition, this vegetable is a good source of antioxidants, flavonoids, phenolic acids and carotenoids [13,14]. Green peppers are harvested before they ripen completely and, recently, the phenological stage and harvest date have been defined as two key factors that significantly influence their bioactive compounds content. The later phenological stages and harvest dates which have been studied have revealed that there could be a greater benefit to health [15].

Beyond the upsides of this crop, as a subtropical crop, pepper fruit is susceptible to chilling injury (CI) at temperatures < 7 °C, leading to superficial pitting, watery stains and seed and calyx browning. The major postharvest problem when pepper fruits are stored at non-chilling temperatures is excessive softening that may cause shrinkage, drying and pathological disorders, such as gray mold caused by *Botrytis cinerea*, which reduce the product quality and acceptability. In fact, handlers and consumers focus a lot of importance on the retention of quality attributes, such as fruit green color, freshness and firmness, during postharvest handling and storage [10]. In addition, other factors such as absence of defects, diseases and shelf-life are also considered [16]. In this sense, the storage or marketable life of harvested green pepper fruits can be extended by various treatments applied to them postharvest. As a postharvest treatment in pepper fruit, SA could delay the softening process, maintaining fruit quality and retaining the nutritional quality of sweet peppers during storage at 25 °C and at 10 °C [10]. Furthermore, a single SA treatment or a combined SA treatment with trisodium phosphate (TSP) was found to enhance cold tolerance through the effects on antioxidant metabolism or inhibiting the CI-induced membrane damage [6,17].

On the other hand, control of postharvest diseases is commonly linked to the use of synthetic fungicides, which is being increasingly legislated in order to avoid their potential risk for consumer health and to promote the use of eco-friendly strategies [18]. Preharvest applications of SA have stimulated the disease resistance against fungal infection of pepper fruits [19–22]; accordingly, it also reduced the decay incidence and postharvest losses during storage. In the scientific literature, studies focusing on the action of SA preharvest application on salinity stress oxidative damage of sweet pepper plants are also incipient [2,23,24]. Nevertheless, few studies have investigated the effect of SA preharvest treatment on increasing crop yield and quality of pepper fruit [25,26]. Both studies are focusing on the effect at one harvest date along the crop cycle [25] and on the quality of red sweet pepper cultivars [26]; however, no information is available about these effects on green pepper fruit. Pepper fruit, as non-climacteric fruit, show a low profile and a gradual decline in its respiration pattern through the ripening process [27]. This physiological behavior has a great importance in the postharvest biology and technology of green pepper fruit. As far as we know, there is no scientific literature regarding the effect of SA preharvest treatment on crop yield throughout the developmental and growth cycle of green pepper fruit as well as on its quality parameters, including functional quality, at harvest and during 21 days of storage at 7 °C, which is the main aim of this research.

2. Materials and Methods

2.1. Plant Material, Treatments and Growth Conditions

For this experiment, pepper plants (*Capsicum annuum* L.) of 'Herminio' cultivar were planted on January 2020 in a commercial plot growing under plastic-roofed greenhouse located in El Raal (Murcia, Spain). The experiment was conducted from February to July 2020 (Table 1). Thus, 180 pepper plants were selected and distributed in randomized complete block design with twelve replicates or blocks in total. Each treatment was performed in three blocks (n = 3) of 15 plants (45 plants per block and treatment). Treatments were a control (plants treated with distilled water) and salicylic acid (SA) (plants treated with the reagent purchased from Sigma, Sigma-Aldrich, Madrid, Spain; CAS Number: 69-72-7) at three concentrations: 0.5, 1 and 5 mM. These doses were chosen according to previous experiments in which 0.5, 1.0 and 2.0 mM doses of SA were applied to sweet cherry and plum trees [28,29] and higher doses (SA 5 mM) have recently been applied on

pomegranate trees [30]. Seven exogenous applications by foliar spray throughout the crop cycle were performed (Table 1), the first treatment was applied before the beginning of the flowering stage. The equidistance among application dates was ca. 21 days due to a staggered flowering cycle, except for the last application that was performed close to the last commercial harvest, being chosen based on the crop cycle duration of this pepper cultivar. Crop management was performed according to the usual crop program designed by the company for the short-term crop cycle of 'Lamuyo' pepper type, in which rockwood was used as the soil substrate and drip irrigation and optimal nutrient levels were applied. The soil texture was sandy loam with a pH of 7.50.

Table 1. Application dates of treatments (control and SA at 0.5, 1 and 5 mM) throughout the developmental and growth cycle of 'Herminio' green pepper fruit.

Treatments	T1	T2	T3	T4	T5	T6	T7
Dates	24 February	17 March	6 April	29 April	19 May	9 June	12 July

Pepper fruits were harvested at the commercial harvest stage when green pepper had reached the phenological stage suitable for its consumption. A total of 10 harvest dates throughout the developmental and growth cycle were performed according to a staggered production and the commercial criteria of harvesting green pepper fruit established by the company. The harvest dates started from April until July: 6 April, 20 April, 4 May, 14 May, 26 May, 4 June, 16 June, 26 June, 6 July and 17 July. The mean temperature for each month was recorded: April (14.58 °C), May (20.06 °C), June (23.33 °C) and July (25.98 °C), using a station close to the experimental greenhouses (38°2′2.64" North, 1°1′18.9" West).

2.2. Crop Yield

Parameters related to crop yield were evaluated for each harvest date along the crop cycle and blocks designed per treatment. Accumulative crop yield was expressed as kg plant^{-1} and number of peppers harvested plant^{-1}. The average fruit weight (g) was analyzed by weighing all harvested pepper fruits individually.

2.3. Experimental Postharvest Storage Design

Pepper fruits harvested on 4 May, this date was chosen according to one previous experiment [15], without visual defects were selected and transferred to the laboratory to carry out a postharvest storage experiment. For each treatment and sampling date, 6 peppers were selected for 3 replicates (18 pepper fruits in total), which were weighted and stored at 7 °C and 85% of relative humidity (RH). Specifically, 72 pepper fruits were stored for each treatment. Pepper fruits were analyzed at harvest (day 0) and at 7, 14 and 21 days of storage. For the experimental postharvest storage, 54 fruits in total were used for the analyses on the three sampling dates (7, 14 and 21 storage days). However, another 18 pepper fruits were stored and weighed to replace rotten pieces of pepper fruit; thus, fruit quality was always analyzed on 18 non-rotten pepper fruits on each sampling date. For each sampling date, weight loss, fruit respiration rate, firmness, color, total soluble solids, titratable acidity, total phenolics content, total antioxidant activity and the incidence of decay were measured. All the analyses were performed on 18 green pepper fruits, this number being representative of each treatment and sampling date.

2.4. Quality Parameters of Green Pepper Fruit

Weight loss was measured for each individual lot by recording the pepper fruit weight at harvest (day 0) and at 7, 14 and 21 days of storage. Accumulative weight loss was expressed as a percentage (%) with respect to pepper fruit weight at day 0. Respiration rate was determined following the protocol described by Giménez et al. [31] with slight modifications. Pepper fruits were placed individually in 2 L capacity glass jars, hermetically sealed for 60 min. Thus, CO_2 concentration was measured using a ShimadzuTM GC-14B

(Kyoto, Japan) gas chromatograph equipped with thermal conductivity (TCD). Results were expressed in mg CO_2 kg^{-1} h^{-1}.

Firmness was determined individually in each pepper fruit using a TX-XT2i Texturometer (Stable Microsystems, Godalming, UK). A flat steel plate, mounted on the machine, measured the equatorial fruit diameter and applied a force that achieved a 5% deformation of this diameter. Results were expressed as a force-deformation ratio (N mm^{-1}). Color was measured on three points of the equatorial pepper fruit perimeter by using a Minolta colorimeter (CFRC400, Minolta Camera Co., Kanto- , Tokio, Japan) using the CIELab coordinates and was expressed as a b* parameter.

Pepper samples of each replicate were combined to obtain a homogeneous sample of juice for each replicate. Total soluble solids (TSS) content was measured in duplicate from the juice obtained from 50 g of pepper fruit, using a digital refractometer (Atago PR-101, Atago Co., Ltd., Tokyo, Japan) at 20 °C and expressed as g kg^{-1} of fresh weight (FW). Titratable acidity (TA) was determined in duplicate from the same juice by automatic titration (785 DMP Titrino, Metrohm, Burladingen, Germany) with 0.1 N NaOH up to a pH of 8.10, using 1 mL of diluted juice in 25 mL distilled H_2O, and results were expressed as g malic acid equivalent kg^{-1} FW.

2.5. Total Phenolics Content and Hydrophilic and Lipophilic Total Antioxidant Activity

All green pepper fruits were cut to remove the peduncle and the seeds at each sampling date and then frozen in liquid N_2 and maintained at −80 °C until functional analysis. Total phenolic content and total antioxidant activity were measured according to the protocol described in green pepper fruit by Dobón-Suárez et al. [15]. Briefly, 5 g of green pepper fruit were homogenised in 10 mL of 50 mM phosphate buffer pH = 7.8 and 5 mL of ethyl acetate. The extracts were centrifugated at 10,000× *g* for 15 min at 4 °C and the upper and lower fractions were used to quantify total lipophilic (L-TAA) and hydrophilic (H-TAA) antioxidant activity, respectively. Total phenolic content was quantified in duplicate on the lower fraction for each extract using the Folin–Ciocalteu reagent, as previously described by García-Pastor et al. [30]. Results for this parameter were expressed as g gallic acid equivalent (GAE) kg^{-1} FW. On the other hand, H-TAA and L-TAA were also determined in duplicate in each extract sample, by using the ABTS-peroxidase system, as described by Dobón-Suárez et al. [15]. Results of both parameters, H-TAA and L-TAA, were expressed as g of Trolox Equivalent (TE) kg^{-1} FW.

2.6. Incidence Decay

Weekly, for each sampling date, the number of decay peppers per treatment stored at 7 °C was recorded. Results were expressed as the percentage (%) of accumulated decay at 21 days of storage with respect to the total number of pepper fruits used in the present experiment (54 pepper fruits in total). In addition, photographs were taken to display the visual aspect of green pepper fruits treated with SA and untreated during 21 days of storage. Photographs of pepper fruits were captured using a digital camera (Nikon D3400) in a light box with white background. The setup conditions of the camera were as follows: light provided by two LEDs of color temperature of 5600 K, flash speed of 1/5 s, ISO-200, focal opening (f) 20, and length 35 mm, according to the described conditions by García-Pastor et al. [32]. The rotten pieces of pepper fruit were assessed, photographed and removed from the experiment.

2.7. Statistical Analysis

Statistical analysis was carried out in three replicates for all analytical determinations. Results were expressed as the mean ± SE. Data were subjected to analysis of variance (ANOVA). The sources of variation were treatments and storage time. Mean comparisons were performed using Tukey's HSD test to determine whether the differences among the treatments or storage time were significant at $p < 0.05$. All analyses were performed using

the SPSS software package v.17.0 for Windows (SPSS, 2001, IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Effect of SA Preharvest Treatment on Crop Yield

Crop yield, expressed as kg per tree, number of fruits per plant and average fruit weight, is presented in Figures 1–3, respectively. Accumulated yield (kg plant^{-1}) was recorded throughout the developmental and growth cycle of the green pepper fruit, specifically on ten harvest dates from 6 April to 27 July in the 2020 experiment (Figure 1). Results showed that kg of green pepper fruits harvested by plant were significantly higher ($p < 0.05$) for the 0.5 and 1 mM SA-treated plants than in controls, although these increases were not observed in 5 mM SA treated plants. From 6 April to 27 July, SA applied at 0.5 mM was the most effective for increasing accumulated yield, followed by 1 mM SA. In this sense, SA preharvest treatments applied at 0.5 and 1 mM significantly increased ($p < 0.05$) the kg of green pepper fruits harvested per plant, reaching 0.87 and 0.29 kg more than control plants, respectively, at the last harvest date (27 July). In addition, a 2.0- and 1.6-fold increase by 0.5 and 1 mM SA treatments, respectively, was observed at the first harvest date (6 April). However, SA applied at the higher concentration (5 mM) showed a significant decrease in the accumulated yield, leading to 0.62 kg less than untreated plants on 27 July.

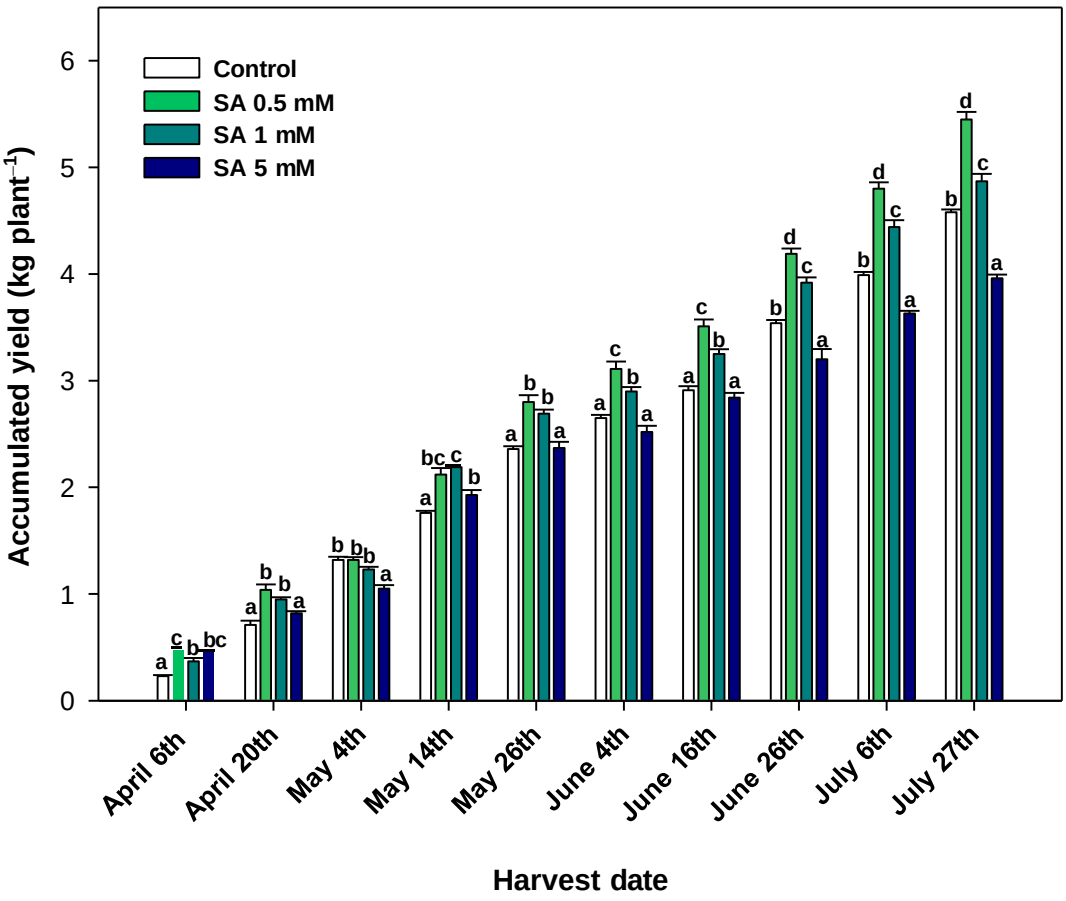

Figure 1. Accumulated yield (kg plant^{-1}) throughout the developmental and growth cycle of green pepper fruit, as affected by salicylic acid (SA) preharvest treatment at 0.5, 1 and 5 mM in the 2020 experiment. Data are the mean ± SE. Different lowercase letters show significant differences among treatments for each harvest date at $p < 0.05$.

The number of pepper fruits per plant was also significantly higher ($p < 0.05$) in the 0.5 mM SA-treated plants than in controls throughout the developmental and growth cycle of green pepper fruit. In addition, this increase was also significant ($p < 0.05$) in the 1 mM SA treated plants compared to untreated plants but generally to a lesser extent than the lowest concentration of SA tested (Figure 2). Nevertheless, in the same way that it was observed for accumulated yield (Figure 1), SA treatment applied at 5 mM significantly reduced ($p < 0.05$) the number of pepper fruits harvested per plant at the end of the crop cycle (27 July), although all treatments with SA significantly increased ($p < 0.05$) this parameter on 6 April (Figure 2).

A drastic downward trend was observed for the average weight of green pepper fruit throughout its developmental and growth cycle (Figure 3). The average fruit weight, taking into account data from 6 April to 27 July, was significantly higher ($p < 0.05$) in green pepper fruit from 0.5 mM SA treated plants than from controls, although no significant ($p \geq 0.05$) effect was generally observed with 1 mM SA treatment along the crop cycle (Figure 3). In relation to the other results discussed above, 5 mM SA-treated plants produced green pepper fruits with a significantly lower ($p < 0.05$) average fruit weight than the other treatments in most of the harvest dates studied, except for the first harvest (6 April).

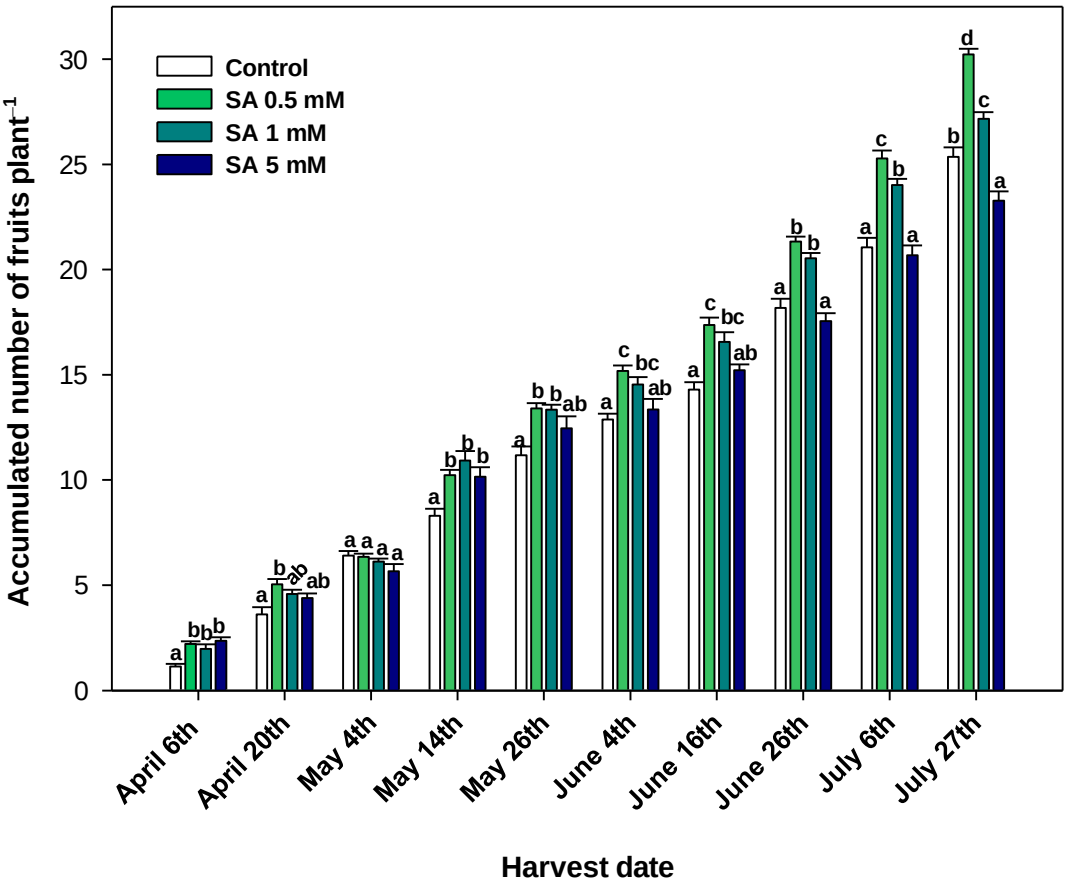

Figure 2. Accumulated number of pepper fruits per plant (number of fruits plant^{-1}) throughout the developmental and growth cycle of green pepper fruit as affected by salicylic acid (SA) preharvest treatment at 0.5, 1 and 5 mM in the 2020 experiment. Data are the mean ± SE. Different lowercase letters show significant differences among treatments for each harvest date at $p < 0.05$.

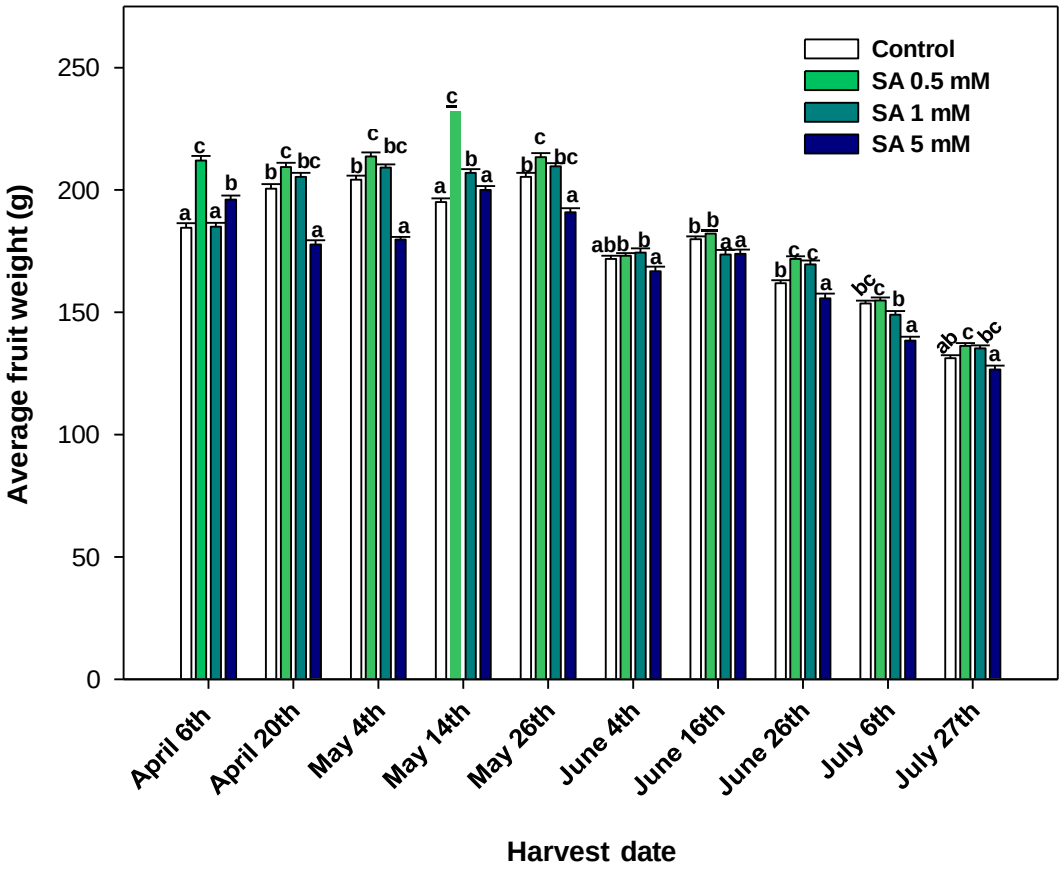

Figure 3. Average fruit weight (g) throughout the developmental and growth cycle of green pepper fruit as affected by salicylic acid (SA) preharvest treatment at 0.5, 1 and 5 mM in the 2020 experiment. Data are the mean ± SE. Different lowercase letters show significant differences among treatments for each harvest date at $p < 0.05$.

3.2. Effect of SA Preharvest Treatment on Weight Loss, Respiration Rate and Physico-Quemical Parameters at Harvest and during Storage

Weight loss increased during postharvest storage in green pepper fruit for all treatments studied, although these increases were significantly reduced ($p < 0.05$) by the SA treatment applied at 0.5 mM after 14 days of storage at 7 °C (Table 2). Thus, peppers treated with SA at the lowest concentration showed the lowest percentage of weight loss at the end of the storage (21 days), although 1 and 5 mM SA treatments did not show significant differences ($p \geq 0.05$) compared to pepper fruit harvested from the control plants. On the other hand, 0.5 mM SA showed the lowest respiration rate at harvest, followed by SA treatments applied at the other concentrations (Table 2). This parameter significantly decreased ($p < 0.05$) from harvest until 21 days of storage at 7 °C in all treatments tested. The effect of SA treatments at 0.5, 1 and 5 mM decreased the respiration rate by 1.33, 1.21 and 1.24-fold compared to the control pepper fruits, respectively, at the end of the storage period, although no significant differences among SA treatments were observed.

Table 2. Effects of salicylic acid (SA) preharvest treatments at 0,5, 1 and 5 mM on weight loss (%), respiration rate (mg CO_2 kg^{-1} h^{-1}) and physico-chemical parameters: firmness (N mm^{-1}), color (b*), total soluble solids (TSS; g kg^{-1}) and total acidity (TA; g kg^{-1}) content of green pepper fruit during 21 days of cold storage at 7 °C [1].

	Days	Control	SA 0.5 mM	SA 1 mM	SA 5 mM
Weight loss (%)	0	-	-	-	-
	7	2.62 ± 0.16 aA	2.29 ± 0.17 aA	2.42 ± 0.19 aA	2.54 ± 0.17 aA
	14	5.24 ± 0.44 bB	3.90 ± 0.24 aB	4.51 ± 0.25 abB	4.72 ± 0.41 abB
	21	7.50 ± 0.52 bC	5.81 ± 0.35 aC	6.62 ± 0.38 abC	6.70 ± 0.49 abC
Respiration rate (mg CO_2 kg^{-1} h^{-1})	0	86.62 ± 2.91 cC	65.02 ± 1.62 aC	73.51 ± 2.63 bC	74.65 ± 2.86 bC
	7	18.43 ± 0.44 cB	12.73 ± 0.44 aB	14.37 ± 0.43 bB	14.67 ± 0.41 bB
	14	17.34 ± 0.75 bB	11.47 ± 0.72 aAB	12.22 ± 0.72 aAB	12.15 ± 0.67 aA
	21	14.15 ± 0.35 bA	10.63 ± 0.39 aA	11.73 ± 0.59 aA	11.43 ± 0.38 aA
Firmness (N mm^{-1})	0	4.77 ± 0.11 aC	5.84 ± 0.11 cD	5.51 ± 0.14 bcC	5.35 ± 0.11 bC
	7	3.31 ± 0.17 aB	4.65 ± 0.17 bC	3.55 ± 0.19 aB	3.69 ± 0.19 aB
	14	2.94 ± 0.15 aB	3.70 ± 0.13 bB	3.20 ± 0.15 abB	3.03 ± 0.18 aB
	21	1.08 ± 0.18 aA	2.20 ± 0.18 bA	1.84 ± 0.11 bA	1.73 ± 0.12 bA
Color (b*)	0	23.57 ± 0.63 aC	21.75 ± 0.69 aC	22.04 ± 0.68 aC	23.43 ± 0.64 aC
	7	20.89 ± 0.51 bB	16.67 ± 0.58 aB	17.22 ± 0.61 aB	18.70 ± 0.63 aB
	14	17.05 ± 0.35 bA	15.34 ± 0.54 aAB	15.70 ± 0.55 abAB	15.68 ± 0.59 abA
	21	16.00 ± 0.38 bA	14.50 ± 0.31 aA	15.00 ± 0.51 abA	15.00 ± 0.51 abA
TSS (g kg^{-1})	0	45.33 ± 1.02 aA	47.16 ± 1.17 aA	46.16 ± 1.42 aA	46.00 ± 1.20 aA
	7	46.66 ± 1.16 aA	49.00 ± 1.73 aA	48.00 ± 1.31 aA	47.66 ± 1.19 aA
	14	47.90 ± 1.42 aA	49.46 ± 1.35 aA	48.66 ± 1.62 aA	48.33 ± 1.85 aA
	21	48.30 ± 1.50 aA	50.83 ± 1.16 aA	50.33 ± 1.23 aA	49.90 ± 1.42 aA
TA (g kg^{-1})	0	1.91 ± 0.07 aB	2.26 ± 0.05 cC	2.17 ± 0.07 bcC	1.96 ± 0.06 abB
	7	1.75 ± 0.04 aB	1.92 ± 0.04 bB	1.90 ± 0.06 abB	1.89 ± 0.07 abB
	14	1.54 ± 0.06 aA	1.87 ± 0.07 bAB	1.81 ± 0.05 bAB	1.75 ± 0.08 abAB
	21	1.35 ± 0.08 aA	1.68 ± 0.06 bA	1.61 ± 0.07 bA	1.52 ± 0.06 abA

[1] Values (mean of three replicates) ± SE followed by different lowercase letters, within the same row, show significant differences ($p < 0.05$) among treatments, according to Tukey's HSD test, for each parameter. Different capital letters in the same column show significant differences among storage days for each treatment at $p < 0.05$.

Fruit firmness at harvest was significantly higher ($p < 0.05$) in fruit from SA treated plants than in controls (Table 2). However, SA applied at 0.5 and 1 mM were the most effective treatments for increasing the firmness levels of pepper fruits at harvest, since a 1.20-fold increase was achieved in those SA treated peppers compared to untreated. During postharvest storage, fruit firmness decreased in green pepper fruits from the control and treated plants, maintaining the differences found at harvest. With respect to color, expressed as b* parameter, no significant differences ($p \geq 0.05$) were observed among treatments at harvest (Table 2). Nevertheless, after 21 days of storage at 7 °C, all pepper fruits showed important color losses, leading to fruit yellowing, which was significantly delayed ($p < 0.05$) by the 0.5 mM SA treatment.

TSS content did not show significant differences ($p \geq 0.05$) neither among treatments nor during storage days (Table 2). However, TA content was ca. 2.2 g kg^{-1} in green pepper fruits treated with SA at 0.5 and 1 mM concentrations (Table 2), being significantly higher ($p < 0.05$) than untreated pepper fruits. During storage, decreased trends in TA were observed for control and SA treated fruit; although significantly higher ($p < 0.05$) levels were maintained in 0.5 and 1 mM SA-treated peppers until the last sampling date.

3.3. Effect of SA Preharvest Treatment on Total Phenolics Content and Total Antioxidant Activity at Harvest and during Storage

Total phenolic content as well as hydrophilic (H-TAA) and lipophilic (L-TAA) total antioxidant activity were significantly increased ($p < 0.05$) during 21 days of postharvest storage at 7 °C regardless of treatment tested (Table 3). Specifically, total phenolics at harvest were significantly higher ($p < 0.05$) in 0.5 and 1 mM SA-treated green pepper fruits, reaching values of 0.850 g kg^{-1} FW, compared to controls (*ca.* 0.700 g kg^{-1} FW). At 21

days of storage, significant differences ($p < 0.05$) on total phenolic content were observed in 0.5 mM SA-treated green pepper fruit compared with control fruits.

Table 3. Effects of preharvest salicylic acid (SA) treatments (0,5, 1 and 5 mM) on total phenolics content (g kg^{-1}) and total antioxidant activity (TAA; g kg^{-1}): hydrophilic (H-TAA) and lipophilic (L-TAA) fractions, of green pepper fruit during 21 days of cold storage at 7 °C [1].

	Days	Control	SA 0.5 mM	SA 1 mM	SA 5 mM
Total phenolic content (g kg^{-1})	0	0.707 ± 0.034 aA	0.854 ± 0.027 bA	0.850 ± 0.036 bA	0.828 ± 0.036 abA
	7	0.800 ± 0.031 aAB	0.928 ± 0.032 bAB	0.900 ± 0.036 abAB	0.914 ± 0.036 abAB
	14	0.870 ± 0.029 aBC	0.987 ± 0.028 bBC	0.952 ± 0.037 abAB	0.950 ± 0.025 abB
	21	0.947 ± 0.026 aC	1.049 ± 0.024 bC	1.017 ± 0.040 abB	0.993 ± 0.037 abB
H-TAA (g kg^{-1})	0	0.750 ± 0.059 aA	1.012 ± 0.049 bA	1.008 ± 0.043 bA	1.004 ± 0.039 bA
	7	1.028 ± 0.053 aB	1.401 ± 0.055 bB	1.287 ± 0.048 bB	1.282 ± 0.044 bB
	14	1.212 ± 0.049 aBC	1.614 ± 0.040 bC	1.522 ± 0.060 bC	1.498 ± 0.054 bC
	21	1.407 ± 0.063 aC	1.866 ± 0.058 cD	1.620 ± 0.036 bC	1.673 ± 0.035 bD
L-TAA (g kg^{-1})	0	0.357 ± 0.022 aA	0.468 ± 0.033 bA	0.381 ± 0.017 abA	0.383 ± 0.020 abA
	7	0.386 ± 0.027 aAB	0.521 ± 0.038 bA	0.395 ± 0.024 aAB	0.392 ± 0.035 abAB
	14	0.430 ± 0.029 aAB	0.647 ± 0.030 bB	0.497 ± 0.041 aBC	0.493 ± 0.043 aAB
	21	0.467 ± 0.030 aB	0.729 ± 0.033 bB	0.539 ± 0.036 aC	0.535 ± 0.050 aB

[1] Values (mean of three replicates) ± SE followed by different lowercase letters, within the same row, show significant differences ($p < 0.05$) among treatments, according to Tukey's HSD test, for each parameter. Different capital letters in the same column show significant differences among storage days for each treatment at $p < 0.05$.

H-TAA at harvest was significantly higher ($p < 0.05$) in pepper fruits harvested from SA-treated plants compared to those from untreated plants, although no significant differences ($p \geq 0.05$) were shown among the three concentrations of SA treatments (Table 3). After the increase of H-TAA during storage at 7 °C, 0.5 mM SA was the most effective treatment increasing this functional parameter at 21 days of storage, followed by SA at 1 and 5 mM, while pepper fruits from control plants had the lowest values. Enhanced L-TAA at harvest was only obtained by 0.5 mM SA treatment (Table 3), since green pepper fruits treated with SA at 1 and 5 mM showed similar values of L-TAA than controls. These differences observed for L-TAA levels among treatments were maintained during the 21 days of storage, with SA applied at the lowest concentration the most effective for increasing TAA attributed to compounds presented on the lipophilic fraction.

3.4. Effect of SA Preharvest Treatment on Visual Aspect and Decay Incidence of Green Pepper Fruit during Storage

Both visual aspect and decay incidence of green pepper fruits were recorded at the end of postharvest storage experiment, as can be observed in Figure 4. The greatest valued visual aspect at 21 days of storage at 7 °C in terms of higher green color presence, absence of wrinkling or drying as well as other visual defects, was presented by green pepper fruits treated with SA (Figure 4A). However, those fruits that showed a greater visual aspect of freshness were obtained from 0.5 mM SA-treated pepper plants. Furthermore, this preharvest treatment was the most effective for reducing the decay incidence during 21 days of storage at 7 °C, since the lowest percentage (%) of decay was recorded for this treatment (Figure 4B). Specifically, the decay incidence for 0.5 mM SA-treated pepper fruits reached a ca. value of 2% at the end of the storage, which was lower than untreated fruits (16.6%) and the other pepper fruits treated with SA at 1 and 5 mM (3.7 and 9.3%, respectively).

Figure 4. (**A**) Visual aspect of 'Herminio' cultivar harvested from one replicate of control plants and SA treated plants with three concentrations: 0.5, 1 and 5 mM, at harvest and after 21 days of storage at 7 °C. (**B**) Heatmap of decay incidence for 'Herminio' cultivar after 21 days of postharvest storage at 7 °C. Colors in the diagram represent the low or high percentage of incidence, ranging from green to red, respectively; and numbers below each color map represent the different treatments (1 = control; 2 = 0.5 mM SA; 3 = 1 mM SA; 4 = 5 mM SA).

4. Discussion

4.1. SA Preharvest Treatment Applied at Low Concentration Tested Increases Crop Yield

In the present experiment, control and SA treated plants were grown in a greenhouse under similar climatic and agronomic conditions, as a result the differences between the control and treated plants was just due to the effects of treatments tested. The increase of accumulated crop yield (kg per plant) was higher with 0.5 mM SA treatment than SA at 1 mM concentration, although a negative effect was observed for 5 mM dose. Results also showed that SA treatments at 0.5 mM increased crop yield due to an increase in the number of harvested fruits per plant, which had a higher average fruit weight. In the same way, the effect of 5 mM SA treatment on reducing crop yield was mainly due to a sharp decrease on the number of harvested fruits per plant as well as due to a lower average fruit weight caused by the treatment. Due to the treatments being performed before the beginning of flowering stage, the effect of SA treatment on increasing or reducing fruit number could be mainly due to a direct consequence on flowering rate, set fruit rate or on pepper fruit abscission from the plants, which naturally occurs during the fruit developmental process. Previous work has studied the action of exogenous application of SA at 0.001 mM on flower induction and fructification in habanero pepper plants [33]. Results demonstrated that the number of flowers per plant observed at 80 days after spraying was 1.70-fold higher in SA treated plants than controls, leading into a 40% higher fructification compared with the control.

On the other hand, our results of SA treatment applied at the lowest concentrations on increasing average fruit weight prove that this plant hormone could increase net photo-assimilate production in pepper plants. In fact, Tucuch-Haas et al. [33] also reported that 0.001 mM SA preharvest treatment significantly increased growth and fresh and dry weight of roots, stems, leaves and fruits of *Capsicum chinense* plant species at harvest, mainly due to a positive effect of SA on increasing the uptake of macronutrients and micronutrients that

are allocated in the plant tissues. Therefore, exogenous application of SA may influence a range of plant processes, including stomatal closure, ion uptake and transport [34], membrane permeability [35] and photosynthetic and growth rates [36]. For instance, in the 'California Wonder' sweet pepper, SA has been reported to increase vegetative growth, photosynthetic pigments, mineral content, and endogenous auxin and cytokinin levels, while decreasing abscisic acid levels [37]. Moreover, an increase on Rubisco activity and total yield by SA treatment were also reported in maize and mustard plants [38]. In addition, a possible action of SA on the reduction of pepper fruit abscission during the crop cycle is not discarded as it was previously reported in pomegranate fruit [32].

Nevertheless, our results reported that SA applied at high concentrations (5 mM) can exert the opposite effect, decreasing the yield in pepper plants. Accordingly, salicylates preharvest treatments in table grapes at high concentration, 5 and 10 mM, delayed berry ripening and reduced crop yield, while ripening was accelerated and crop yield increased at lower concentrations [39]. Thus, for the first time, results on crop yield of 'Herminio' pepper plants are reported and depend on applied concentration of SA. Similarly, pepper fruit grown under moderately salt-stressed greenhouse conditions showed positive results in terms of productivity and fruit quality with SA application at a low concentration (0.001 mM) [23]. To our knowledge, the lowest concentration of SA tested in the present experiment (0.5 mM) could be a useful tool to increase crop yield in green pepper plants.

4.2. SA Preharvest Treatment Applied at Low Concentration Tested Improves Fruit Quality Parameters and Functional Quality at Harvest and during Storage

The loss of water in sweet peppers is one of the problems that is generated during storage, leading to fruit firmness changes [40]. Consumers have become more critical in the last decade with fruit quality parameters, taking into account flavor and firmness [41]. In this sense, SA preharvest application at 0.5 mM was the most effective treatment significantly reducing the weight loss of pepper fruits at 21 days of storage at 7 °C. Nevertheless, all SA treatments efficiently increased fruit firmness both at harvest and at the end of postharvest storage. Other authors have observed a significant improvement by 3 mM SA-foliar application on 'Yolo Wonder' pepper cultivar in fruit firmness at harvest [25]. The general positive effects delaying losses of fruit weight and firmness during the postharvest storage experiment of 'Herminio' pepper fruits as a result of applying SA could be attributed to its role on the activities of cell wall modifying enzymes, such as polygalacturonase (PG) and pectin methyl esterase (PME), according to the results reported by Rao et al. [10]. In this study, the authors observed that levels of these enzyme activities were found to be decreased by the SA and $CaCl_2$ postharvest treatments, suggesting that SA and $CaCl_2$ delays softening of *Capsicum* fruit and supports brittleness and firmness retention of the fruit flesh. Srivastava and Dwivedi [42] also reported that SA treatment suppresses the cell wall degrading enzyme activities. On the other hand, a key reduction of fruit respiration rate was observed both at harvest and after 21 days of storage by all SA treatments compared to untreated pepper fruits. Perhaps, similar to research reported by Rao et al. [10] in 'Indra' sweet pepper cultivar, the fruits treated with SA could present reduced PG and PME activities that may be due to the antisenescent action and inhibitory effect of SA on ethylene biosynthesis. This effect could delay the activity of enzymes responsible for ripening and resistance to pathogen incidence; therefore, cell wall degradation could be prevented by this preharvest treatment, which in turn facilitated the reduced water loss and lesser fruit respiratory gas exchange. In fact, Rao et al. [10] concluded that SA at 1 and 2 mM applied as a postharvest treatment led to a lower percentage of shrinkage in the treated pepper fruits. Similar results can be observed in 'Herminio' green pepper fruit (Figure 4A), where shrinkage symptoms were observed in control pepper fruits at the end of postharvest storage.

Furthermore, SA foliar application did not affect the color of green pepper fruit at harvest. Nevertheless, losses of green color during storage were delayed by all SA treatments leading to less yellowish pepper fruits and, specially, for those fruits treated with SA at 0.5 mM concentration (Figure 4A). This result of preharvest SA treatment on

avoiding the pepper fruit discoloration during storage could be a consequence of the treatment delaying the fruit senescence process. In this sense, a previous study concluded that SA applied as postharvest treatment extended the shelf-life of sweet pepper fruits by up to 71 days stored at 10 °C [10]. Despite no significant differences being observed on TSS content, all SA treated pepper fruits showed higher levels of TA at harvest. However, the TA content after 21 days of storage was only significantly higher in those pepper fruits harvested from 0.5 and 1 mM SA-treated plants. Other authors have reported that vitamin C, TSS and TA were increased at harvest in 'California Wonder' sweet pepper fruit also as a response to 0.1 mM SA [37]. Hanieh et al. [43] reported that SA application at 0.7 mM also increased TA but decreased TSS in 'Cadia' sweet pepper. Several studies concluded that content of vitamin C, TA and TSS, and total sugars in sweet pepper fruit are cultivar-dependent and influenced by growth conditions [44,45]. Therefore, we can conclude that SA preharvest treatment did not affect the on-plant ripening process of pepper fruit, although it led to pepper fruits with higher levels of organic acids at harvest and during storage.

Phenolics content is a suitable indicator to evaluate environmental stress tolerance and improve plant metabolism, inducing stress tolerance in plants through light or antioxidant protection [46]. For the first time, the effect of SA preharvest treatment of green pepper plants on phenolic content at harvest and during fruit storage has been reported in the present study. Our results showed that SA applied at the three studied concentrations increased the total phenolics content at harvest. However, it would be worth highlighting that 0.5 mM SA was the most effective treatment for improving total phenolic content after 21 days of storage at 7 °C. In Chinese cabbage, Thiruvengadam et al. [47] reported that SA increased the expression of genes codified by enzymes, such as chalcone synthase (CHS) and chalcone isomerase (CHI), which are involved further downstream in the pathway of flavonoids. Other authors have hypothesized on the effects of salicylate treatments on enhancing total phenolic concentration could be due to the activation of phenylalanine ammonia lyase (PAL) activity, which is the main enzyme involved in the biosynthetic phenolic pathway, by these treatments [32,39].

Ultimately, the antioxidant capacity is given by compounds present on hydrophilic and lipophilic fractions. Hydrophilic compounds are mainly ascorbic acid or vitamin C, glutathione (GSH) and phenolic compounds, mainly flavonoids, while the lipophilic ones are mainly chlorophylls, carotenoids and vitamin E [15]. Regarding H-TAA, all preharvest treatments with SA enhanced the TAA of those compounds presented in the hydrophilic fraction at harvest. Nevertheless, the 0.5 mM SA was the most effective one on this functional improvement at the end of postharvest storage, followed by 1 and 5 mM SA treatments. Previous studies have shown that SA at a low concentration (0.001 mM) positively increased vitamin C content in pepper fruits grown in a moderately salt-stressed greenhouse at harvest [23]. Under salt-stress conditions, higher content of ascorbic acid could maintain relatively lower levels of reactive oxygen species (ROS) in pepper fruit, resulting in less damage caused by these ROS since ascorbic acid as an antioxidant plays an important role and protect the plant during oxidative damage by scavenging free radicals and ROS [48]. Other authors have also reported that preharvest treatments with salicylates on pomegranate trees increased total phenolic compound content, as well as ascorbic acid, leading to increases in H-TAA [32]. Therefore, we hypothesize that foliar application of SA to pepper plants could increase the vitamin C content of green pepper fruits, leading to an enhanced H-TAA, as has been observed in terms of total phenolic content.

Chlorophylls are lipophilic-nature pigments, which change during pepper development on the plant and are responsible for the characteristic green color of each pepper cultivar. Nevertheless, chlorophyll degradation by chlorophyllase, which is the enzyme catalyzing the conversion of chlorophyll to its degradation product chlorophillide, and loss of green color of 'Herminio' pepper fruits are direct consequences of postharvest storage, which are directly as a result of specific relative humidity [49]. Our results showed that SA preharvest treatment at 0.5 mM increased L-TAA of green pepper fruits at harvest and

after postharvest storage. Accordingly, other authors reported that chlorophyll content decreased under drought or salt stress in bell pepper cultivars, but SA spray increased this content [50]. The increase of L-TAA could be mediated by the role of SA on increasing chlorophyll content at harvest and on delaying its deterioration during storage at 7 °C, since the greatest visual aspect in terms of green color was recorded on both sampling dates (at harvest and after 21 days of storage; Figure 4A) in those peppers treated in preharvest with SA at a dose of 0.5 mM. Both antioxidant activity fractions could be related to potential health functionality against various chronic non-communicable diseases [51]. Therefore, it is advisable that the green pepper fruits treated with SA at the lowest concentration (0.5 mM) could improve green pepper fruit quality and their content on antioxidant compounds with beneficial health effects, both at harvest and during storage.

4.3. SA Preharvest Treatment Applied at Low Concentration Tested Induces Fruit Tolerance against Decay Incidence during Storage

The lowest percentage (%) of decay was recorded in those green pepper fruits harvested from 0.5 mM SA treatment, followed by SA treatments at 1 and 5 mM concentrations. The induction of green pepper fruit tolerance by SA foliar application could be related to the stimulation of peroxidase and polyphenoloxidase activities, as was reported by Mekawi et al. [21]. These authors found that salicylic acid at 8 mM was the most effective treatment against grey mold caused by *B. cinerea* and for maintaining naturally infected pepper fruits. Accordingly, Li and Zou [52] observed that SA application significantly increased the accumulation of hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), PAL activity, expression level of PR gene (pathogenesis related protein) in tomato plants, improving fruit resistance against *B. cinerea*.

5. Conclusions

The commercial harvest stage of green pepper fruit is prior to full developed physiological stage, which directly influences its postharvest quality. In this sense, this is the first report showing that foliar application of SA in preharvest to green pepper plants has a significant effect on crop yield, fruit quality parameters and functional quality at harvest and after 21 days of storage at 7 °C. The lowest concentration of SA tested (0.5 mM) showed the best results since this treatment increased crop yield, in terms of kg per plant, number of fruits harvested per plant and average fruit weight and fruit quality parameters as well as bioactive compound content at harvest. In addition, this treatment delayed various losses of physico-chemical and functional traits that normally occur during postharvest storage of pepper fruit at non-chilling temperatures, leading to fruit quality maintenance after 21 days of storage. Finally, SA preharvest treatment applied at 0.5 mM was the most effective tool in order to induce pepper fruit tolerance against decay incidence during storage. Thus, SA applied at 0.5 mM could be a safe, useful and natural preharvest strategy to increase crop yield and green pepper fruit quality parameters at harvest and to maintain them during storage. However, more studies are needed in order to elucidate the effect of SA application on the flowering biology of pepper plants that could affect crop yield over the year.

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4.3. Publication 3 — Research article

PUBLICATION 3 (Open access)

Antioxidant metabolism insights into ripening and senescence delay of green pepper fruit through the salicylic acid preharvest treatment

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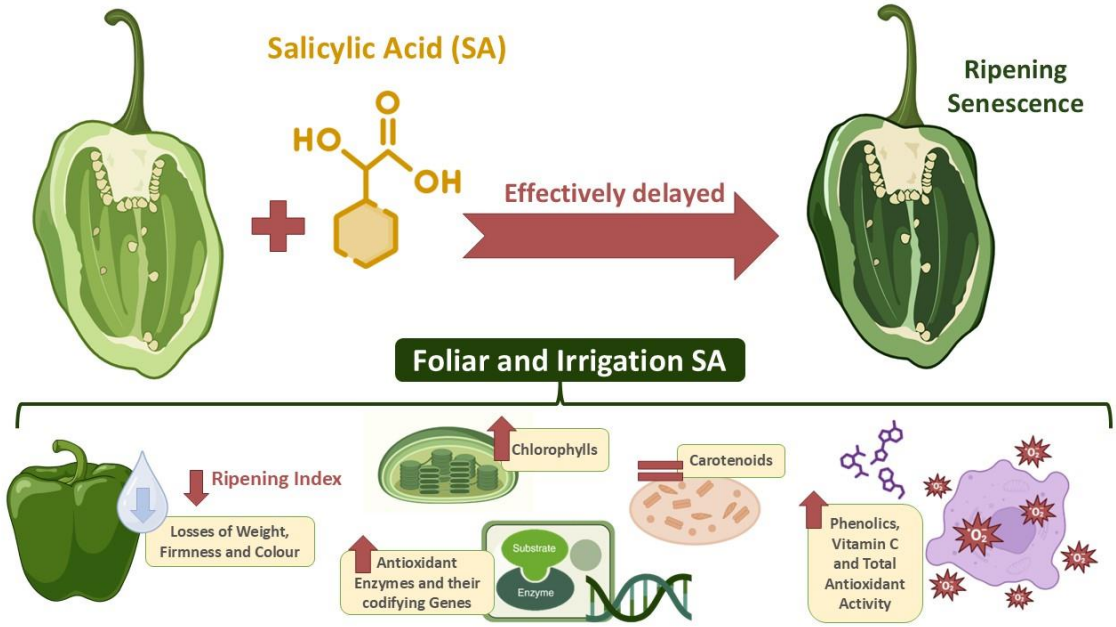

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Antioxidant metabolism insights into ripening and senescence delay of green pepper fruit through the salicylic acid preharvest treatment

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Introduction: The systematic investigation of the biochemical and molecular bases of salicylic acid (SA) in the postharvest physiological process of green pepper fruit remains unclear.

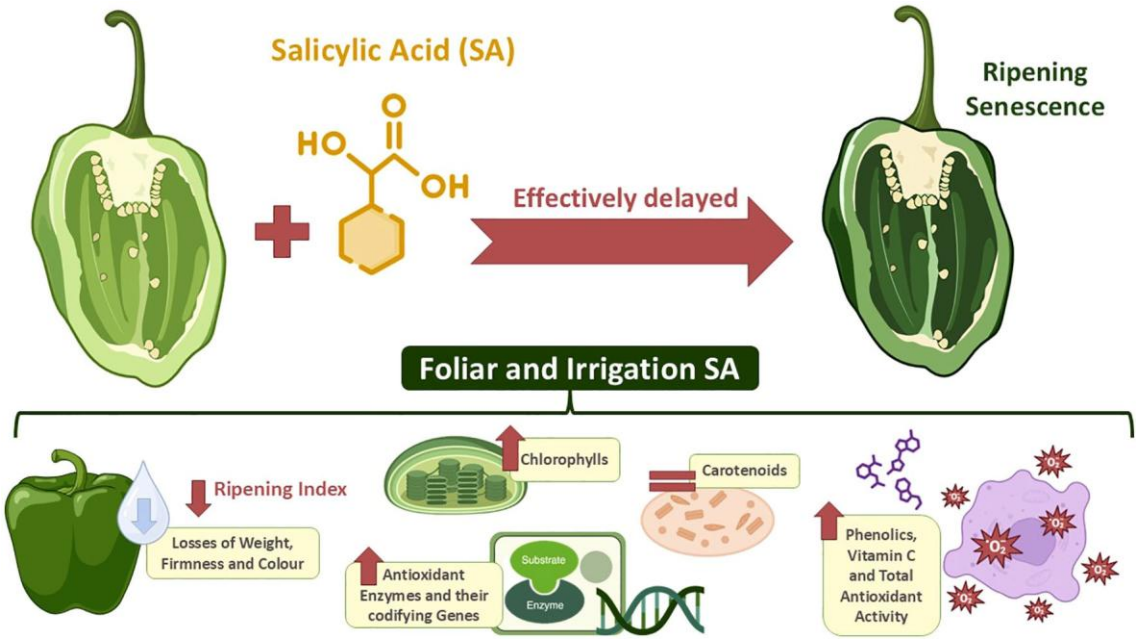
Methods: Accordingly, this study aims to analyze the effects of 0.5 mM-SA preharvest treatments, applied by foliar spraying or irrigation, on the ripening and senescence of green pepper fruit for 28 days of storage at 7 °C.

Results: The study revealed that the preharvest application of SA, either by foliar spraying or irrigation, significantly delayed losses of weight, firmness and color during postharvest. Additionally, both treatments increased the total soluble solids and total acidity content, which lead to a significantly reduced ripening index after storage. These results were evidenced by a slowing down of the ripening and senescence processes, accompanied by the stimulation of the antioxidant enzymes in those SA-treated green pepper fruits. Furthermore, a significant increase in chlorophylls, phenolics, ascorbic acid and dehydroascorbic acid content was observed. The SA treatments also enhanced the total antioxidant activity, in both hydrophilic and lipophilic phases. These positive effects were mediated by the upregulation of the relative response of the *CaAPX, CaPOD, CaPAL, CaDHAR2* genes at harvest.

Discussion: These findings reinforce the existing knowledge gap regarding the impact of foliar spraying or irrigation SA on the intricate interplay between metabolites and genes related to the antioxidant system in regulating the bell pepper fruit ripening and senescence. The impact of both applications exhibited comparable results; however, the irrigation was identified as the most advantageous due to its ease applicability and cost effectiveness in comparison.

KEYWORDS

Capsicum annuum L., quality losses, bioactive compounds, antioxidant capacity, antioxidant enzymes, relative gene expression



GRAPHICAL ABSTRACT
An antioxidant metabolism approach delaying ripening and senescence of green pepper fruit by applying foliar or irrigation salicylic acid (SA) preharvest treatment.

1 Introduction

Bell pepper (*Capsicum annuum* L.) is an economically important vegetable crop which has a recent worldwide popularity among consumers in human diet due to its nutritional value, as an excellent source of biologically active compounds (vitamins, carotenoids, flavonoids, phenolic acids and other phytochemicals) with health-related properties, the crispness, and the versatility to be consumed as a fresh vegetable in salads, cooked meals or dehydrated for spices (Mariṅet al., 2004; Navarro et al., 2006; Serrano et al., 2010; Raybaudi-Massilia et al., 2017; Soare et al., 2017; Ge et al., 2020). Consumers have become more critical in the last decade with their purchasing decisions which are commonly focused not only in physical and sensory traits, such as color, size, pericarp thickness, firmness and flavor, but also in nutritional and nutraceutical characteristics (Rodríguez-Burruezo et al., 2010; Jiménez-Garcıáet al., 2018). In Spain, the total production of chili peppers and peppers (*Capsicum annuum* L. and *Piper nigrum* L, respectively) has steadily increased to more than 1.58-fold over the past 10 years, reaching over 1.5 million tons in the 2022 season (FAOSTAT, 2024). The primary postharvest challenges that result in substantial quality deterioration and diminished acceptability of bell peppers are as follows: Water loss, which leads to significant softening and shrinkage due to turgor pressure loss, starch degradation, and chemical modifications in the cell wall related to pectin by the action of softening enzymes, such as polygalacturonase (PG), pectin methyl esterase (PME), cellulase, and ß-galactosidase (Li Z. et al., 2024). On the other hand, peppers can experience chilling injury (CI) when stored at temperatures below 7-10°C. This leads to symptoms such as surface pitting, watery stains, browning of the seed and calyx, and fruit decay. This, in turn, can result in reduced marketability (El-Ramady et al., 2015; Charoenphun et al., 2024). Finally, there are pathological disorders, for example grey mold, which is mainly caused by *Botrytis cinerea* (Cheema et al., 2018).

Nowadays, global demand for high-quality vegetable products is rapidly increasing. However, climate change is negatively affecting agricultural areas and water resources which are decreasing (Sobczak et al., 2023). Bell pepper crop is sensitive to temperature fluctuations during the developmental and growth cycle, showing a lack of tolerance to high temperature since its fruit setting is drastically reduced when the day temperature rises above 32°C and/or the night temperature is above 20°C (Erickson and Markhart, 2002). This abiotic stress in the plant induces the production and accumulation of reactive oxygen species (ROS) leading to membrane breakdown and cellular turgor loss that can prevent plant growth and development (Preet et al., 2023). The process of scavenging ROS in plants as part of the antioxidant defense system and osmoprotectants comprises various antioxidant enzymes, including superoxide dismutase (SOD), which converts free superoxide (O_2) radicals to hydrogen peroxide (H_2O_2) and oxygen, ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD). The latter enzymes have shown to play a pivotal role in the detoxification of H_2O_2 through its decomposition into water and oxygen. Furthermore, the role of radical scavenging metabolites in ROS scavenging mechanisms is also of significance. In this sense, different phytohormones can positively influence the crop yield and reduce negative environmental impacts. On the other hand, fruit senescence and postharvest disease infection can result in quality and economic

losses (Zhang and Jiang, 2019). Some plant hormones have been widely studied to improve the postharvest quality of fruit and vegetables (Zhang et al., 2020).

As the major endogenous component in signal transduction systems, SA plays an efficient role in plant growth and development, flowering, and fruit ripening, as well as in regulating photosynthesis (Natasha et al., 2020; Hadjipieri et al., 2021). On the other hand, SA is crucial in stimulating the systemic acquired resistance (SAR) in plants by regulating numerous biochemical and physiological functions related to tolerance to both biotic and abiotic stresses and modifying the antioxidant system (Khan et al., 2015). Therefore, SA and its derivatives elicit a wide range of metabolic and physiological processes in plants, which have great potential in reducing postharvest losses in horticultural crops. SA leads to the synthesis of proteins affecting several metabolic processes by regulating their gene expression (Janda et al., 2020). The mechanisms by which SA generates these improvements could be related to the protection of cell membranes, the increase in carbon metabolism, and antioxidant system, and the regulation of stress defense proteins (Kang et al., 2012; Sharma et al., 2017). However, its specific action mechanism is still not well understood. Indeed, different studies have demonstrated the hormonal interactions between SA and jasmonic acid (JA) and several different stress-link compounds taking place under abiotic stresses, highlighting the complexity of hormonal signaling cascades (Dempsey and Klessig, 2017; Devireddy et al., 2021; Huntenburg et al., 2022). Furthermore, the attenuating effects of SA in plants depend on the concentration used, the method of application, and the plant development stage (Nóbrega et al., 2020).

In recent years, the foliar application of exogenous SA to crops has shown to be effective in the regulation of biotic and abiotic stresses, increasing the yield of green pepper fruit by reducing stress-induced growth inhibition as well as fruit quality traits (Elwan and El-Hamahmy, 2009; Jiménez-Garcıáet al., 2018; Ibrahim et al., 2019; Veloso et al., 2021; Dobón-Suárez et al., 2021b; Ghahremani et al., 2023; Sobczak et al., 2023; Preet et al., 2023; Rodrigues da Silva et al., 2023). In addition, SA treatment was found to alleviate chilling injury in pepper fruits through enhancing antioxidant metabolism, fatty-acid desaturation efficiency and water retention (Fung et al., 2004; Ge et al., 2020; Hanaei et al., 2022). However, appropriate concentrations and methods of elicitor application need to be determined to improve the effectiveness of this practice under different growing conditions (Munshi et al., 2020; Sobczak et al., 2023). A recent review concluded that preharvest spraying provides better results than postharvest treatments, but the specific results need deeper research to be conducted focusing on the effect of spraying frequency time and growing environment on postharvest storage quality of fruit (Chen S. et al., 2023). Furthermore, Chen S. et al. (2023) stated that there is a lack of information about the metabolic mechanism of exogenous treatments with SA and its derivatives affecting fruit quality parameters, which is an emerging field that needs to be explored. In fact, most of the metabolomic and transcriptomic studies of SA available are related to the functions, biosynthesis or transcriptional regulations of this plant hormone for establishing resistance to many pathogens in plants (Ding and Ding, 2020). For instance, exogenous SA application bolstered resistance to *Colletotrichum viniferum* (Lin et al., 2024), *Ralstonia solanacearum* (Li N. et al., 2024), *Podosphaera pannosa* (Yang et al., 2022), *Xanthomonas campestris pv. campestris* (Sun et al., 2022), *Colletotrichum* (Shi et al., 2019), cucumber green mottle mosaic virus (Liu et al., 2023), and *Penicillium expansum* (Zhang et al., 2024) in grapes, tomato, roses, cabbage, tea, watermelon, and apples, respectively. Recently, Perez-Aranda et al. (2024) demonstrated that the expression of the *CaPR1* (Pathogenesis-related protein 1) in *Capsicum annuum* seedlings, a marker gene employed as indicator of SA pathways activation, is down-regulated with SA elicitation (1, 2.5 and 5 mM) and the cross-talk between jasmonic acid/ethylene and SA mediated signal pathways for the regulation of this gene. Other studies have revealed that the transcriptional activation of SA signaling pathway, rather than its biosynthesis, plays a crucial role in the chilling and freezing tolerance of cucumber and potato fruit, respectively (Sim et al., 2024; Chen L. et al., 2023). However, Nguyen et al. (2024) reported that the ripening quality of mango mirrored the induced SA and jasmonic acid (JA) endogenous levels after liquid methyl salicylate (MeSA) fumigants in postharvest and correlated with the high expression of biosynthetic-related genes. In this sense, in-depth study of both foliar spraying and irrigation methods to pepper plants has received little scientific attention, and the systematic investigation of the mechanism and molecular bases of SA effects in the postharvest physiological process of green pepper fruit remains unclear, which restricts the possibilities for improvement of green pepper fruit quality using preharvest elicitation tools. Therefore, this paper aims to compare the effect of SA preharvest treatment applied by foliar spraying or irrigation on ripening and senescence of green pepper fruit during postharvest storage from an antioxidant metabolism approach to provide deep insight into a practical application of SA on extending the storage shelf-life.

2 Materials and methods

2.1 Plant materials, treatments and experimental design

Pepper plants (*Capsicum annuum* L., 'Lamuyo' type), 'Herminio' cultivar, were planted in January 2021 in a commercial plot growing under plastic-roofed greenhouse located in El Raal (Murcia, Spain). The optimal concentration of salicylic acid (SA) was chosen according to the best results observed in our latest study about the evaluation of SA foliar application on crop yield and quality parameters of green pepper fruit during 21 days of storage at 7°C (Dobón-Suárez et al., 2021a). In this study, SA applied at 0.5 mM showed the best results since this treatment increased crop yield, in terms of kg per plant, number of fruits harvested per plant, average fruit weight, fruit quality parameters and bioactive compound content at harvest. In addition, this treatment delayed losses of physio-chemical and functional traits that normally occur during postharvest storage of pepper fruit at non-chilling temperatures, maintaining fruit quality after 21 days of storage. Finally, SA preharvest treatment applied at 0.5 mM was the

most effective tool to induce pepper fruit tolerance against decay incidence during storage. Therefore, SA was applied in the present study at 0.5 mM following two different commercial practices: *1)* Foliar spraying [Foliar SA] and *2)* Irrigation [Irrigation SA], while the control plants were treated only with distilled water as a spray [Control]. SA reagent (CAS Number: 69-72-7) was purchased from Sigma (Sigma-Aldrich, Madrid, Spain). Solutions for all treatments were supplemented with Tween 20 [0.05% (*v/v*)]. The foliar spray was carried out with a manual pump, while the root application was performed in the automatic irrigation system was carried out automatically. Plants received irrigation and fertilization according to normal agricultural practices designed by the company for the short-term crop cycle of 'Lamuyo' pepper type, in which rockwood was used as the soil substrate and drip irrigation and optimal nutrient levels were applied. The soil texture was sandy loam with a pH of 7.50.

The experiment was conducted from February to July 2021. The experimental design was completely randomized. Thus, 135 pepper plants were selected and distributed in randomized complete block design with nine replicates or blocks in total. Each treatment was performed in three blocks (n = 3) of 15 plants (45 plants per treatment). Seven exogenous SA applications by foliar spraying or irrigation throughout the crop cycle were performed in the morning (8-9 a.m.), the first treatment being applied before the beginning of the flowering stage. Treatments were applied seven times at a 21-d interval until the harvest date with a total amount of SA supplied of 0.48 g L^{-1}. The equidistance among application dates was *ca.* 21 days due to a staggered flowering cycle, except for the last application that was performed close to the last commercial harvest, being chosen based on the crop cycle duration of this pepper cultivar and our previous experience (Dobón-Suárez et al., 2021a). Application dates of treatments (Control, Foliar SA, and Irrigation SA) throughout the developmental and growth cycle of 'Herminio' green pepper fruit were as follows: T1 (22 February), T2 (15 March), T3 (29 March), T4 (19 April), T5 (17 May), T6 (7 June) and T7 (10 July). Pepper fruits were harvested at the commercial harvest stage when green pepper had reached the phenological stage suitable for its consumption (Dobón-Suárez et al., 2021b). A total of 10 harvest dates throughout the growth cycle were performed according to a staggered production and the commercial criteria of harvesting green pepper fruit established by the company. The harvest dates started from April until July: 6 April, 20 April, 4 May, 14 May, 26 May, 4 June, 16 June, 26 June, 6 July and 17 July. The mean temperature for each month was recorded: April (16.00°C), May (19.30°C), June (19.70°C) and July (27.20°C), using a station close to the experimental greenhouse (38°2'2.64" North, 1°1'18.9" West). Relative humidity (RH) fluctuated between 66 to 89% during the experiment. The crop yield was measured in terms of accumulative crop yield, expressed as kg per plant and number of peppers harvested per plant, for each harvest date along the crop cycle and blocks designed per treatment. In addition, the average fruit weight (g) was calculated by weighing and counting all harvested pepper fruits individually, according to Dobón-Suárez et al. (2021a). These results are presented in Supplementary Figure 1. The uniform-sized pepper fruits harvested on 20 April were immediately transferred to the research laboratory of Postharvest Group of Fruit and Vegetables and then, they were graded for their uniformity in shape and color, and those fruits free from visual defects and blemishes were selected to carry out a postharvest storage experiment.

For each treatment 90 peppers fruits similar in shape, size and color were selected and weighted individually and stored at 7°C and 85% of RH. Thus, 18 pepper fruits were analyzed at harvest (day 0) and 72 pepper fruits in total were stored for each treatment during 21 days of storage. For the postharvest storage experiment, pepper fruits were analyzed after 7, 14, 21 and 28 days of storage. Specifically, 90 pepper fruits were used in total for the analyses of each treatment in the four sampling dates (0, 7, 14, 21 and 28 storage days). For each sampling date, weight loss, firmness, color (hue°), total soluble solids (TSS), total acidity (TA) and ripening index (RI) were measured as quality parameters of green pepper fruits during postharvest storage. From a metabolomic approach, the content of chlorophyll a and b, total phenolics, total carotenoids, ascorbic acid (AA) and dehydroascorbic acid (DHA) was quantified at harvest and after 28 days of storage at 7°C in freeze-dried samples composed of both flesh and skin tissues. Furthermore, the hydrophilic-total antioxidant activity (H-TAA), lipophilic-total antioxidant activity (L-TAA) and the antioxidant enzymes activities of ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) were also determined in freeze-dried material at harvest and after 28 days of postharvest storage. Finally, a genetic approach was also addressed since the relative expression of *CaAPX* [*L*-ascorbate peroxidase (APX) gene], *CaCAT* [catalase (CAT) gene], *CaPOD* [peroxidase (POD) gene], *CaPAL* [phenylalanine ammonia-lyase (PAL) gene] and *CaDHAR2* [dehydroascorbate reductase 2 gene] genes was also analyzed in freeze-dried samples of green pepper fruits at harvest and at the end of the storage period. All analyses were performed on 3 replicates of 6 fruits for each treatment and sampling date studied (18 pepper fruits in total).

2.2 Evaluation of quality parameters during postharvest storage

2.2.1 Weight loss, firmness and color (hue°) of green pepper fruits

Green pepper fruits were initially weighed at harvest (day 0) and after 7, 14, 21 and 28 days of storage. The difference between the initial and final weight of pepper fruit was considered as accumulative weight loss during each storage interval and was expressed as a percentage (%) on a fresh weight basis with respect to pepper fruit weight at harvest. Firmness was evaluated individually in each pepper fruit as deformation force using a digital TX-XT2i Texturometer (Stable Microsystems, Godalming, UK). The machine had a flat steel plate to measure the equatorial fruit diameter and to apply a force that achieved a 5% deformation of its diameter, according to the protocol described by Dobón-Suárez et al. (2021a). Results were expressed as a force-deformation ratio (N mm^{-1}). After that, 6 pepper fruits from each replicate (18 peppers from each treatment) were used to measure individually the color. Surface color changes of green pepper fruits were reported in hue angle (hue°) parameter (arctan b*/a*), according to García

Pastor et al. (2021). It was measured at three points of the fruit equatorial diameter by using a Minolta Colorimeter CFRC400 (Minolta Camera Co., Kantō, Tokio, Japan).

2.2.2 Total soluble solids, total acidity and ripening index of green pepper fruits

A homogeneous sample was prepared from each replicate and treatment by blending the fruit in a blender. The sample was thoroughly mixed, and a few drops were taken on prism of a portable digital refractometer (Atago PR-101, Atago Co., Ltd., Tokyo, Japan) to measure the content of total soluble solids (TSS) of each sample in duplicate at 20°C. Results were expressed as g kg^{-1} in fresh weight basis (FW). As described by Dobón-Suárez et al. (2021a), total acidity (TA) was determined in duplicate from the same sample by titrating 1 mL of diluted juice in 25 mL of distilled H_2O with 0.1 N NaOH up to a pH of 8.10 using an automatic titration (785 DMP Titrino, Metrohm, Burladingen, Germany). Results were expressed as g of malic acid equivalent kg^{-1} FW. Ripening index (RI) was then calculated as the ratio of TSS/TA.

2.3 Metabolomic analysis at harvest and after 28 days of storage

2.3.1 Quantification of bioactive compounds

Bioactive compounds were quantified in green pepper fruits at harvest (day 0) and after 28 days of storage at 7°C. The chlorophyll a and b, and total carotenoids were extracted according to previously described methods (Knee, 1972; Xie et al., 2023) with some modifications. Approximately 0.20 g of fine freeze-dried powder for the three biological replicates (n = 3) were manually grounded in a mortar and pestle and mixed with 5 mL of acetone extract solution containing 0.1% BHT to prevent the pigment from oxidizing. Next, the mixed extraction was ultrasonically extracted for 15 min and then centrifuged at 10,000 *g* for 10 min at 4°C to obtain the supernatant. The sample was repeatedly extracted until the residue was colorless. Acetone solution containing 0.1% BHT was used for a constant volume of collected supernatants (25 mL) to the subsequent estimation of chlorophyll and total carotenoid contents. Based on the methods reported by Lichtenthaler and Wellburn (1983), the absorbance of the extracts was detected at 470, 645, and 662 nm by spectrophotometric absorbance (UV-1900i- UV- V IS Spectrophotometer, Shimadzu Corporation, Germany) to quantify the chlorophyll and total carotenoid contents, which were calculated from the equations: $C_a = 11.75A_{662} - 2.35A_{645}$, $C_b = 18.6lA_{645} - 3.96A_{662}$ and $C_{TC} = (1000A_{470} - 2.27C_a - 81.4C_b)/227$. C_a, C_b and C_{TC} indicate the content of chlorophyll a, chlorophyll b and total carotenoids (g kg^{-1} DW), respectively. The total chlorophyll content was calculated as the sum of the chlorophyll a and chlorophyll b contents (g kg^{-1} DW). A_{662}, A_{645}, A_{470} represent absorbances at 662 nm, 645 nm and 470 nm, respectively.

Ascorbic (AA) and dehydroascorbic (DHA) acids were measured in the freeze-dried powder for each replicate (n = 3), according to the methodology of Peña-Estévez et al. (2016) with slight modifications. Thus, 0.20 g of fine powder was homogenized manually in a mortar and pestle and mixed with 5 mL of a methanol: water (5:95) solution containing 0.1 mM citric acid, 0.05 mM ethylenediamine tetracetic acid disodium salt, and 4 mM NaF. Then, the extract was filtered through a four-layer cheesecloth and the pH was adjusted to 2.35-2.40 with 2 N HCl. The mixed extraction was centrifuged at 10,000 *g* for 15 min at 4°C and the supernatant was purified through a methanol-activated C18 cartridge (Sep-Pak cartridges C18, Waters, Dublin, Ireland) and filtered through a 0.45 mm PFTE filter. For DHA derivatization, 750 mL of extract was mixed with 250 mL of 7.7 M 1,2-phenylenediamine in an HPLC amber vial. The mixture was allowed to react for 37 min and then 20 mL were injected onto a Luna (250 mm × 4.6 mm, 5 mm particle size) C18 column (Phenomenex, Macclesfield, UK) with a C18 security guard (4.0 mm × 3.0 mm) cartridge system (Phenomenex) using an HPLC system (1200 Infinity series, Agilent Technologies, Waldbronn, Germany). The mobile phase was 50 mM KH_2PO_4 containing 5 mM hexadecyl trimethylammonium bromide and 5% methanol (pH 4.59) with an isocratic flow of 1 mL min^{-1}. Absorbance was recorded at 261 nm for AA (Rt = 9.4 min) and at 348 nm for DHA (Rt = 4.5 min), and both values were quantified by comparison with AA and DHA standard areas (Sigma-Aldrich, Darmstadt, Germany). Total vitamin C was defined as the sum of both AA and DHA content. Results (mean ± SE) were expressed as g kg^{-1} of dry weight (DW).

The quantification of total phenolic compounds (TPC) was carried out from the hydrophilic phase obtained in the total antioxidant activity extraction, as previously described by Dobón-Suárez et al. (2021b). Briefly, 5 g of green pepper fruits were homogenized with 10 mL of 50 mM phosphate buffer pH = 7.8 and 5 mL of ethyl acetate using a homogenizer (Ultraturrax, T18 basic, IKA, Berlin, Germany) for 30 s. The extracts were centrifuged at 10,000 *g* for 10 min at 4°C and the supernatant was used to quantify the total phenolic content in duplicate in each extract by using the Folin-Ciocalteu reagent (Sayyari et al., 2011). Results were expressed as g gallic acid equivalent (GAE) kg^{-1} of fresh weight (FW) and are the mean ± SE of three replicates.

2.3.2 Total antioxidant capacity: hydrophilic and lipophilic fractions

Total antioxidant capacity was determined in both hydrophilic and lipophilic fractions at harvest (day 0) and after 28 days of storage at 7°C after cutting the pepper fruit, removing its peduncle and seeds, and being frozen with liquid N_2 and stored at -20°C. As previously reported in green pepper fruit (Dobón-Suárez et al., 2021b), 5 g of frozen samples were extracted with 10 mL of 50 mM phosphate buffer pH = 7.8 and 5 mL of ethyl acetate. The extracts were homogenized using a homogenizer (Ultraturrax, T18 basic, IKA, Berlin, Germany) for 30 s and then, centrifugated at 10,000 *g* for 15 min at 4°C. Both upper and lower fractions were used to quantify the hydrophilic (H-TAA) and lipophilic (L-TAA) total antioxidant activity, respectively. Both antioxidant fractions were measured in duplicate using a reaction mixture in which $ABTS^+$ radicals are generated and monitored at 730 nm. Results were expressed as g of Trolox equivalent (TE) kg^{-1} FW and are the mean ± SE (n = 3).

2.3.3 Assays of antioxidant enzymes

The antioxidant activity of ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) enzymes were also determined in freeze-dried powder (flesh + skin tissues) maintained at -80°C both at harvest (day 0) and after 28 days of postharvest storage. APX, CAT and POD enzymes were extracted by homogenizing 0.20 g of fine powder with 5 mL of phosphate buffer 50 mM, pH 6.8, containing 1% (*w/v*) of polyvinylpyrrolidone (PVP) and ethylenediamine-tetracetic acid 1 mM. After centrifugation at 10,000 *g* for 15 min at 4°C, the supernatant was used to quantify each enzyme activity in duplicate, as reported elsewhere (García Pastor et al., 2021). Antioxidant enzyme activities were expressed as units of enzymatic activity (U min^{-1} g^{-1}) of dry weight (DW) with one enzymatic unit (U) being defined as a 0.01 decrease of ascorbate at 290 and 240 nm min^{-1} for APX and CAT, respectively, and a 0.01 increase of absorbance at 470 nm min^{-1} for POD. Results were the mean ± SE of three replicates (n = 3).

2.4 Gene expression analysis at harvest and after 28 days of storage

Plant RNA was extracted from 0.03 g of freeze-dried samples (flesh + skin tissues of green pepper fruit) to analyze the relative expression of targeted genes at harvest (day 0) and after 28 days of storage. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Dusseldorf, Germany) according to manufacturer's instructions, in which a DNase treatment was recommended on the eluted RNA by using Baseline-ZERO DNase (Epicentre/Lucigen USA). RNA quantification was carried out by the spectrophotometric absorbance using an Implen Nanophotometer® (IMPLEN, Munich, Germany). RNA extracts were maintained at -80°C. The single-strand cDNA was synthesized from 500 ng of total RNA using the PrimeScript RT Master Mix (Perfect Real Time) kit (Takara Bio, Japan) in a Mastercycler Nexus X2 (Eppendorf, Germany) PCR machine, following the manufacturer's protocol. This synthesis and the subsequent qPCR for the expression of the targeted genes were carried out by Genomic Centre of the Complutense University of Madrid (Madrid, Spain). Total RNA (15-40 ng per reaction) from three biological replicates and treatments was used as the template for the OneStep qPCR reactions.

Two housekeeping genes, ubiquitin (*CaUBI*) and actin (*CaACT*), were selected as reference genes in *Capsicum annuum* L (Li et al., 2020; Seo et al., 2020; Ge et al., 2020). The relative expression of five genes was evaluated as targeted genes: *L*-ascorbate peroxidase gene (*CaAPX*), catalase gene (*CaCAT*), peroxidase gene (*CaPOD*), phenylalanine ammonia-lyase gene (*CaPAL*) and dehydroascorbate reductase 2 gene (*CaDHAR2*). The gene-specific primers used are listed in Table 1. Gene sequences were obtained from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). The amplicon length for each primer was 123 pb for *CaUBI*, 130 pb for *CaACT*, 238 pb for *CaAPX*, 104 pb for *CaCAT*, 131 pb for *CaPOD*, 676 pb for *CaPAL* and 136 pb for *CaDHAR2*. The primers for qRT-PCR (Table 1), purchased from Merck (Sigma-Aldrich, Darmstadt, Germany). The reactions were prepared in duplicate on 384-well plates using PowerUp SYBR® Green Master Mix (Applied Biosystems, California) with the primers at a concentration of 300 nM in a reaction volume of 10 µL. The qPCR analysis was performed in a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, California) with an initial step at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Additionally, the quality of amplicons was controlled by a melt curve analysis step showing no side products. Data obtained from the qPCR was treated with the QuantStudio Reat-Time PCR software (Applied Biosystems, California). Relative targeted gene expression in treated green pepper fruit was normalized using the expression levels of the *CaUBI* and *CaACT* genes and was calculated regarding control fruit using three biological replicates (n = 3).

2.5 Statistical analysis

The experiment was conducted using a randomized design with three replicates (n = 3). Data are expressed as mean ± standard error (SE). Statistical comparisons of the means were performed using one-way analysis of variance (ANOVA) of SPSS software package v. 17.0 for Windows (SPSS, 2001, IBM Corporation, Armonk, NY, USA). The source of variation was treatments. Mean separation was analyzed using Tukey's HSD test to determine whether the differences among treatments were significant at $p < 0.05$. The heatmap analysis was conducted with Microsoft Excel® for Windows (Excel, 2016, Microsoft Corporation, Redmond, Washington, USA).

TABLE 1 Genetic details of primers for the reference and targeted genes[†].

Gene	Forward Primer Sequence (5′-3′)	Reverse Primer Sequence (5′-3′)	NCBI Reference
CaUBI	GGCATGTCTGGGACTTTTGC	AGACCCGTTCCTTGACAACC	AY486137.1
CaACT	ACCCTGTGCTTCTCACTGAAG	GCATAAAGAGACAACACCGCC	AY572427.1
CaAPX	ACTGGTGGACCGAATGGTTC	GTAACCGCCCTTCCTTTGGA	NM_001324587.1
CaCAT	TATCCGATCCCCGAGCAACT	CACAGTGAGACGAGAAGCG	AF227952.1
CaPOD	AACAGGGAAACCCGAATGGG	TTTGGTGCAGCCCTCTTCTC	FJ596178
CaPAL	ATGCTCTTAGAACGTCGCCC	AAGACGTATTCCCTGTCCACG	NM_001325423
CaDHAR2	GTTGATTTGAGCTTGGCCCC	TCTGGAAAGACTCACGCTCG	KJ950368.1

[†]Based on National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/).

3 Results

3.1 Foliar and irrigation SA delays quality losses during postharvest storage

SA applied by foliar spraying and irrigation significantly reduced ($p < 0.05$) weight loss in green pepper fruit, 'Herminio' cv., after 28 days of storage at 7°C compared to untreated fruits. Specifically, irrigation SA showed a 4.81% of weight loss than the 6.56% achieved in control treatment, with a 1.36-fold reduction, at 28 storage days (Figure 1A). SA treated green pepper fruits showed the highest firmness levels at harvest (≈ 5.80 N mm^{-1}) compared with control (4.42 N mm^{-1}), although no significant differences ($p \geq 0.05$) were observed between foliar and irrigation SA treatments (Figure 1B). The losses of firmness were significantly delayed ($p < 0.05$) by both SA treatments compared to untreated pepper fruits during postharvest storage. Thus, green pepper fruits treated with SA in preharvest had 1.19-fold in firmness values at 28 days of storage as compared with control (≈ 3.14 *vs.* 2.63 N mm^{-1}, respectively). The increase was similar for both foliar application and irrigation at the end of storage (Figure 1B). The highest values of color, expressed in terms of hue°, were observed in those pepper fruits treated with foliar and irrigation SA at harvest (≈ 130 hue°), although no significant differences ($p \geq 0.05$) were showed between

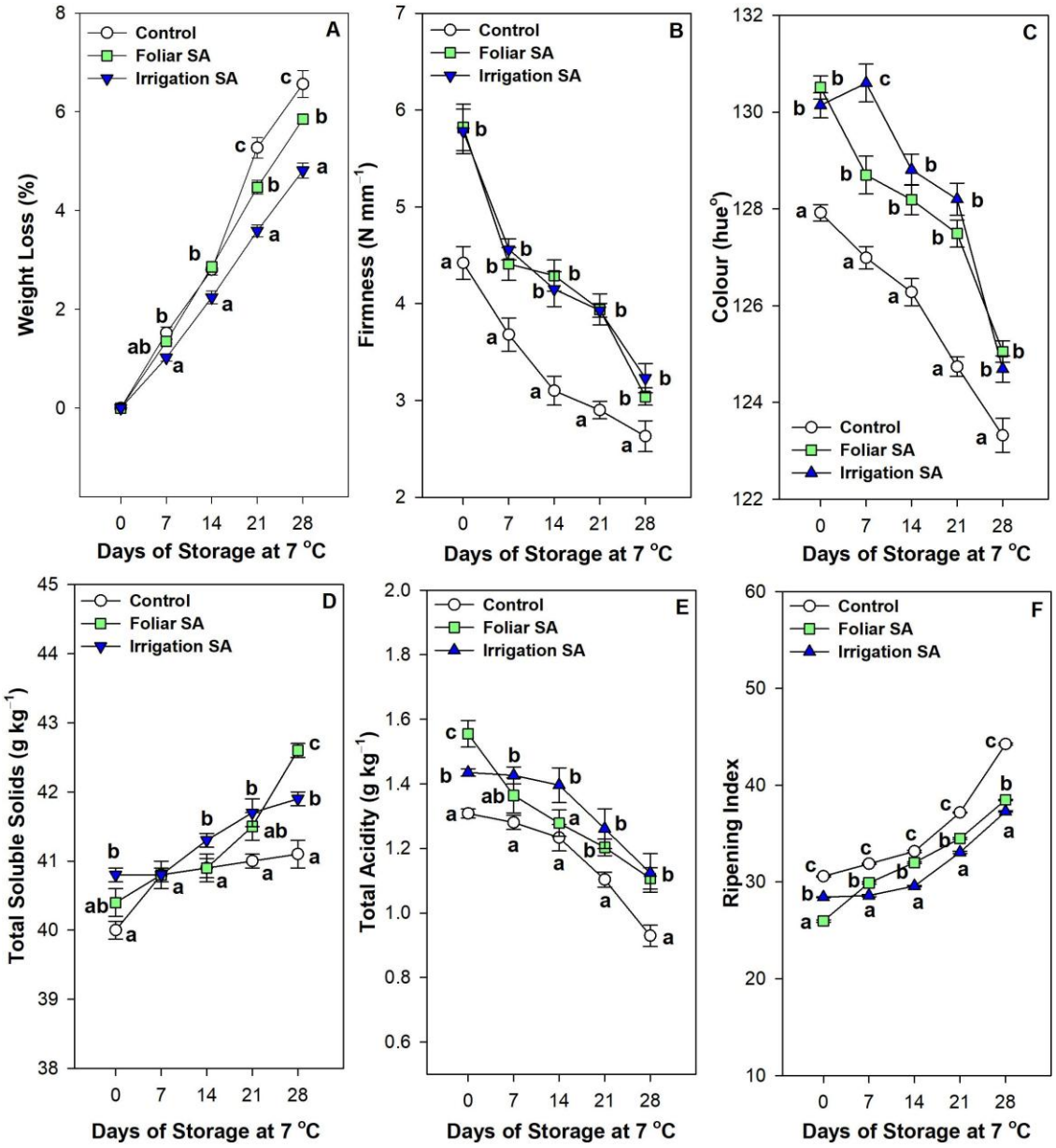


FIGURE 1
Effect of salicylic acid (SA) applied by foliar spraying [Foliar SA] and irrigation [Irrigation SA] on weight loss (%) (A), firmness (N mm^{-1}) (B), color (hue°) (C), total soluble solids (g kg^{-1}) (D), total acidity (g kg^{-1}) (E) and ripening index (F) of green pepper fruit during 28 days of storage at 7°C. Different lowercase letters indicate significant differences at p < 0.05 according to Tukey's HSD test among treatments for each sampling date and parameter tested.

both application methods (Figure 1C). On the other hand, color changes during postharvest storage were significantly delayed ($p < 0.05$) by both SA treatments and hue° values were higher in pepper fruits from SA treated plants (≈ 125 hue°) than in control (≈ 123 hue°) during the whole storage period, without significant differences between both SA treatments. It is well known, a higher hue° value showed a more intense dark green color in the pepper fruit (Figure 1C; Supplementary Figure 5).

Irrigation SA-treated green pepper fruits had a significantly higher ($p < 0.05$) content of total soluble solids (40.80 g kg^{-1}) than control fruits at harvest (40.00 g kg^{-1}), although those peppers treated with the foliar method did not show any significant differences ($p \geq 0.05$) compared to untreated fruits (Figure 1D). Total soluble solids increased during postharvest storage at 7°C in all treatments, although the highest values were reached in foliar SA-treated fruits at 28 days of storage followed by those pepper fruits treated by irrigation methods (42.60 and 41.90 g kg^{-1}, respectively). Thus, control pepper fruits showed the lowest values of total soluble solids of 41.10 g kg^{-1} at the end of postharvest storage at 7°C (Figure 1D). The highest levels of total acidity were observed in foliar SA treatment at harvest followed by irrigation SA treatment (1.56 and 1.44 g kg^{-1}, respectively; Figure 1E). Total acidity decreased during postharvest storage until it reached the lowest values (0.93 g kg^{-1}) in untreated fruits. However, those green pepper fruits treated with SA showed a content of total acidity around 1.12 g kg^{-1} at the end of storage, although no significant differences ($p \geq 0.05$) were appreciated between both treatments. Therefore, both SA applied by foliar spraying and irrigation delayed losses of total acidity in green pepper fruit stored at 7°C for 28 days (Figure 1E). Ripening index was significantly higher ($p < 0.05$) in control pepper fruits at harvest (30.58) and after 28 days of postharvest storage (44.24) than SA treated ones (Figure 1F). When comparing both application methods studied for the SA application in preharvest, foliar SA treatment significantly showed ($p < 0.05$) the lowest ripening index with a value of 25.98 at harvest while the irrigation SA method was the most effective treatment to delay the ripening during postharvest storage (ripening index of 37.28; Figure 1F).

3.2 Foliar and irrigation SA enhances the bioactive compound content and the antioxidant capacity at harvest and during postharvest storage

The chlorophyll a and b content, as well as the total chlorophyll content calculated as the sum of both individual forms, followed the same pattern to all treatments for both studied times (Figure 2; Supplementary Figure 2). Chlorophyll a was the major chlorophyll pigment in green pepper fruit, 'Herminio' cv., since its content was 2-fold higher than chlorophyll b. Both SA treatments significantly increased ($p < 0.05$) the content of both chlorophylls than control at harvest (≈ 5.58 *vs.* 4.27 g kg^{-1} for chlorophyll a and ≈ 2.71 *vs.* 2.02 g $kg^{-1}$for chlorophyll b) and after 28 days of storage (≈ 5.32 *vs.* 4.01 g kg^{-1} for chlorophyll a and 2.54 *vs.* 1.93 g kg^{-1} for chlorophyll b), although no significant differences were observed between foliar and irrigation SA treatments ($p \geq 0.05$) (Figures 2A–D; Supplementary Figures 2A, B).

Results showed that foliar and irrigation SA treatments reduced the rate of decline on chlorophyll a content (5.02 and 4.30%,

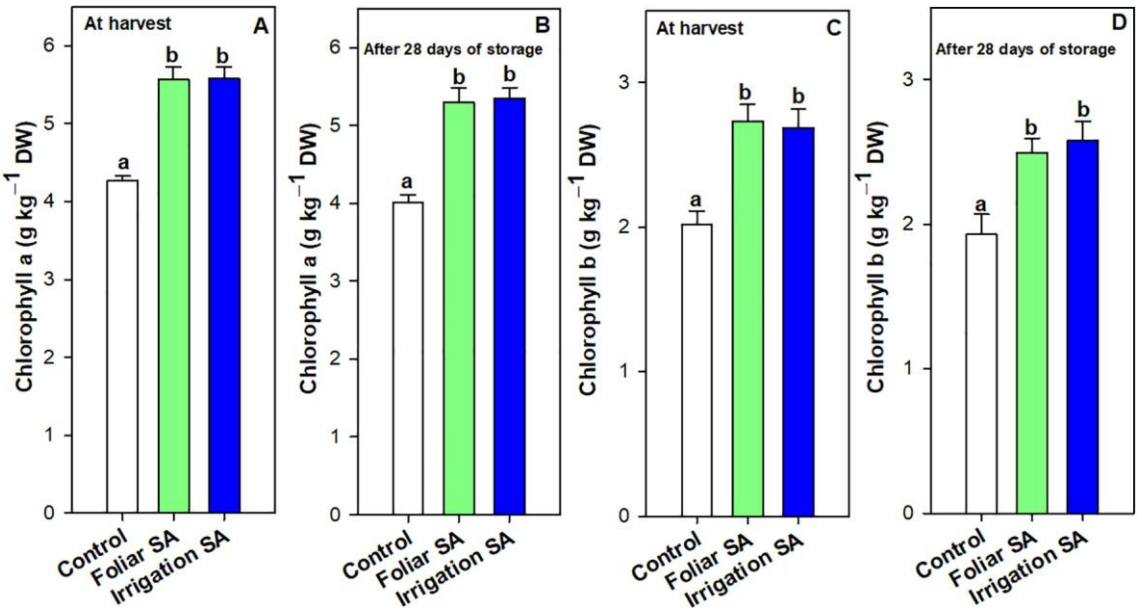


FIGURE 2
Effect of salicylic acid (SA) applied by foliar spraying [Foliar SA] and irrigation [Irrigation SA] on chlorophyll a and chlorophyll b content (g kg^{-1} DW) of green pepper fruit at harvest [A, C, respectively] and after 28 days of storage at 7°C [B, D, respectively]. Different lower case letters indicate significant differences at p < 0.05 according to Tukey's HSD test among treatments for each parameter tested at harvest or after 28 days of storage..

respectively) after 28 days of storage compared to control (6.08%). However, only those green pepper fruits irrigated with SA showed the lowest rate of decline on chlorophyll b content. Thus, both SA treatments enhanced the total chlorophyll content in green pepper fruits at harvest (Supplementary Figures 2A) and after 28 days of storage (Supplementary Figures 2B) in the same way ($p \geq 0.05$) than untreated fruits. This result could influence the maintenance of the intense dark green color of pepper fruit during postharvest, as it can be observed in Supplementary Figure 5. Total phenolic content was significantly enhanced ($p < 0.05$) at harvest and after 28 days of storage in those green pepper fruits treated with SA (0.71 and 0.91 g kg^{-1}, respectively) compared to control fruits (0.64 and 0.69 g kg^{-1}, respectively), although no significant differences ($p \geq 0.05$) were appreciated between both application methods (Figures 3A, D). The significant differences between SA treated and untreated pepper fruits were higher at the end of the storage, showing an increase of 1.32-fold on total phenolics caused by SA treatment (Figure 3D). Compared with the values at harvest, the increase rate of phenolics compounds (21.97%) was enhanced by both SA applications during postharvest storage than control fruits (7.24%).

Ascorbic acid (AA) and dehydroascorbic acid (DHA) content were enhanced with the SA preharvest application than control treatment both at harvest (≈ 0.30 *vs.* 0.16 g kg^{-1} for AA and 0.76 *vs.* 0.58 g kg^{-1} for DHA) and after 28 days of storage (≈ 0.19 *vs.* 0.10 g kg^{-1} for AA and

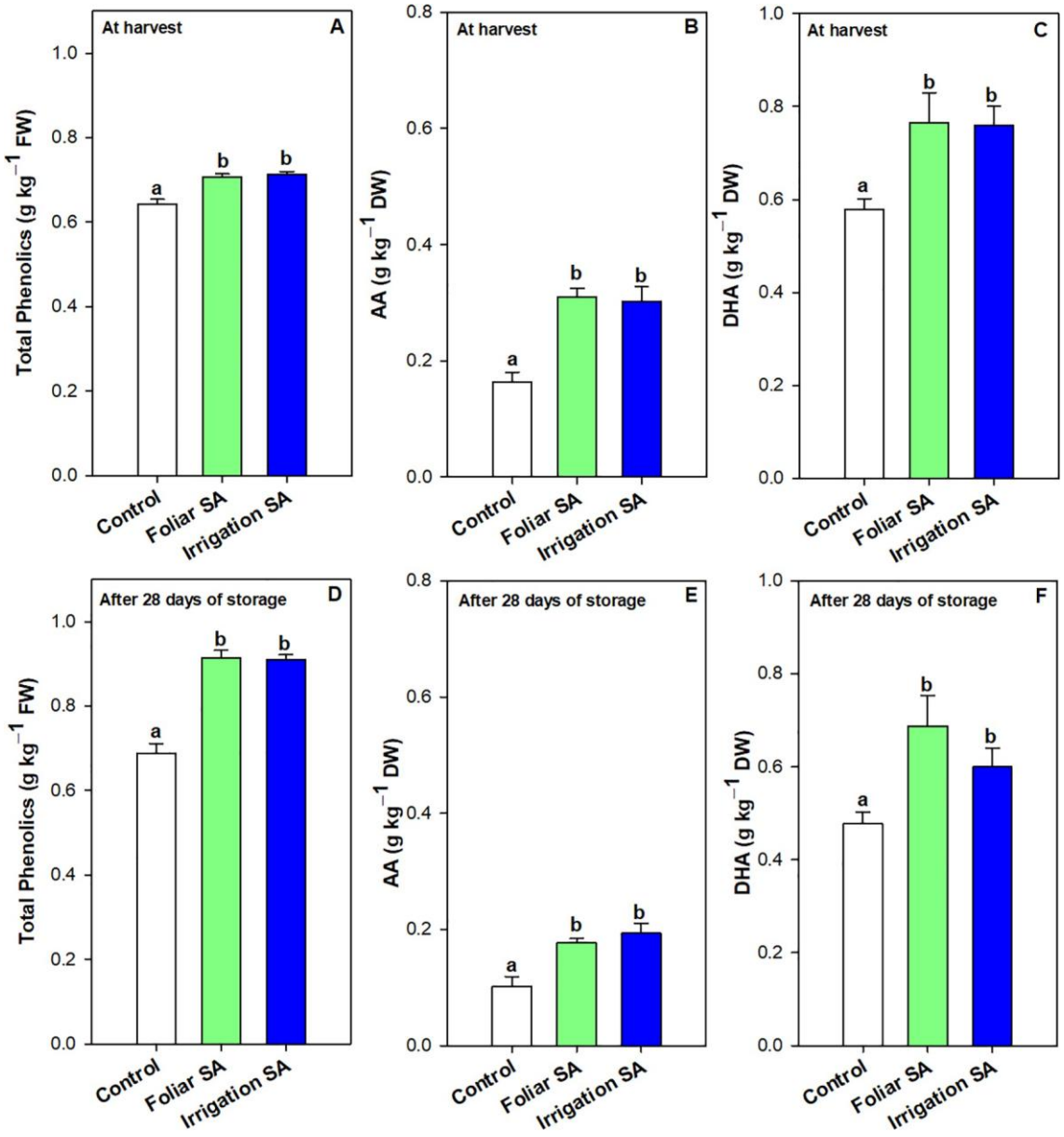


FIGURE 3
Effect of salicylic acid (SA) applied by foliar spraying [Foliar SA] and irrigation [Irrigation SA] on total phenolic (g kg^{-1} FW) and ascorbic acid (AA) and dehydroascorbic acid (DHA) content (g kg^{-1} DW) of green pepper fruit at harvest [A–C, respectively] and after 28 days of storage at 7°C [D–F, respectively]. Different lower case letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test among treatments for each parameter tested at harvest or after 28 days of storage.

0.65 *vs.* 0.48 g kg^{-1} for DHA). Nevertheless, no significant differences ($p \geq 0.05$) were observed between both SA application ways. The DHA content was 2-fold higher than AA content in green pepper fruit and both forms of vitamin C were degraded during storage for all treatments. However, SA treatment delayed this functional degradation by 44% (Figures 3B, C, E, F). In fact, the irrigation SA treatment showed a decrease rate on AA content of 36.66% than control green pepper fruits (37.50%) from harvest until 28 days of storage at 7°C. Nevertheless, those pepper fruits harvested from plants treated with SA by foliar spraying presented the lowest percentage of decrement on DHA content (9.21%) compared with the control ones (17.24%). Total vitamin C, expressed as the sum of both AA and DHA forms, was also significantly enhanced ($p < 0.05$) by the two SA treatments studied in the same proportion (Supplementary Figures 3A, B). Hydrophilic (H-TAA) and lipophilic (L-TAA) total antioxidant activity was significantly improved ($p < 0.05$) with SA preharvest treatments compared to control both at harvest (≈ 1.38 *vs.* 1.10 g kg^{-1} for H-TAA and 0.51 *vs.* 0.36 g kg^{-1} for L-TAA) and after 28 days of storage (≈ 1.55 *vs.* 1.23 g kg^{-1} for H-TAA and 0.59 *vs.* 0.50 g kg^{-1} for L-TAA) (Figures 4A–D). Specifically, foliar SA application was the most effective treatment stimulating the H-TAA at harvest compared to other treatments (Figure 4A). However, these significant differences ($p < 0.05$) between both application methods of SA were not observed after 28 storage days (Figure 4B). The increment of H-TAA from harvest to the end of the postharvest period was highest in those green pepper fruits irrigated with SA compared to control treatment (11.92 *vs.* 9.83%). Similarly, no significant differences ($p \geq 0.05$) were appreciated between foliar and irrigation SA methods on L-TAA (Figures 4C, D). Finally, carotenoids content did not show any significant differences ($p \geq 0.05$) among treatments (Supplementary Figures 4A, B).

3.3 Foliar and irrigation SA modulates antioxidant enzyme activities and the relative antioxidant systems-based gene expression at harvest and during postharvest storage

Foliar and irrigation SA treatments significantly stimulated ($p < 0.05$) the APX activity compared to control at harvest (≈ 354 and 372 U min^{-1} g^{-1}, respectively, *vs.* 279 U min^{-1} g^{-1}) and after 28 days of storage (≈ 468 and 501 U min^{-1} g^{-1}, respectively, *vs.* 327 U min^{-1} g^{-1}), although no significant differences ($p \geq 0.05$) were appreciated between both application methods (Figures 5A, D). This effect could be related to the upregulation of the relative *CaAPX* gene expression detected by foliar and irrigation SA treatments at harvest (relative expression of 2.70 and 3.57, respectively), disappearing this effect after 28 days of storage at 7°C (Figures 6A, F).

CAT activity was only significantly stimulated ($p < 0.05$) in those green pepper fruits treated with SA by irrigation at harvest (≈ 286 U min^{-1} g^{-1}) and no significant differences ($p \geq 0.05$) were observed between foliar SA and control treatments (≈ 271 and 247 U min^{-1} g^{-1}, respectively) (Figure 5B). Nevertheless, this difference was accentuated during postharvest storage and both SA preharvest treatments significantly increased ($p < 0.05$) the activity of CAT antioxidant enzyme than control fruits in the same way (≈ 116 *vs.* 48 U min^{-1} g^{-1}) (Figure 5E). When the effect of SA was studied on the relative *CaCAT* gene expression, no significant differences ($p \geq 0.05$) were observed on the modulation of this targeted gene either at harvest nor during postharvest storage (Figures 6B, G). Foliar and irrigation SA treatment significantly activated ($p < 0.05$) the POD enzyme at harvest reaching values of 575.46 and 741.02 U min^{-1} g^{-1}, respectively, compared with control (356.75 U min^{-1} g^{-1})

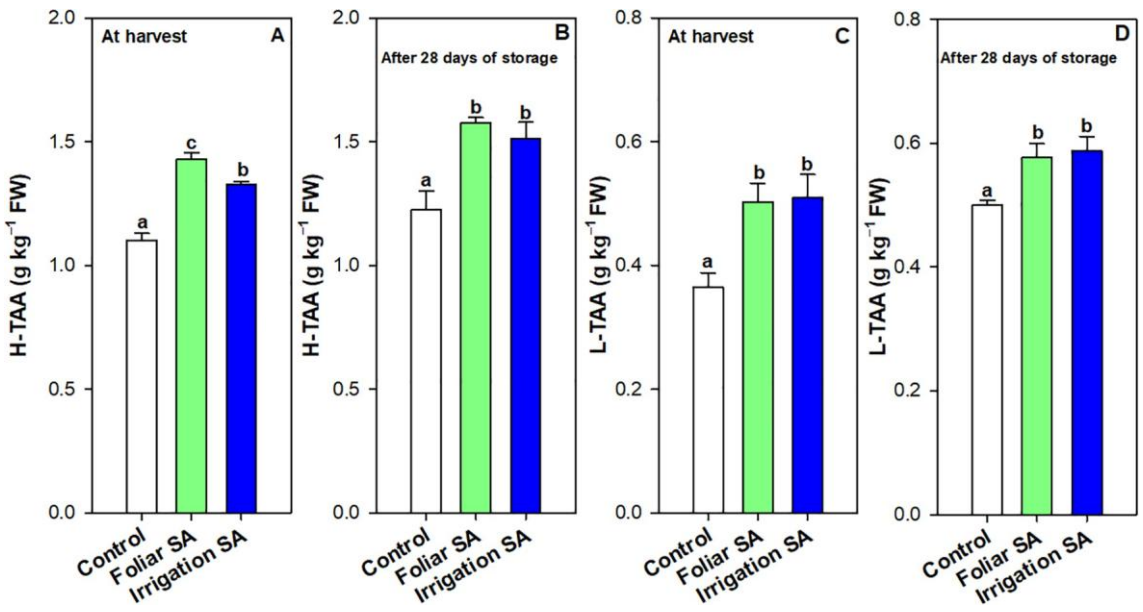


FIGURE 4
Effect of salicylic acid (SA) applied by foliar spraying [Foliar SA] and irrigation [Irrigation SA] on hydrophilic total antioxidant activity (H-TAA) and lipophilic total antioxidant activity (L-TAA) (g kg^{-1} FW) of green pepper fruit at harvest [A, C, respectively] and after 28 days of storage at 7°C [B, D, respectively]. Different lower case letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test among treatments for each parameter tested at harvest or after 28 days of storage.

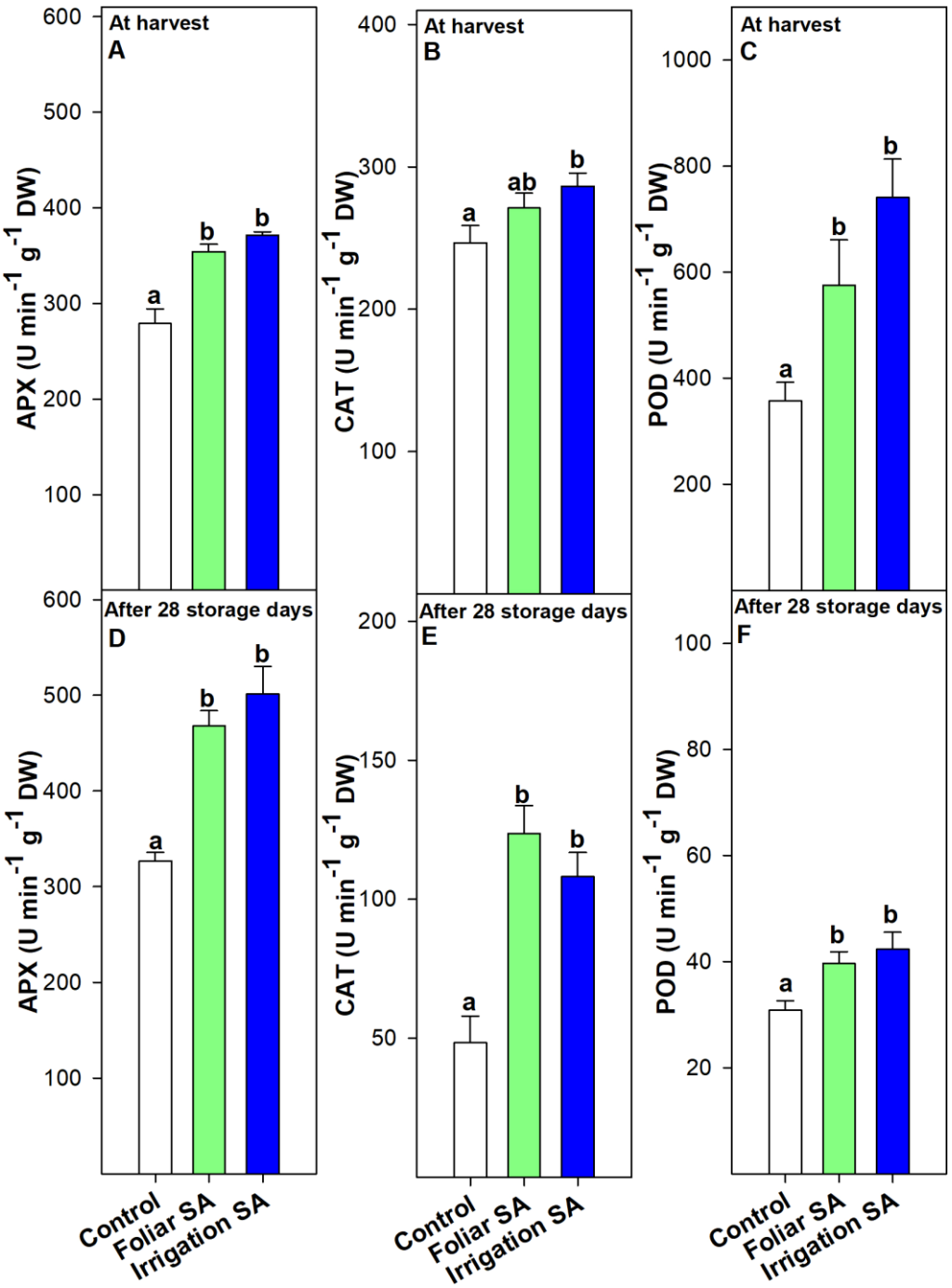


FIGURE 5
Effect of salicylic acid (SA) applied by foliar spraying [Foliar SA] and irrigation [Irrigation SA] on ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) activities (U min^{-1} g^{-1} DW) of green pepper fruit at harvest [A–C, respectively] and after 28 days of storage at 7°C [D–F, respectively]. Different lower case letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test among treatments for each parameter tested at harvest or after 28 days of storage.

(Figure 5C). This effect was also observed after 28 days of storage since those foliar and irrigation SA-treated green pepper fruits exhibited values of POD of 39.65 and 42.34 U min^{-1} g^{-1}, respectively, compared with untreated fruits (30.85 U min^{-1} g^{-1}) (Figure 5F). In this sense, SA applied by foliar spraying and irrigation significantly upregulated ($p < 0.05$) the relative *CaPOD* gene expression at harvest by 1.94 and 1.50, respectively (Figure 6C). Thus, the highest effect was observed for the preharvest foliar treatment, and it was maintained after 28 storage days only in those green pepper fruits treated with foliar SA with a relative expression of 1.34 (Figure 6H). The relative *CaPAL* gene expression was only upregulated at harvest with a value of 1.73 after the foliar SA application, while both foliar and irrigation SA treatments upregulated the relative *CaDHAR2* gene expression at

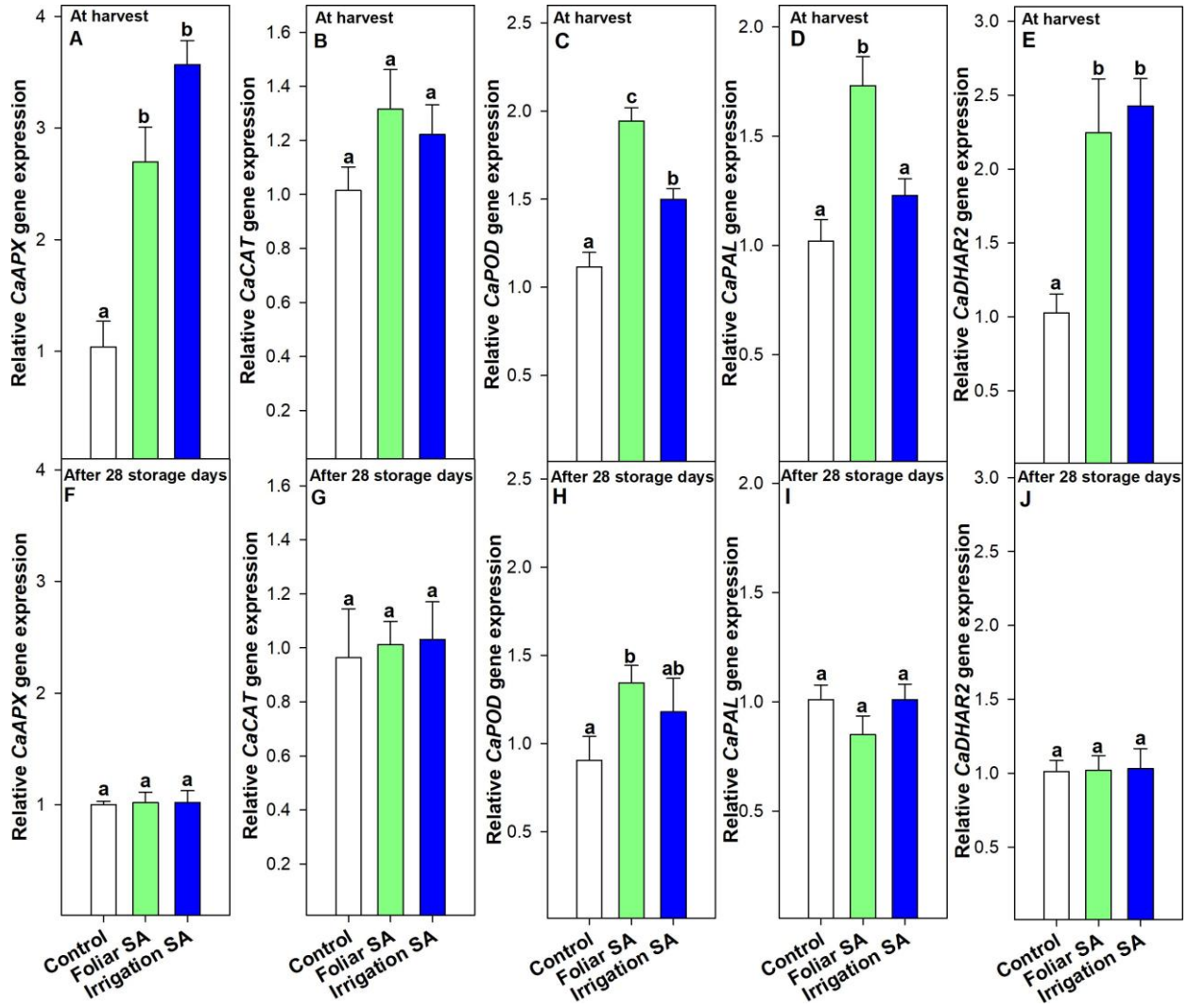


FIGURE 6
Effect of salicylic acid (SA) applied by foliar spraying [Foliar SA] and irrigation [Irrigation SA] on the relative *CaAPX*, *CaCAT*, *CaPOD*, *CaPAL*, and *CaDHAR2* genes expression of green pepper fruit at harvest [A–E, respectively] and after 28 days of storage at 7°C [F–J, respectively]. Different lower case letters indicate significant differences at $p < 0.05$ according to Tukey's HSD test among treatments for each parameter tested at harvest or after 28 days of storage.

harvest in green pepper fruits (relative expression of 2.25 and 2.43, respectively) (Figure 6E). However, SA did not modulate ($p \geq 0.05$) the expression of these two targeted genes after 28 days of storage at 7°C (Figures 6I, J).

4 Discussion

Bell pepper (*Capsicum annuum* L.) is a model for studying the ripening and senescence processes of non-climacteric fleshy fruit, during which numerous physiological changes occur, the most noticeable being the color change caused by chlorophyll degradation and the synthesis of new pigments such as carotenoids (Chaki et al., 2015; Ma et al., 2021). Nevertheless, numerous changes occur during the ripening of bell pepper fruit, including alterations in flavor, aroma, and texture, which are regulated by both external and internal factors (Palma et al., 2011; Klie et al., 2014). For instance, phenological stages and harvest dates are two key factors that significantly influence some nutritional and functional traits of green pepper fruit, as it was unveiled by Dobón-Suárez et al. (2021b). Fruit ripening and senescence involve complex and highly coordinated molecular and biochemical processes that include ripening-associated genes, transcription factors, enzymes, repressors, signaling molecules, and metabolic pathways in both climacteric and non-climacteric fruits (Cherian et al., 2014; Fuentes et al., 2019). These processes influence fruit quality on one hand and postharvest losses on the other.

As fruit ripens or undergoes senescence, it becomes more susceptible to fungal pathogens (Alkan and Fortes, 2015), leading to green pepper fruit deterioration. Cold storage is widely adopted to prevent premature ripening and senescence since this fruit is highly perishable at ambient temperatures. However, green bell pepper is susceptible to chilling injury (CI) at temperatures below 7°C (Lim et al., 2007). Consequently, common strategies to slow

down senescence and preserve fruit quality include both pre- and postharvest management practices and technological tools. Many factors, however, such as various plant hormones and biotic and abiotic stresses are known to influence bell pepper fruit ripening (Sun et al., 2015; Cheng et al., 2016). Recently, numerous studies have shown that salicylic acid (SA) preharvest treatment applied by foliar spraying influences the ripening and senescence of fruit species, such as sweet cherry (Giménez et al., 2014), table grape (Champa et al., 2014; Gomes et al., 2021), jujube fruit (Shanbehpour et al., 2020), pomegranate fruit (García-Pastor et al., 2020), lemon (Serna-Escolano et al., 2021) and green pepper (Dobón-Suárez et al., 2021a), showing activation of the antioxidant system and a delay in fruit senescence, as it was highlighted by Chen S. et al. (2023). The present study showed that SA preharvest treatment applied by both foliar spraying and irrigation enhanced the fruit quality of green pepper fruit resulting in a slowdown of the ripening and senescence processes during postharvest storage at 7°C (Supplementary Figure 5). As it can be observed in Figure 7, this fact could be modulated throughout the stimulation of the antioxidant system accompanied by an increase on the content of secondary metabolites and the activity of antioxidant enzymes that is mediated by the upregulation of their codifying gene expressions.

Fruit quality is related to the ripening and senescence of fruit during storage, which is involved in fruit softening, weight loss, color change, ethylene production and respiration rate (Gao et al., 2020). Salicylic acid (SA) regulates growth in plants, playing an efficient role in growth and development, flowering and fruit ripening, and photosynthesis (Natasha et al., 2020). Many studies have also indicated that SA and its derivatives play an important role in regulating the physiological metabolism of fruit to achieve optimal fruit quality and to maintain it during postharvest (Valverde et al., 2015; Giménez et al., 2016; Hanif et al., 2020; Amiri et al., 2021; Chen S. et al., 2023). Over the past ten years, biochemical data have also indicated that the bell pepper fruit ripening process is influenced by the metabolism of reactive oxygen species (ROS) and nitrogen oxygen species (NOS) (Palma et al., 2015; Corpas and Palma, 2018; Corpas et al., 2018), which reflects the profound biochemical and molecular changes taking place during ripening (Camejo et al., 2015). The accumulation of the reactive oxygen species (ROS) including hydroxyl radical, superoxide and hydrogen peroxide during fruit ripening can cause oxidative damage leading to membrane lipid breakdown and loss of cellular turgor, triggering cell death and damage in fruit tissue (Champa et al., 2014). The beneficial effect of SA has been recently related to its capacity to improve photosynthesis and

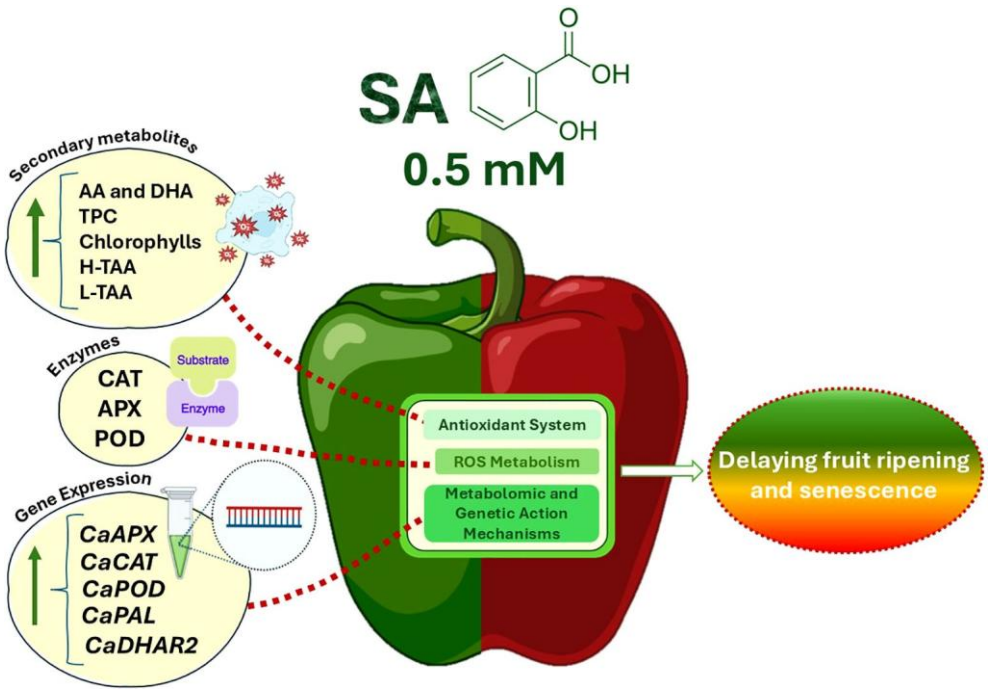


FIGURE 7
A hypothetical working model illustrates the role of preharvest 0.5 mM SA treatment delaying green pepper fruit ripening and senescence during storage at 7°C using a regulatory network model for some targeted metabolites and genes. Green arrows represent stimulation of metabolites or functional parameters and an up-regulation of the gene expression. SA preharvest treatment at 0.5 mM increases secondary metabolites (ascorbic acid and dehydroascorbic acid, total phenolic content, carotenoids and chlorophylls) and stimulates both the hydrophilic and lipophilic total antioxidant activity, as well as antioxidant enzymes (catalase, ascorbate peroxidase and peroxidase), reducing probably ROS accumulation, thereby delaying fruit ripening and senescence. This metabolomic modulation has been mediated by increasing the expression of *CaAPX*, *CaCAT*, *CaPOD*, *CaPAL* and *CaDHAR2* genes by SA [For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article]. Abbreviations: SA (salicylic acid), AA (ascorbic acid), DHA (dehydroascorbic acid), TPC (total phenolic content), H-TAA (hydrophilic-total antioxidant activity), L-TAA (lipophilic-total antioxidant activity), CAT (catalase), APX (ascorbate peroxidase), POD (peroxidase), *CaAPX* [*L*-ascorbate peroxidase (APX) gene], *CaCAT* [catalase (CAT) gene], *CaPOD* [peroxidase (POD) gene], *CaPAL* [phenylalanine ammonia-lyase (PAL) gene] and *CaDHAR2* [dehydroascorbate reductase 2 gene], ROS (Reactive Oxygen Species).

the activity of antioxidant enzymes, leading to maintaining the balance between the production and elimination of ROS (Batista et al., 2019). In the present study, results show a significant effect of both foliar and irrigation SA on activating the APX and POD by upregulating the relative *CaAPX* and *CaPOD* gene expression, respectively, at harvest (Figures 5A, C, 6A, C). Results showed that SA treatment applied by foliar spraying was more effective on increasing *CaPOD* gene expression than SA, irrigation treatment, although this effect was not reflected in a higher POD activity in those foliar SA-treated peppers than the irrigated ones (Figure 5C). Nevertheless, SA treatment applied by irrigation was the most effective in stimulating CAT activity at harvest (Figure 5B), although this effect was not mediated through the upregulation of the relative *CaCAT* gene expression (Figure 6B). The effect of SA treatment on activating the antioxidant enzymes activity at harvest has been demonstrated in the present study and results show that this plant growth regulator could have a potential effect modulating the antioxidant enzymes-gene expression. These results propose that the enzymatic antioxidants can offset the damaging effects of ROS metabolism on cell structure (Gomes et al., 2021; Serna-Escolano et al., 2021), which results in a slowdown in ripening process and an increase on quality traits at harvest (Figure 1). In fact, the ripening index (RI) was deeply correlated (negatively, Pearson) with firmness, color, TSS, TA, chlorophylls, phenolics, antioxidant capacity and antioxidant enzymatic system, except with the carotenoids content which also showed a similar correlation pattern (Figure 8A). Some studies corroborate these findings regarding the activation of enzymatic systems by the application of SA and its derivatives [Methyl salicylate (MeSA) and Acetylsalicylic acid (ASA)], leading to the high antioxidant activity in sweet cherry (Giménez et al., 2014; Zhang et al., 2011), pomegranate fruit (García-Pastor et al., 2020), citrus (Zhu et al., 2016), papaya (Hanif et al., 2020) and banana (Xu et al., 2019).

Phenolic compounds have antioxidant activity, which not only scavenge free radicals and reduce oxidative damage to fruits, but also contribute to fruit flavor and quality maintenance. Their biosynthesis is involved in phenylpropanoid pathway of plant secondary metabolites. Phenylalanine ammonia-lyase (PAL) is the first step to catalyze the conversion of phenylalanine to cinnamic acid which is further converted to phenolic acid (Oraei et al., 2019). The enzyme activity of PAL could be enhanced by SA (Zhou et al., 2018), increasing phenolic content in oranges (Amiri et al., 2021) and table grapes (Blanch et al., 2020). In the present study, SA treatment applied by foliar spraying upregulated the relative *CaPAL* gene expression at harvest (Figure 6D), which led to a significant increase in the total phenolic content (Figure 3A). However, the irrigation-SA treatment also showed an enhancement in phenolic compounds as compared with control (Figure 3A), although these findings were not corroborated by the analysis of the *CaPAL* targeted gene (Figure 6D). In this sense, previous transcriptomic and metabolic profiling of watermelon uncovered the role of SA pretreatment up-regulating the expression of flavonoid biosynthesis genes, thus increasing the total flavonoid content (Liu et al., 2023). Furthermore, transcriptome analysis and exogenous SA treatment demonstrated that SA (NPR1) is involved in the positive regulation of flavonoid biosynthesis (Wu et al., 2021).

On the other hand, SA treatment increased ascorbic acid content which is an essential plant antioxidant vital for defense against oxidative stress, leading to alleviating damages induced by ROS accumulation. In this sense, the content of both AA and DHA was quantified at harvest (Figures 3B, C), as well as the relative *CaDHAR2* gene expression which is involved in the biosynthesis of the cytoplasmic enzyme DHAR2, namely dehydroascorbate reductase, implicated on the catalyzation of the glutathione (GSH)-dependent reduction of dehydroascorbate and had a direct role in regenerating ascorbic acid (Figure 6E). Results suggest that both SA applications (foliar and irrigation) effectively enhanced the AA and DHA content, and consequently, the total vitamin C content, throughout the upregulation of the *CaDHAR2* gene expression at harvest, although no significant differences were appreciated between both methods (Figures 6E, 3B, C; Supplementary Figure 3A). The form of AA can neutralize radicals to retard oxidative reactions triggering the ripening process in plant tissues (Huang et al., 2017; Sangprayoon et al., 2019). Other studies corroborate these findings hypothesizing that the application of SA could enhance the antioxidant ability by inhibiting the ascorbic acid oxidase (AAO) enzyme, which would affect the ascorbate-glutathione cycle positively, leading to the high content of AA in orange fruit (Amiri et al., 2021; Hanif et al., 2020; Wei et al., 2011). Other reports indicated that SA, MeSA, or ASA treatments could lead to high content of DHA in fruit, which is oxidized from AA by the AAO enzyme in pomegranate fruit and table grapes (García-Pastor et al., 2020; Hazarika and Marak, 2022).

Bell pepper ripening is characterized by important visual and metabolic changes regulated by transcription factors, with color changes caused by chlorophyll degradation and biosynthesis of new pigments such as carotenoids (Chaki et al., 2015). In the present study, both SA treatments applied by foliar spraying and irrigation significantly influenced the content of these pigments at harvest (Figures 2A, C; Supplementary Figure 2A), the highest levels of chlorophylls a and b were recorded in those green pepper fruits harvested from 0.5 mM SA-treated plants (Supplementary Figure 5). Multiple biological functions have been reported for chlorophylls as lipophilic-nature pigments. Strictly related to their antioxidant capabilities, two main mechanisms can be described: Their direct free-radical-scavenging activity and the metabolic activation of detoxification pathways, as was reported by Pérez-Gálvez et al. (2020). Accordingly, exogenous SA increased chlorophyll content under drought and salinity conditions (Tang et al., 2017; Ghassemi-Golezani et al., 2018). Some studies showed that there was a positive correlation between TPC and TAA (Amiri et al., 2021; Wei et al., 2011), considering also the contribution of ascorbic acid. The present study shows the positive correlation observed between total phenolics and AA and DHA content (Figure 8A). In this sense, the H-TAA (Figure 4A) was significantly improved by SA preharvest treatments at harvest, although the highest stimulation was achieved with foliar spraying. Since no significant differences were recorded between the two application methodologies on total phenolics or vitamin C content (Figures 3A–C; Supplementary Figure 3A), probably other hydrophilic antioxidant compounds, such as glutathione (GSH), could be highly influenced by foliar SA treatment. As opposed to H-TAA, the analyses of the L-TAA at harvest showed that this

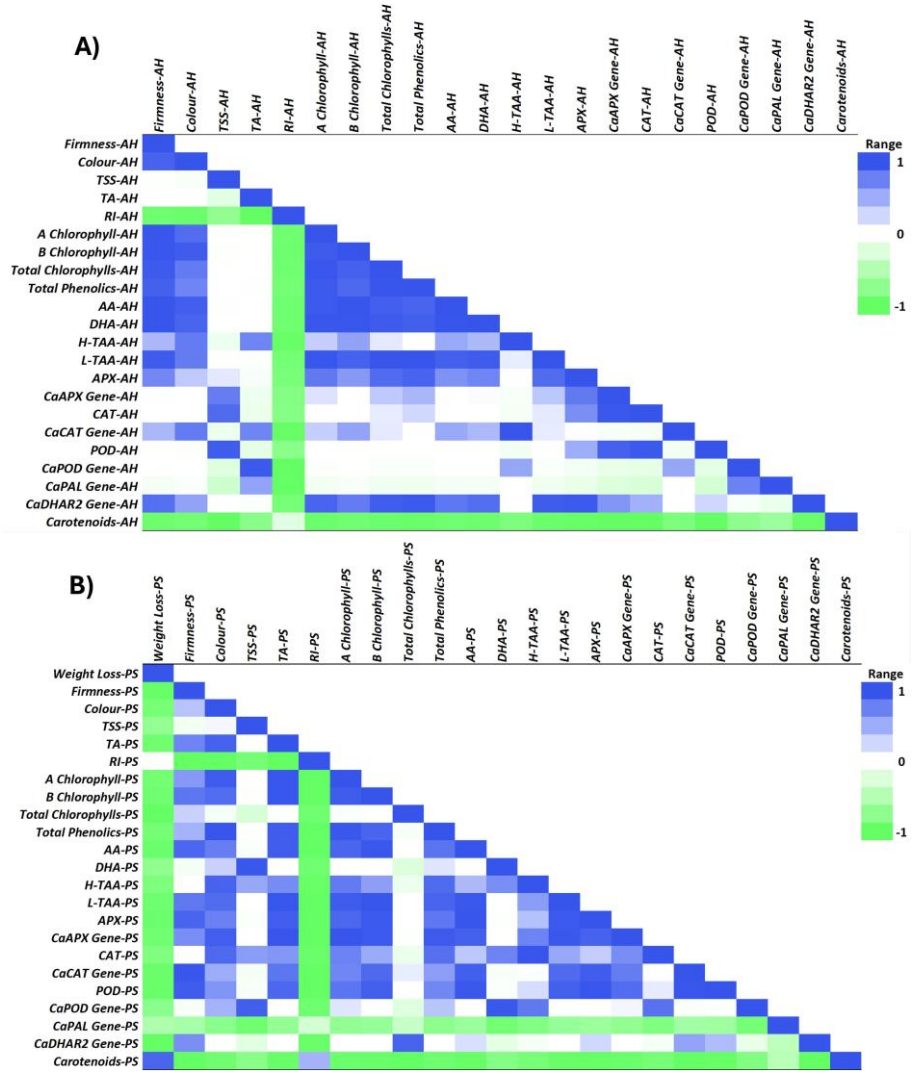


FIGURE 8
Pearson correlation heatmap of green pepper fruit qualities, metabolites and antioxidant system and targeted genes at harvest (AH) (A) and after 28 days of postharvest storage (PS) at 7°C (B). Fruit quality includes weight loss, firmness, color, total soluble solids (TSS), total acidity (TA) and ripening index (RI). Metabolites and antioxidant systems include chlorophyll a, chlorophyll b, total chlorophylls, total phenolics, ascorbic acid (AA), dehydroascorbic acid (DHA), hydrophilic-total antioxidant activity (H-TAA), lipophilic-total antioxidant activity (L-TAA), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and carotenoids. Targeted genes based on the antioxidant enzymatic system including the relative expression of *CaAPX* gene, *CaCAT* gene, *CaPOD* gene, *CaPAL* gene and *CaDHAR2* gene. The range runs from -1 = green to 1 = blue, which represents the correlation coefficient between the fruit qualities and metabolomic and genetic parameters of the antioxidant system run from -1 to 1.

parameter was stimulated by both SA applications, although no significant differences were observed between both methodologies (Figure 4C). This functional increment with the application of SA at 0.5 mM is corroborated by a previous study in 'Lamuyo' green pepper fruit and could be related to the enhancement chlorophyll a and b content by the application of SA that has been demonstrated in the present study (Figures 2A, C; Supplementary Figures 2A, 5). Preharvest applications of SA have been reported to increase the antioxidant capacities of grapes (Champa et al., 2014; Gomes et al., 2021), Indian jujube (Shanbehpour et al., 2020), lemon (Serna-Escolano et al., 2021), pomegranate fruit (García-Pastor et al., 2020), and sweet cherry (Giménez et al., 2014). Accordingly, SA preharvest treatment has a beneficial impact on the quality of green pepper fruit, 'Herminio' cv., at harvest which results in a slowdown in the ripening process (Figure 1; Supplementary Figure 5). At harvest, quality traits such as color and firmness were positively correlated and both showed a deep correlation (positively, Pearson) with the antioxidant compounds and the activity of the antioxidant enzymes, although no correlation was observed with the targeted genes (Figure 8A). Specifically, the relative expression of *CaDHAR2* gene was highly correlated with firmness, color, chlorophylls, phenolics, AA, DHA, L-TAA, APX and the expression of *CaAPX* gene (Figure 8A; positively, Pearson). The action mechanism proposed in the present study is the following: *1)* SA induces the synthesis of secondary metabolites and enhances the antioxidant systems by stimulating the phenylpropanoid biosynthesis pathway, and *2)* SA

activates the antioxidant enzymes, acting on the modulation of the relative antioxidant systems-based genes expression and regulating the balance between the antioxidant system and ROS metabolism, contributing to antioxidant metabolism insights (Figure 7).

The senescence delay triggered by the foliar and irrigation application of 0.5 mM SA extended the shelf-life of 'Lamuyo' green pepper fruit since the preharvest treatment reduced fruit quality losses (Figure 1; Supplementary Figure 5). The contribution to extending shelf-life and delaying fruit senescence for 28 days at 7°C could be attributed to the enhancement of both total phenolics (Figure 3D) and antioxidant capacity from hydrophilic and lipophilic phases (Figures 4B, D) with both SA applications which might reduce the oxidative damage (Figure 7). Regarding the functional increment related to phenolics content after harvest, results showed the highest increase rate in those green pepper harvested from SA-treated plants. However, the highest increment on H-TAA after 28 storage days was observed in those pepper fruits irrigated with SA, leading to a higher stimulating effect of the antioxidant system in those pepper fruits treated with SA. Similar findings applying salicylates in preharvest have been obtained in sweet cherry (Valverde et al., 2015; Zhang et al., 2011), pomegranate fruit (García-Pastor et al., 2020), lemon (Serna-Escolano et al., 2021) and plum (Davarynejad et al., 2015). Other contributing aspects to the delay in senescence could be related to the higher levels of two forms of ascorbic acids (AA and DHA; Figures 3E, F) quantified in those green pepper fruits treated with foliar and irrigation SA. Moreover, foliar and irrigation SA treatments lead to higher increase rate on DHA and AA content, respectively, compared to control from harvest until 28 days of storage. In this sense, Ruoyi et al. (2005) indicated that this effect is mediated by the inactivation of the AAO enzymatic activity, which might have caused a delay in the senescence of peppers in the later stages of the storage period. The inhibition of AAO was also advantageous in keeping vitamin C and for anti-browning in sweet pepper (Rao et al., 2011). Recently, the coordinated regulatory network of ncRNAs involved in the ripening of bell pepper fruit has been analyzed (Zuo et al., 2019), providing a theoretical basis for deciphering novel mechanisms of fruit ripening in future studies. In the present study, the increase in secondary metabolites, such as phenolics or vitamin C, mediated by SA was not correlated with the upregulation of the relative *Ca*PAL gene expression after 28 days of storage at 7°C, as it can be observed in Figure 8B. Similarly, both SA applications did not modulate the response of *CaDHAR2* gene after 28 storage days (Figure 6J). This finding could be related to the fact that SA was applied in preharvest upregulating both genes at harvest, and probably during the crop cycle, although the modulation effect is lost during postharvest storage.

On the other hand, a delay in senescence is commonly associated with a delay in color changes and this effect was observed in the present study from a metabolomic approach, where chlorophylls a and b content were significantly higher in both foliar and irrigation SA-treated pepper fruits than control (Figures 2B, D; Supplementary Figure 2B), as it was previously discussed. In addition, the rate of decline on chlorophyll a and b from harvest date until the end of postharvest storage was lower in both SA treatments, especially with the irrigation method, compared to control. This result shows a preserving effect on green color maintenance after postharvest storage associated with the SA treatments, as it can be observed in Supplementary Figure 5. After 28 days of storage at 7°C, a similar effect was observed on the relative expression of *CaAPX* and *CaCAT* genes which was no upregulated by SA treatments (Figures 6F, G), although an enzymatic activity stimulation was observed compared to untreated pepper fruit because of both foliar and irrigation SA treatments (Figures 5D, E). Nevertheless, SA treatment applied by foliar spraying positively modulated the relative expression of *CaPOD* gene after 28 storage days and, therefore, stimulated the activity of the POD enzyme (Figures 5F, 6H). Induction POD activity, which is an important oxyradical detoxification enzyme in plant tissues, has been demonstrated in the present study and may generally facilitate conditions that can delay senescence in green peppers fruits (Zhou et al., 2011). Furthermore, the only relative expression of those antioxidant enzyme activities-related genes that was upregulated after 28 days of storage was for the POD enzyme (Figure 6H). These results are in accordance with those reported by Rao et al. (2011) in which 1- and 2-mM SA postharvest treatments extended the shelf-life of sweet pepper fruit (*Capsicum annum* L., cv. Indra). Senescence is associated with the defensive system, including antioxidant enzymes, such as POD. In the present study, POD was highly correlated (positively, Pearson) with firmness, color, TA, chlorophylls, phenolics and AA content, L-TAA, APX and the relative expression of both *CaAPX* and *CaCAT* genes (Figure 8B). An efficient antioxidant system can postpone the senescence process even though antioxidative activity in fruits decreases with ageing (Zheng et al., 2007). Antioxidants can delay, retard or prevent oxidation processes by reacting with free radicals, chelating metals and acting as oxygen scavengers, a triplet as well as singlet form and transferring hydrogen atoms to the free radical structure. Similar results were recently reported where the incorporation of SA foliar spraying and caraway oil coating resulted in the highest antioxidant enzyme activity and the lowest chilling injury in treated pepper fruits stored under cold conditions (Hanaei et al., 2022). The results of the present study reinforce the knowledge gap about the effect of SA applied by foliar spraying and irrigation on the complex interaction of metabolites and genes in regulating the bell pepper fruit ripening and senescence processes.

Finally, whilst not the primary focus of this study, it has been demonstrated that the foliar and irrigation application of SA on bell pepper plants has the capacity to enhance fruit quality without exerting a detrimental effect on productivity. The results suggest that both foliar and irrigation SA treatments improve the accumulated crop yield, expressed in terms of kg per plant, throughout the crop cycle of 'Lamuyo' green pepper fruit, 'Herminio' cv., as it can be observed in Supplementary Figure 1. However, no significant differences were appreciated between the two application methodologies ($p \geq 0.05$). The role of SA in improving fruit yield may have been due to the translocation of more photoassimilates to fruits, thereby increasing fruit weight and reducing negative environmental impacts. These findings confirm those observed in a previous study where an increase of 0.5 kg yield was recorded in the last harvest date after the foliar application of SA (Dobón-Suárez et al., 2021a). Other studies also observed a higher productivity with the preharvest application of exogenous SA in different types of pepper plants (Sobczak et al., 2023; Preet

et al., 2023; Ghahremani et al., 2023; Ibrahim et al., 2019; Elwan and El-Hamahmy, 2009).

In summary, the results of the present study demonstrated that both SA preharvest methods tested (foliar and irrigation) were effective in enhancing crop yield, fruit quality at harvest, and delaying ripening and senescence of green pepper fruit throughout the antioxidant metabolism. The effect of both SA applications was found to be similar in most of the parameters analyzed, although it should be highlighted that they showed differences as follows: Firstly, foliar spraying of SA led to a positive modulation of the relative expression of *CaPOD* and *CaPAL* genes at harvest, although this stimulation was only upregulated for the *CaPOD* gene after 28 days of storage. Secondly, irrigation with SA led to an enhancement of H-TAA at harvest and TSS content after postharvest storage. Thirdly, the irrigation method of SA showed a significant increase in CAT activity and delayed weight loss and ripening index. However, regarding their impact on the field and on the agri-food industry, it should be emphasized that the irrigation method was the most beneficial due to the ease and cost-effectiveness with which it could be applied in comparison to foliar spraying.

5 Conclusions

In our study, we found that salicylic acid (SA) preharvest treatment, both by foliar spraying and irrigation, improved the fruit quality of green peppers, resulting in a slowing of the ripening and senescence processes during postharvest storage, while stimulating the antioxidant system, accompanied by an increase in the content of secondary metabolites and activity of antioxidant enzymes, mediated by the upregulation of the relative response of genes responsible for their biosynthesis. The mechanism of action proposed in the present study is as follows: *1)* SA induces the synthesis of secondary metabolites and enhances the antioxidant systems by stimulating the phenylpropanoid biosynthesis pathway, and *2)* SA activates the antioxidant enzymes by acting on the modulation of the relative expression of antioxidant system-based genes and regulating the balance between the antioxidant system and ROS metabolism, contributing to the antioxidant metabolism insights. The results of the present study fill the knowledge gap on the effect of SA applied by spraying and irrigation on the complex interaction of metabolites and genes in regulating the ripening and senescence processes of pepper fruit. Finally, both foliar spray and irrigation SA applications showed a great effect on fruit quality and crop yield. However, in terms of beneficial effects on the field and for the agri-food industry it should be highlighted the irrigation method due to the easier way and the reduced cost of application compared to the foliar application.

Data availability statement

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

Author contributions

AD-S: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Visualization. MG-P: Methodology, Software, Writing – review & editing, Visualization. VS-E: Funding acquisition, Methodology, Visualization, Writing – review & editing. MJG: Funding acquisition, Software, Visualization, Writing – review & editing. DV: Methodology, Writing – review & editing, Visualization. MS: Data curation, Writing – review & e diting, Visualization. MG-P: Conceptualization, Data curation, Supervision, Writing – original draft, Writing – review & editing, Visualization. PZ: Conceptualization, Funding acquisition, Supervision, Writing – review & editing, Visualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2025.1475068/full#supplementary-material

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4.4. Publication 4 — Research article

PUBLICATION 4 (Original manuscript)

Methyl jasmonate fumigation enhances crop yield and delays physiochemical quality changes by modulating the secondary metabolism in green bell

Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., Zapata, P.J.

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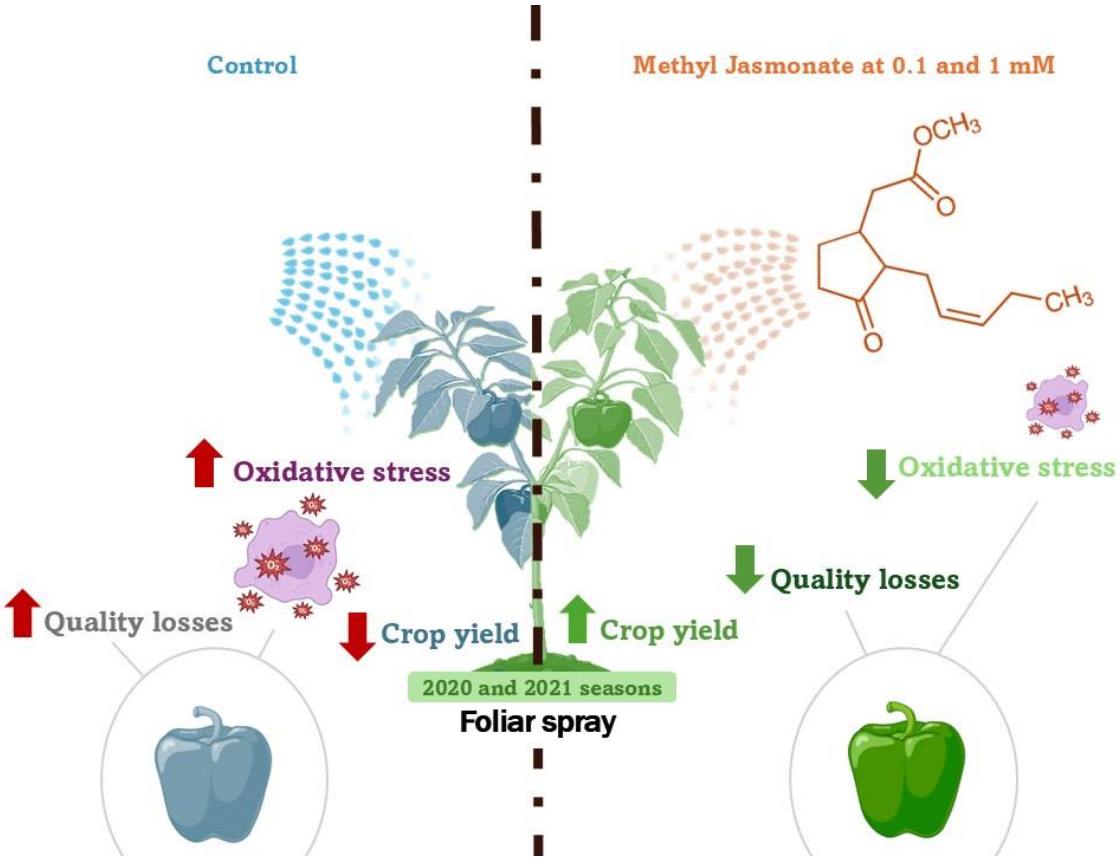
Graphical abstract:



Methyl jasmonate fumigation enhances crop yield and delays physiochemical quality changes by modulating the secondary metabolism in green bell pepper

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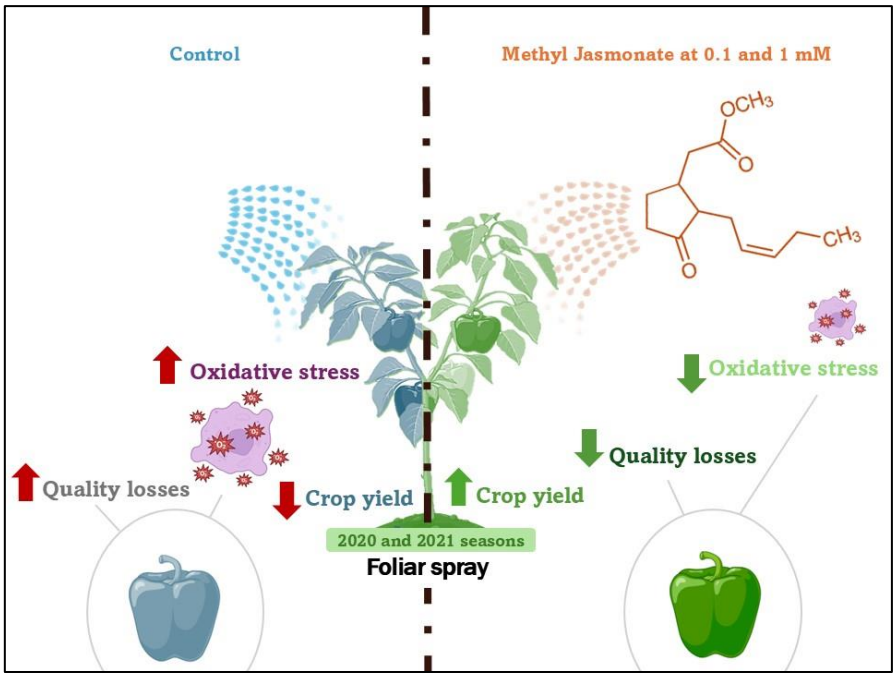
Abstract

BACKGROUND: The green bell pepper (*Capsicum annuum* L.), of the 'Lamuyo' type, is a highly valued vegetable due to its excellent organoleptic and nutritional properties. However, these properties are subject to deterioration during postharvest storage, which in turn limits the shelf-life of the pepper fruit. The present study examined the impact of preharvest treatment with 0.1- and 1-mM methyl jasmonate (MeJA) administered via foliar spraying on crop yield and both physiochemical and functional fruit quality at harvest and throughout a 28-day postharvest storage period at 7 °C. **RESULTS:** The study was conducted over two seasons (2020 and 2021) on green pepper fruit. The findings indicated that the application of MeJA had a favourable impact on crop yield in both growing seasons. Additionally, the treated peppers demonstrated a diminished percentage of weight loss and respiration rate, accompanied by augmented firmness, hue angle values, total soluble solids, and total acidity in comparison to the controls. Furthermore, MeJA treatments increased the levels of the antioxidant system by stimulating the activities of ascorbate peroxidase, catalase and peroxidase enzymes, as well as increasing the phenolic content and the total antioxidant activity. This could lead to an enhancement of the health benefits after the consumption of these treated peppers. **CONCLUSION:** The results demonstrated that the most efficacious concentration for enhancing yield and quality parameters by stimulating the secondary metabolism of peppers was 0.1 mM, a finding that was corroborated in the second season of 2021. The effects of MeJA on green pepper fruit may have significant commercial implications, as it has the potential to enhance quality at harvest and maintain it during postharvest storage. This may result in a delay in the onset of quality losses and the oxidative stress associated with fruit senescence, thereby extending the shelf-life of the fruit after prolonged storage at optimal temperatures.

Keywords: *Capsicum annuum* L., antioxidant enzymes, phenolics, postharvest, quality losses.



Graphical abstract. The preharvest application of methyl jasmonate (MeJA) at concentrations of 0.1 and 1 mM, administered via foliar spraying to green bell pepper (*Capsicum annuum* L.) plants, resulted in enhanced crop yield and the postponement of quality deterioration and oxidative stress levels in treated pepper fruits following prolonged storage at optimal temperatures.

1.Introduction

Bell peppers (*Capsicum annuum* L.), a member of the Solanaceae family, represent a significant agricultural and commercial crop, cultivated across tropical and subtropical regions globally.[1,2] The cultivated varieties of pepper fruit exhibit variation in fruit shape (block, conical, oblong), fruit size, and colour.[3] The most prevalent sweet pepper varieties are those with fleshy, block-type fruit that is green (unripe) and red (ripe) colour. The high level of consumer acceptance can be attributed to the functional compounds present in peppers, including polyphenols, flavonoids, vitamin C, and carotenoids.[2,4] These parameters exhibit considerable variation in peppers depending on the physiological and maturity stage.[3,5,6] Several factors, including the genetic environment (temperature, light, water and nutrient availability), production techniques used (including growth regulators, harvest date) and postharvest storage conditions,[7,8] have been identified as influencing these parameters. These metabolites may have beneficial effects on human health, and some may exert antioxidant activity, as they may be able to scavenge free radicals and oxygen.[9] Conversely, a multitude of factors impact the quality of the pepper fruit, including fruit weight and firmness, as well as the soluble solids content.[10] A strong positive correlation was observed between total soluble solids (TSS) and sugar content, which is frequently employed as an indicator of ripeness.[11] Furthermore, the formation of non-structural carbohydrates, which are utilised for the synthesis of phenolic compounds and antioxidants, has been associated with the sugar content of plants.[12]

In recent years, there has been a notable increase in the production of bell peppers. Nevertheless, postharvest losses of approximately 40 % per annum are still recorded for this crop.[13] Pepper is a highly perishable vegetable with a limited shelf-life. Therefore, it is essential to ensure proper handling and adequate care for the maintenance of postharvest quality.[14] The primary factors that negatively impact the postharvest quality of bell peppers during transportation, postharvest handling, and storage at 7 °C (the optimal temperature to prevent chilling injury) are water loss and fruit softening.[1,15,16] Consequently, bell peppers are vulnerable to a range of postharvest deterioration phenomena, including flaccidity, wilting, shrinkage, mechanical damage, fungal infections and deterioration.[17] These factors collectively influence consumer acceptance of fruit. The accelerated ageing process observed in peppers is attributed to the generation of reactive oxygen species (ROS) and a reduction in antioxidant enzyme activity. These factors have a deleterious impact and are responsible for the

induction of oxidative damage to cell components.[18] Oxidative stress has been demonstrated to exacerbate enzymatic browning, which can ultimately result in a loss of shelf-life. This is due to the peroxidizing of membrane lipids and the subsequent activation of softening-related enzymes.[19,20] It is crucial to enhance the activities of redox enzymes, including catalase (CAT) and ascorbate peroxidase (APX), among others, in order to extend the shelf-life of fruit and vegetable crops.[21,22,23]

A variety of postharvest technologies, including chemical and non-chemical treatments, have been employed to preserve the quality of sweet peppers during storage.[13,24] However, the recent focus of postharvest research on peppers has been on the development of non-polluting and non-chemical treatments for the control of postharvest infections and the maintenance of metabolic processes in pepper fruits. In this regard, recent studies have documented a range of approaches for enhancing the quality and shelf-life of pepper fruits through the utilisation of methodologies or materials, including organic manures, salicylic acid, cytokinin, chitosan, calcium and potassium thiosulfate, during the preharvest growth phase.[25-29] Regarding elicitors, the action mechanism in the preservation of fruits and vegetables involves the activation of the plant's defence responses. This, in turn, results in the production of various defence molecules, including phytochemicals, antioxidants and enzymes, which help to protect the fruits and vegetables from microbial and oxidative degradation. Methyl jasmonate (MeJA) is a plant growth regulator derived from jasmonic acid (JA). It is involved in several biological processes and defence responses to biotic and abiotic stresses.[30,31] Previous studies have demonstrated that MeJA is a dose-dependent signalling agent. For instance, García-Pastor et al.[32] found that high concentrations (5 and 10 mM) applied as preharvest treatments in table grapes delayed the ripening process and reduced the fruit weight. Conversely, lower concentrations (1, 0.1 and 0.01 mM) were observed to promote accelerated ripening.[32] Furthermore, Otálora et al.[33] demonstrated that the ameliorative impact of MeJA on heat stress was dependent on the specific variety of pepper plants, with the emergence of specific traits.[33] This evidence suggests that the response of pepper cultivars to MeJA application differs.

On the other hand, recent findings indicate that the exogenous application of MeJA, either alone or in combination with low-temperature conditioning, effectively alleviates the chilling injury of bell pepper.[1,2,34] These treatments have resulted in the enhancement of cold resistance of green bell pepper fruit by regulating membrane lipid composition, glutathione metabolism and the antioxidant system, as revealed by multi-omics insights. Moreover, Pu et al.[35] have demonstrated that MeJA can mitigate the damage caused to pepper leaves by low temperature and low light, safeguarding the integrity of the cell membrane and enhancing the resilience of pepper seedlings in this environment.[35] Notwithstanding these encouraging findings, there is currently no information available regarding the effects and preharvest application of MeJA for the purpose of enhancing the quality and shelf-life of bell peppers under optimal stored conditions and at a non-chilling injury temperature. Moreover, the precise mechanisms by which these beneficial effects are achieved remain unclear. This study aims to evaluate the impact of preharvest fumigation with methyl jasmonate (MeJA) on crop yield under greenhouse conditions, fruit quality and shelf-life of bell peppers. Furthermore, it seeks to provide practical recommendations for growers to enhance the postharvest performance of this valuable crop.

2. Materials and methods

2.1. Plant material, experimental design and yield evaluation

The investigation was conducted in a commercial plot located in El Raal (Murcia, Spain), cultivated under greenhouse conditions with a plastic covering. The experimental procedure was carried out during both the 2020 and 2021 growing seasons. In January 2020, plants of the *Capsicum annuum* L. cultivar 'Herminio', designated 'Lamuyo', were planted. The duration of the experiment was six months, spanning the period from February to July in both 2020 and 2021, as evidenced in **Table 1**. Subsequently, 135 pepper plants were randomly selected and distributed across blocks to complete the experimental design. A total of 45 plants were included in each treatment, with three randomised blocks of fifteen plants (n = 3). The pepper plants were treated via foliar spray application with distilled water containing 1 mL L^{-1} Tween-20, which served as the control group. In 2020, two concentrations of methyl jasmonate (MeJA) solutions, containing 1 mL L^{-1} Tween-20, were employed: 0.1 and 1

mM (reagent from Sigma-Aldrich, Madrid, Spain, CAS Number: 39924-52-2). The optimal dose of MeJA (0.1 mM) was then replicated in 2021. The concentrations were selected based on previous research experiments conducted on other fruits, including sweet cherries, plums, table grapes, pomegranate and lemon fruit.[32,36-39]

A total of seven foliar spray applications were conducted throughout the crop cycle, as detailed in **Table 1**. The initial treatment was administered prior to the commencement of the flowering phase. The equidistance among application dates was approximately 21 days due to a staggered flowering cycle, except for the final application, which was conducted in proximity to the final commercial harvest. The application was selected based on the duration of the crop cycle of the specific pepper cultivar in question. The crop was cultivated in accordance with the established crop programme for the 'Lamuyo' pepper variety, which was overseen by the company. The plants were irrigated using drip systems with the requisite nutrient levels, in accordance with standard practice. The soil exhibited a pH value of 7.50 and a sandy loam texture. The green pepper fruit were harvested at the optimal phenological stage, thus ensuring their suitability for commercial consumption.[5] A total of ten harvest dates were conducted throughout the developmental and growth cycle of 2020 and 2021. These dates were selected in accordance with a staggered production schedule and the commercial criteria for harvesting green pepper fruit established by the company. The harvesting of the peppers commenced in April and continued until July in both the 2020 and 2021 seasons (**Table 1**).

Table 1. The number and date of application and harvest of treatments (control and MeJA at 0.1 and 1 mM) applied by foliar spraying to 'Herminio' green pepper plants throughout the 2020 and 2021 growing seasons.[Y]

2020				2021			
Application number	**Application date**	**Harvest number**	**Harvest date**	**Application number**	**Application date**	**Harvest number**	**Harvest date**
A1	24th February	**H1**	06th April	**A1**	22nd February	**H1**	07th April
A2	17th March	**H2**	20th April	**A2**	15th March	**H2**	22nd April
A3	06th April	**H3**	04th May	**A3**	29th March	**H3**	04th May
A4	29th April	**H4**	14th May	**A4**	19th April	**H4**	14th May
A5	19th May	**H5**	26th May	**A5**	17th May	**H5**	27th May
A6	09th June	**H6**	04th June	**A6**	07th June	**H6**	03rd June
A7	12nd July	**H7**	16th June	**A7**	10th July	**H7**	11st June
		H8	26th June			**H8**	23rd June
		H9	06th July			**H9**	05th July
		H10	27th July			**H10**	21st July

[Y] Abbreviations: The letters "A" and "H" are used to denote the application and harvest, respectively.

The mean daily temperatures for each month were recorded: April (14.58 ^{o}C), May (20.06 ^{o}C), June (23.33 ^{o}C) and July (25.98 ^{o}C) in 2020; and April (16.00 ^{o}C), May (19.30 ^{o}C), June (19.70 ^{o}C) and July (27.20 ^{o}C) in 2021, being recorded at a station situated in close proximity to the experimental greenhouses (38^{o}2'2.64" North, 1^{o}1'18.9" West). The relative humidity (RH) exhibited fluctuations between 66 and 89 % throughout the course of the experiments. Crop yield was assessed at each harvest date throughout the crop cycle. The experimental design involved the establishment of blocks for each treatment in both growing seasons (2020 and 2021). The

yield of green peppers was quantified until the conclusion of the final harvest. The accumulative yield was expressed in terms of kg plant^{-1}.

2.2. Postharvest storage at optimal temperature

The green pepper fruits were subjected to analysis at harvest and following a 28-day postharvest storage period. The experimental postharvest storage design was conducted for both green pepper seasons in 2020 and 2021. The former occurred on 4 May 2020, while the latter took place on 22 April 2021. The dates were selected in accordance with the optimal phenological stage.[5] Green peppers, selected at random, without regard to any imperfections in appearance or internal quality, were extracted from each block per treatment and immediately transported to the laboratory. This procedure ensures the acquisition of a homogeneous sample from the different blocks, thereby ensuring the sample's representativeness of the commercial harvest. A total of 18 green peppers were selected for each treatment and sampling date (6 peppers per replicate, with a total of three replicates; n = 3), after which they were weighed and stored at 7 °C and 85% RH. The green pepper fruits were subjected to analysis at harvest (day 0) and at 7, 14, 21 and 28 days of storage. Accordingly, 90 green pepper fruits were utilised for postharvest storage per treatment. A series of measurements was taken for each sampling date, including weight loss, respiration rate, firmness, colour (hue°), total soluble solids (TSS), total acidity (TA), total phenolic content (TPC), total antioxidant activity (TAA) and the enzymatic activity of the antioxidant enzymes. A total of 18 green pepper fruits were selected for analysis, representing the various treatments and sampling dates.

2.3. Weight losses, respiration rate and physiochemical traits

The weight loss (WL) of the treatments tested was determined by weighing each green pepper fruit individually at harvest (day 0) and at subsequent sampling dates (7, 14, 21 and 28 days) following storage. The WL was expressed as a percentage (%) with respect to the initial weight of the green pepper fruit. The respiration rate (RR) was determined at room temperature by placing individual green pepper fruits in 2 L capacity glass jars, which were hermetically sealed for a period of 60 min. Subsequently, 1 mL of the atmosphere within the holder was withdrawn and employed for the quantification of carbon dioxide (CO_2) in a gas chromatograph (Shimadzu™ GC-14B) equipped with a thermal conductivity detector (TCD). The RR was then expressed as mg of CO_2 kg^{-1} h^{-1}.[26] The firmness of each pepper fruit was assessed individually using a TX-XT2i texturometer (Stable Microsystems, Godalming, UK). The texturometer employed a flat steel plate to determine the deformation produced by force on the equatorial fruit diameter, expressed as a percentage of the initial measurement. The results were expressed as a force-deformation ratio (N mm^{-1}). The colour of the equatorial region of the green pepper fruit was quantified at three points along the fruit's perimeter using a Minolta colorimeter (CFRC400, Minolta Camera Co., Kanto, Tokyo, Japan). The results were expressed as the hue angle (hue°) parameter, with the CIELab coordinates employed for this purpose. The data regarding firmness and colour are the mean ± SE of three replicates (n = 3) of ten pepper fruits. Subsequently, one half of each of the six green peppers from each replicate was chopped and blended to create a uniform juice sample. The TSS were then determined in duplicate using a digital refractometer (Atago PR-101, Atago Co. Ltd., Tokyo, Japan) at 20 °C, with the results expressed as g kg^{-1} (mean ± SE) of fresh weight (FW). The TA was also determined in duplicate in the same juice by automatic titration (785 DMP Titrino, Metrohm, Burladingen, Germany) with 0.1 N NaOH up to pH 8.10, using 1 mL of diluted juice in 25 mL distilled H_2O. The results (mean ± SE) were expressed as g malic acid equivalent kg^{-1} of FW.

2.4. Total phenolic content and total antioxidant activity of the hydrophilic and lipophilic fractions

On each sampling date, a subset of the 18 green peppers from each treatment was selected (6 peppers from 3 replicates) and processed. The remaining portion of each pepper was then cut into small pieces, with the peduncle and seeds removed, and subsequently frozen in liquid nitrogen and stored at -80 °C until the functional and enzymatic analysis could be conducted. The total phenolic content (TPC) and total antioxidant activity (TAA) in both fractions (hydrophilic (H-TAA) and lipophilic (L-TAA)) were extracted and quantified in accordance with

the methodology previously reported by Dobón-Suárez et al.[26] for 'Lamuyo' green pepper fruit. In summary, 10 mL of 50 mM phosphate buffer (pH = 7.8) and 5 mL of ethyl acetate were used to homogenize 5 g of green pepper fruit. Subsequently, the extracts were subjected to centrifugation at 10,000 x g for 15 min at 4 °C. The total phenolic content (TPC) and the hydrophilic total antioxidant activity (H-TAA) were quantified using the lower fraction, while the lipophilic total antioxidant activity (L-TAA) was determined in the upper extract. The results (mean ± SE) were expressed as g kg^{-1} of FW.

2.5. Antioxidant-enzymatic system: Ascorbate peroxidase, catalase and peroxidase activities

The ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) enzymes were analysed in freeze-dried green pepper powder samples (flesh + skin tissues) stored at -80 ^{o}C. Samples were taken at harvest (day 0) and at regular intervals throughout the postharvest storage period (days 7, 14, 21 and 28). The activity of APX, CAT and POD were determined by homogenising 0.20 g of fine powder with 5 mL of phosphate buffer (50 mM, pH 6.80) containing 1 % (*w/v*) polyvinylpyrrolidone and 1 mM ethylenediaminetetraacetic acid (EDTA). The resulting homogenate was subjected to centrifugation at 10,000 × *g* for 15 min at 4 °C. The supernatant was then employed in antioxidant enzyme assays in accordance with the methodology previously described by García-Pastor et al.,[40] with minor modifications. Antioxidant enzyme activities were expressed as units of enzymatic activity (U min^{-1} g^{-1}) of dry weight (DW). One enzymatic unit (U) was defined as a 0.01 decrease of ascorbate at 290 and 240 nm min^{-1} for APX and CAT, respectively, and a 0.01 increase of absorbance at 470 nm min^{-1} for POD. The results are presented as the mean ± SE of three replicates (n = 3).

2.6. Statistical analysis

All analytical determinations were subjected to statistical analysis, with three replicates (n = 3) being carried out for each parameter analysed. The results were expressed as the mean ± SE. The data were subjected to an analysis of variance (ANOVA). The sources of variation were the treatments and storage time. The mean comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test to ascertain whether the observed differences among the treatments or storage time were statistically significant. The resulting differences were represented as *, **, and *** symbols when $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. No statistically significant differences were identified when the probability value (p) was equal to or greater than 0.05 and thus represented as NS. All analyses were conducted using the SPSS software package, version 17.0 for Windows (SPSS, 2001, IBM Corporation, Armonk, NY, USA).

3. Results and discussion

3.1. The application of MeJA foliar spraying resulted in a notable increase in the accumulated yield of green pepper plants during two growing seasons

The accumulated yield of the green pepper crop was evaluated over two growing seasons (2020 and 2021) and expressed in kg per plant (**Figures 1A and 1B**). The results showed that the accumulated yield for both seasons was significantly higher in the MeJA-treated plants than in the controls, with the first harvest showing a greater difference (**Figure 1 and Table 2**). In the first season, preharvest MeJA treatments were applied, resulting in an improvement in yield per plant. The control plants had a total accumulated production of 4.42 ± 0.07 kg per plant. In contrast, the MeJA treated plants at 0.1 and 1 mM reached a production of 5.20 ± 0.08 and 4.89 ± 0.09 kg, respectively. These values obtained in the MeJA-treated plants represent a 0.78- and 0.47-fold increase in total production compared to the untreated plants. However, the most effective treatment in terms of total yield increase was MeJA at 0.1 mM (**Figure 1A and Table 2**). This concentration was applied in the 2021 season to confirm the effect of the lowest dose of MeJA tested on the accumulative yield parameter. The plants previously treated with MeJA at 0.1 mM showed a yield of 5.01 ± 0.07 kg per plant compared to 4.09 ± 0.054 kg per plant in the control plants (**Figure 1B and Table 2**).

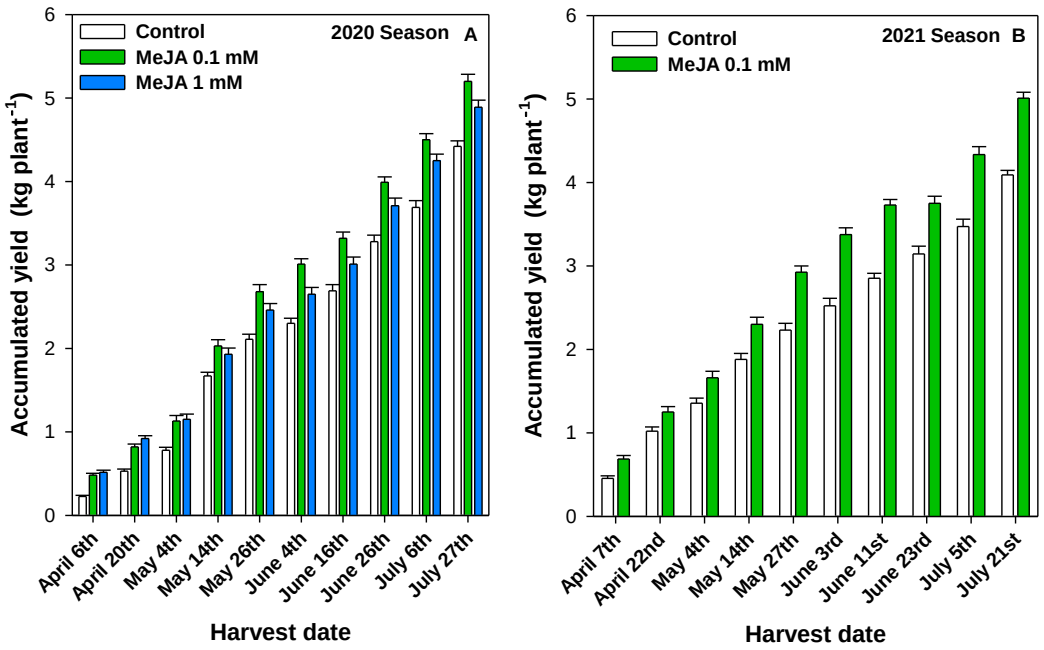


Figure 1. Accumulated yield (kg plant^{-1}) throughout the developmental and growth cycle of 'Lamuyo' green pepper plants, as affected by foliar spraying with methyl jasmonate (MeJA) at 0.1 and 1 mM in the 2020 **(A)** and at 0.1 mM in the 2021 **(B)** seasons. Data are the mean ± SE.

Table 2. Analyses of variance (ANOVA) for crop yield (kg plant^{-1}), weight loss (%), respiration rate (mg CO_2 kg^{-1} h^{-1}), physiochemical parameters [firmness (N mm^{-1}), colour (hue°), total soluble solids (TSS; g kg^{-1}) and total acidity (TA; g kg^{-1})] and the antioxidant system [total phenolic content (TPC; g kg^{-1} FW), hydrophilic-total antioxidant activity (H-TAA; g kg^{-1} FW), lipophilic-total antioxidant activity (L-TAA; g kg^{-1} FW), catalase (CAT; U min^{-1} g^{-1} DW), ascorbate peroxidase (APX; U min^{-1} g^{-1} DW), and peroxidase (POD; U min^{-1} g^{-1} DW) using the storage days and the treatment as factors for each growing season (2020 and 2021).$^{\Upsilon}$

	Factor			
	2020 season		2021 season	
Parameters	**Storage days**	**Treatment**	**Storage days**	**Treatment**
Crop yield (kg plant $^{-1}$)		469.85*** MeJA 0.1 mM = C MeJA 1 mM = B Control = A		851.70*** MeJA 0.1 mM Control
Weight loss (%)	275.81*** 7 = a 14 = b 21 = c 28 = d	12.15*** MeJA 0.1 mM = A MeJA 1 mM = A Control = B	1089.75*** 7 = a 14 = b 21 = c 28 = d	46.44*** MeJA 0.1 mM Control
Respiration rate (mg CO_2 kg^{-1} h^{-1})	1340.35*** 0 = c 7 = b 14 = bc 21 = bc 28 = a	10.33*** MeJA 0.1 mM = A MeJA 1 mM = A Control = B	953.66*** 0 = d 7 = c 14 = bc 21 = ab 28 = a	40.30*** MeJA 0.1 mM Control
Firmness (N mm^{-1})	79.31*** 0 = d 7 = c 14 = b 21 = a 28 = a	5.82** MeJA 0.1 mM = B MeJA 1 mM = B Control = A	43.30*** 0 = c 7 = c 14 = b 21 = b 28 = a	31.20*** MeJA 0.1 mM Control
Colour (hue^{o})	28.26*** 0 = c 7 = c 14 = b 21 = b 28 = a	54.41*** MeJA 0.1 mM = A MeJA 1 mM = B Control = C	30.15*** 0 = c 7 = b 14 = b 21 = b 28 = a	8.57** MeJA 0.1 mM Control

TSS (g kg^{-1} FW)	89.13*** 0 = a 7 = b 14 = c 21 = d 28 = e	47.86*** MeJA 0.1 mM = C MeJA 1 mM = B Control = A	38.25*** 0 = a 7 = ab 14 = ab 21 = b 28 = c	30.25*** MeJA 0.1 mM Control
TA (g kg^{-1} FW)	21.36*** 0 = c 7 = c 14 = b 21 = b 28 = a	2.36 NS MeJA 0.1 mM = A MeJA 1 mM = A Control = A	92.26*** 0 = d 7 = c 14 = b 21 = b 28 = a	15.64*** MeJA 0.1 mM Control
TPC (g kg^{-1} FW)	153.39*** 0 = a 7 = b 14 = c 21 = d 28 = e	37.07*** MeJA 0.1 mM = C MeJA 1 mM = B Control = A	44.26*** 0 = a 7 = b 14 = c 21 = d 28 = d	54.81*** MeJA 0.1 mM Control
H-TAA (g kg^{-1} FW)	1318.77*** 0 = a 7 = b 14 = c 21 = d 28 = e	122.98*** MeJA 0.1 mM = C MeJA 1 mM = B Control = A	191.97*** 0 = a 7 = b 14 = b 21 = c 28 = d	139.07*** MeJA 0.1 mM Control
L-TAA (g kg^{-1} FW)	119.03*** 0 = a 7 = b 14 = c 21 = d 28 = e	79.89*** MeJA 0.1 mM = B MeJA 1 mM = A Control = A	284.32*** 0 = a 7 = b 14 = b 21 = c 28 = d	236.90*** MeJA 0.1 mM Control
APX (U min^{-1} g^{-1} DW)	114.04*** 0 = b 7 = c 14 = c 21 = b 28 = a	295.56*** MeJA 0.1 mM = C MeJA 1 mM = B Control = A	39.89*** 0 = b 7 = c 14 = c 21 = b 28 = a	2020.08*** MeJA 0.1 mM Control
CAT (U min^{-1} g^{-1} DW)	11.74*** 0 = b 7 = a 14 = b 21 = b 28 = c	210.56*** MeJA 0.1 mM = B MeJA 1 mM = C Control = A	17.04*** 0 = a 7 = b 14 = b 21 = b 28 = b	7241.87*** MeJA 0.1 mM Control
POD (U min^{-1} g^{-1} DW)	66.40*** 0 = b 7 = cd 14 = d 21 = c 28 = a	60.87*** MeJA 0.1 mM = C MeJA 1 mM = B Control = A	63.01*** 0 = b 7 = c 14 = c 21 = b 28 = a	1466.79*** MeJA 0.1 mM Control

$^{\Upsilon}$ NS = not significant; *, ** and *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; data were previously tested for normality test. Different lowercase letters indicate significant differences among storage days for each parameter and growing season tested. Capital letters show significant differences among treatments for each parameter in the 2020 season.

In both growing seasons, the MeJA-treated and control peppers were grown in a commercial greenhouse under similar climatic conditions and agronomic treatments, so the differences between the control and treated plants were only due to the preharvest treatments tested. A total of seven applications and ten harvests were carried out continuously from the beginning of April to the end of July, and the results for accumulated yield (kg plant^{-1}) for each treatment studied are shown (**Table 1 and Figure 1**). The increase in accumulated yield was significantly higher ($p < 0.001$) in MeJA-treated plants than in the control in both growing seasons, with the 0.1 mM concentration being the most effective in improving this parameter (**Table 2**). In addition, there is no information on the effect of MeJA fumigation on the total yield of pepper plants; therefore, for the first time, results are reported on the effect of MeJA on the yield of 'Lamuyo' pepper plants, depending on the concentration applied. To the best of our knowledge, the lowest concentration of MeJA tested in the present experiment (0.1 mM) could be a useful tool to increase the yield of green pepper plants grown under greenhouse conditions.

The application of MeJA has been demonstrated to enhance plant resistance to abiotic stress by triggering a cascade of physiological and metabolic changes that reduce oxidative damage by increasing antioxidant enzyme activity.[41] A recent study demonstrated that pepper (*Capsicum annuum* L.) seedlings sprayed with 0.2 mM MeJA exhibited a significantly increased dry matter mass compared to the untreated controls under low temperature/low light (LL) stress.[35] This finding is consistent with the observation made by Yu et al.[42] that plants with greater tolerance exhibited higher photosynthetic quantum yield. This was found to be correlated with the results of

exogenous MeJA pretreatment of the photosystem II (PSII) reaction centre, which demonstrated an increase in the quantum yield of photosynthetic electron transfer, a decrease in the quantum yield due to heat dissipation and a decrease in the non-regulated quantum yield. PSII, one of the most susceptible components of photosynthesis, results in a considerable decline in the electron transport chain under abiotic stress.[43] This indicates that MeJA regulates PSII quantum yield and alleviates photoinhibition in pepper seedlings under LL conditions. Furthermore, the MeJA treatment resulted in an increase in photochemical efficiency, electron transport flux and PSII quantum efficiency to PSI, which serves as an important indicator for the assessment of plant health under conditions of adversity.[44] Nevertheless, Kurowska et al.[45] reported that an exogenous treatment with a high dose of MeJA (0.5 mM for 120 h) resulted in a reduction in photosynthetic efficiency in barley seedlings. This was attributed to a decline in PSII parameters, which was associated with the downregulation of the *HvPsbR* gene, which encodes one of the extrinsic oxygen-evolving complex (OEC) proteins. Other studies have also reported that the application of higher doses of MeJA, such as 5, 10 and 20 mM, has a negative impact on crop yield, significantly decreasing fruit weight and size while also delaying the ripening process.[32,46]

In the present experiment, methyl jasmonate (MeJA) fumigation was conducted prior to the onset of the flowering stage. The elicitation process was maintained throughout the development and growth cycle of the fruit, spanning from February to July. This was achieved through the application of the elicitor approximately every 21 days in both growing seasons (**Table 1**). In addition to alleviating some negative effects on crop yield caused by biotic or abiotic stress, the elicitor MeJA may also increase the accumulated yield per plant by directly affecting the flowering rate, fruit set rate, or abscission of pepper fruits from the plants, which occurs naturally during the fruit development process. Furthermore, an increase in Rubisco activity has been reported in previous studies on pepper fruits treated with other elicitors, including salicylic acid (SA),[26,47-50] have also demonstrated similar results. Similar results regarding an increase in yield have been reported in other fruit species with lower concentrations of MeJA as a preharvest treatment (1, 0.5, 0.25 and 0.1 mM) in plum, artichoke, table grape, pomegranate fruit and tomato. These findings are supported by the studies of Martínez-Esplá et al.,[36,51] García-Pastor et al.,[32,37] and Baek et al.,[52] respectively, which indicate that MeJA may be a dose-dependent compound.

3.2. The application of MeJA foliar spraying effectively delays the deterioration in physiochemical quality of green pepper fruit after prolonged storage at optimal temperatures

In the present study, 'Lamuyo' bell pepper plants were subjected to seven fumigation applications with MeJA solutions at concentrations of 0.1 and 1 mM during the 2020 season, and 0.1 mM during the 2021 season (**Table 1**). A number of fruit quality parameters were evaluated at harvest and subsequently during the storage period. There was a significant increase ($p < 0.001$) in fruit weight loss over time in both growing seasons (**Figures 2A and 2D, and Table 2**). The rate of this increase exhibited significant variation contingent on the foliar spraying treatment in both seasons ($p < 0.001$; **Table 2**).

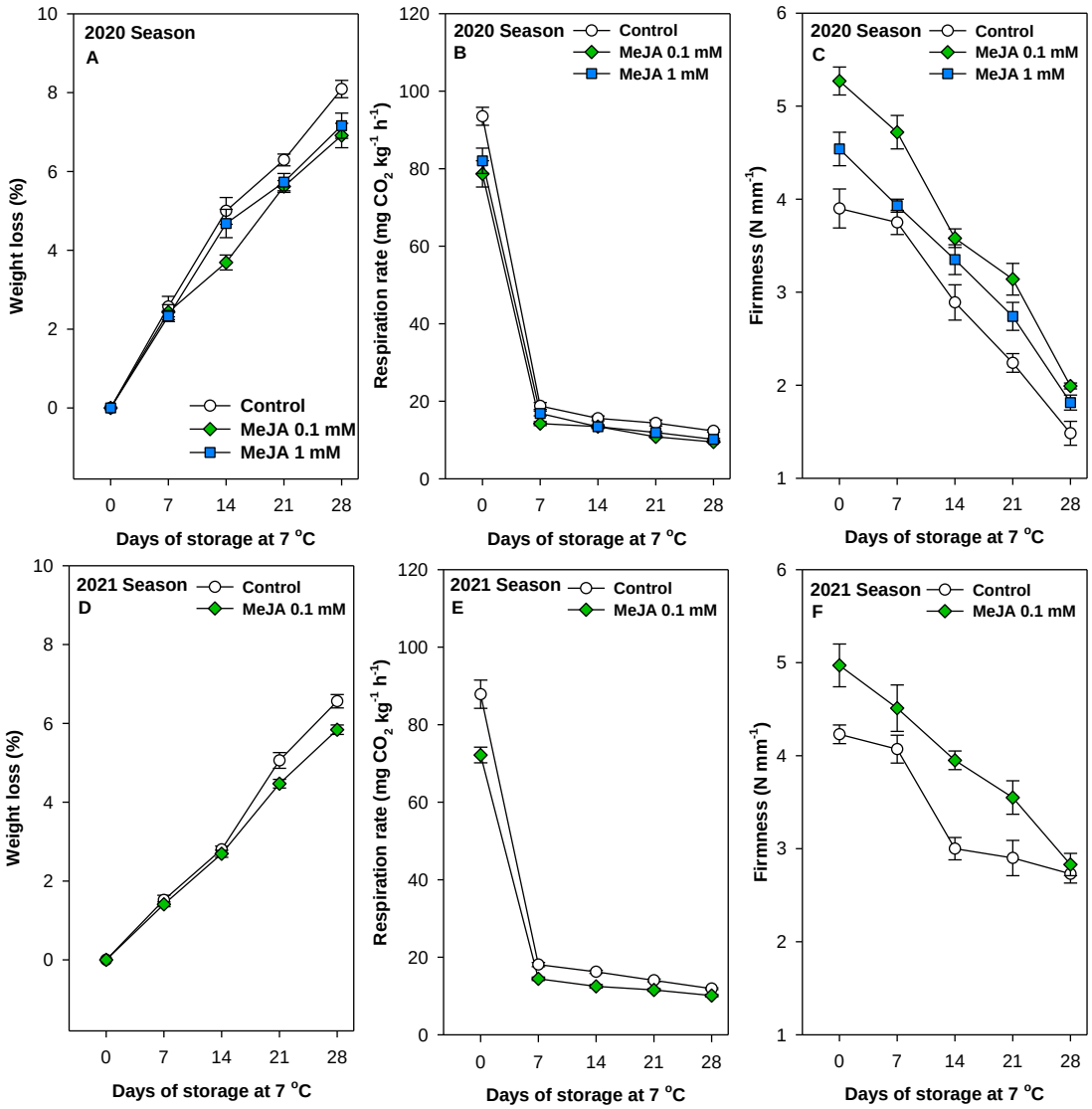


Figure 2. Effects of preharvest methyl jasmonate (MeJA) treatments at 0.1 and 1 mM in the 2020 (**A, B, and C**) and at 0.1 mM in the 2021 (**D, E, and F**) seasons on weight loss (%; **A and D**), respiration rate (mg CO_2 kg^{-1} h^{-1}; **B and E**) and firmness (N mm^{-1}; **C and F**) of 'Lamuyo' green pepper fruit during 28 days of cold storage at 7 ºC. Data are the mean ± SE.

The fruits of the control treatment exhibited the highest rate of weight loss, while the lowest rate was observed in the MeJA-treated pepper fruits, which demonstrated a 1.17- and 1.12-fold reduction at the end of the storage period in the 2020 and 2021 seasons, respectively (**Figures 2A and 2D**). However, no significant differences were observed between the two concentrations of MeJA tested (**Table 2**). Therefore, the foliar spraying application of MeJA at 0.1 and 1 mM effectively delayed the weight loss after 28 days of storage at 7 °C compared with the control (**Figure 2A**), with these results being confirmed for the 2021 growing season (**Figure 2D**). Conversely, the respiration rate demonstrated a significantly ($p < 0.001$) reduced pattern from harvest until 28 storage days at optimal temperature in both growing seasons (**Figures 2B and 2E, and Table 2**). The application of MeJA via foliar spraying at concentrations of 0.1 and 1 mM resulted in a significant ($p < 0.001$) reduction in the respiration rate in comparison to the control, although no significant differences were observed between the two concentrations during the 2020 season (**Figure 2B and Table 2**). The same reduction in behaviour was observed for the 0.1 mM MeJA treatment in the 2021 season, demonstrating a significant ($p < 0.001$) impact of this dose on the minimization of this indicator of fruit metabolic activity at both the harvest and 28-day storage period (**Figure 2E and Table 2**). A significant decline ($p < 0.001$) in fruit firmness was observed

during the storage period, with a particularly pronounced rate of decline evident in the 2020 growing season (**Figures 2C and 2F, and Table 2**). In this season, green pepper fruit fumigated with MeJA at 0.1 and 1 mM exhibited significantly higher firmness ($p < 0.01$; 26 and 14 %, respectively) than the control at harvest (**Figure 2C and Table 2**). This effect was maintained during the storage period, although no significant differences were observed between the two doses (**Table 2**). Furthermore, the delay of softening was observed in green pepper fruits treated with MeJA at 0.1 mM during the 2021 season. This treatment significantly increased firmness levels ($p < 0.001$) compared to control, except at 28 days (**Figure 2F and Table 2**).

The green colour of pepper fruits, expressed as hue angle, demonstrated a significant ($p < 0.001$) decline over the 28-day postharvest storage period at 7 °C, across both seasons, for all treatments tested (**Figures 3A and 3D, and Table 2**). In the 2020 season, this physical parameter was significantly ($p < 0.001$) higher in those pepper fruits treated with MeJA compared with untreated peppers, particularly for the 0.1 mM concentration (**Figure 3A and Table 2**). In the subsequent season, comparable outcomes were documented, with the 0.1 mM MeJA fumigation demonstrating a statistically significant ($p < 0.01$) elevation in hue angle values in comparison to the control fruits at harvest and throughout the postharvest storage period (**Figure 3D and Table 2**). The content of TSS demonstrated a significant ($p < 0.001$) increase during the cold storage period for both growing seasons under study (**Figures 3B and 3E, and Table 2**). The green pepper fruits harvested from plants treated with MeJA exhibited a significantly ($p < 0.001$) higher content of total soluble solids at harvest and after 28 days of cold storage, particularly in the 0.1 mM concentration (**Figures 3B and 3E, and Table 2**). However, the accumulation of these values was more pronounced in the 2020 season. Conversely, total acidity exhibited a notable ($p < 0.001$) decline across all treatments throughout the postharvest storage period in both seasons (**Figure 3C and 3F, and Table 2**). In the 2020 season, there were no significant differences ($p \geq 0.05$) in total acidity content between MeJA-treated and untreated green pepper fruits throughout the 28-day storage period (**Figure 3C and Table 2**). However, in the 2021 season, TA was significantly ($p < 0.001$) higher in fruits treated with 0.1 mM MeJA, and this difference was maintained throughout the experiment (**Figure 3F and Table 2**).

Bell peppers are a highly perishable vegetable, exhibiting a limited shelf-life and a high susceptibility to disease. It is crucial to emphasise the importance of appropriate handling and adequate care to maintain postharvest quality.[14,53] The primary factors that negatively impact the postharvest quality of peppers during transportation, short-term storage, and marketing and sales are water loss, softening and chilling injury.[15,16,54] Other factors that contribute to postharvest deterioration include physiological disorders, disease, and mechanical damage.[55] The fruit is susceptible to several adverse effects, including flaccidity, wilting, shrivelling, fungal infections, and deterioration, which collectively contribute to its relatively short shelf-life.[17] Such factors frequently impact consumer acceptance of the fruit. It is therefore imperative to enhance the shelf-life of bell peppers to reduce postharvest losses and to enhance food security and sustainability.

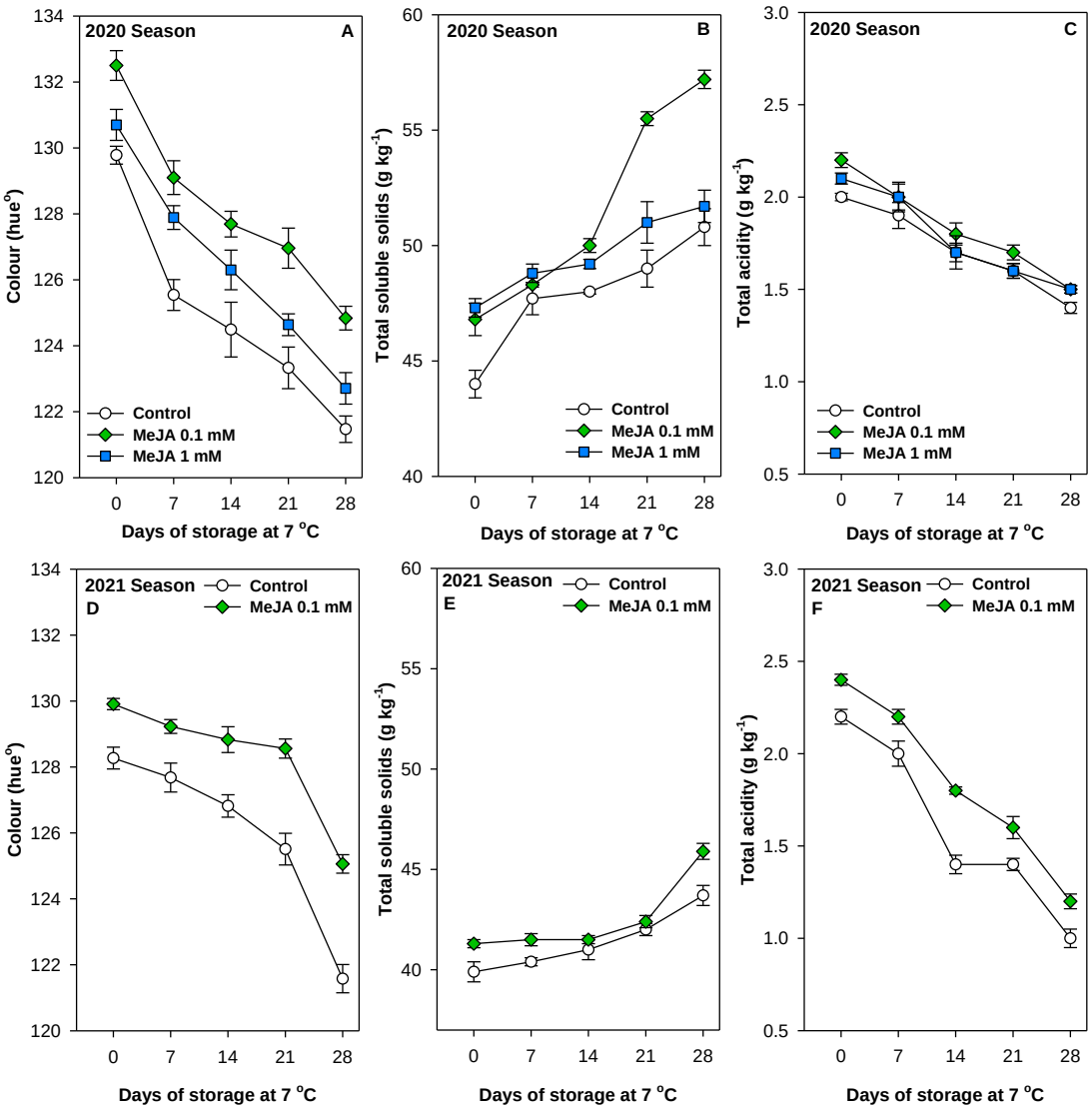


Figure 3. Effects of preharvest methyl jasmonate (MeJA) treatments at 0.1 and 1 mM in the 2020 (**A, B, and C**) and at 0.1 mM in the 2021 (**D, E, and F**) seasons on colour (hue°; **A and D**), total soluble solids (g kg^{-1}; **B and E**) and total acidity (g kg^{-1}; **C and F**) of 'Lamuyo' green pepper fruit during 28 days of cold storage at 7 °C. Data are the mean ± SE.

A number of metabolic and physical characteristics associated with fruit quality were evaluated at harvest and throughout the subsequent storage period. The findings revealed that preharvest fumigation with MeJA significantly delayed weight loss, respiration rate, and softening of pepper fruit during a 28-day storage period at 7 °C (**Figure 2**). To the best of our knowledge, this is the inaugural report on the impact of diverse preharvest MeJA applications on the quality parameters of bell peppers (*Capsicum annuum* L.) during postharvest storage. The findings provide insights into the underlying mechanisms of hormonal field application and its influence on the storability of pepper fruits. The preharvest application of MeJA has a significant impact on fruit ripening, with the effect varying according to the concentration used, in both climacteric and non-climacteric fruits. The loss of water in sweet peppers is a significant issue that arises during storage (**Figures 2A and 2D**), mainly caused by transpiration through fruit skin and leading to alterations in fruit firmness and a decline in quality.[56] Previous reports indicate that MeJA applications during the postharvest period of different fruits have an impact on the fruit weight loss in treated blueberries,[57] apricots,[58] oranges[59] and strawberries.[60] Furthermore, recent studies have indicated that the fumigation of MeJA can significantly delay losses in fruit weight during prolonged storage periods, thereby maintaining the quality of pomegranates, strawberries, lemons and blackberries.[36,37,61-63] This is

associated with the maintenance of cell integrity.[62] MeJA, an ester of jasmonic acid, has been demonstrated to activate an antioxidant defence mechanism against free radicals and retard membrane peroxidation.[64] This could be the reason for the reduced weight loss previously reported in raspberries.[63] Conversely, Karaman et al.[65] reported that the preharvest application of MeJA significantly increased weight loss of plum at the end of the storage period. This phenomenon can be attributed to the fact that MeJA causes the emission of ethylene, a gaseous hormone involved in plant development and ripening, in some species of vegetables, particularly at specific developmental stages.

The results of the experiments indicate that the treatments resulted in a notable decline in fruit respiration rate (**Figures 2B and E**) and fruit softening (**Figures 2C and 2F**). This reduction in respiration and the observed loss of weight (**Figures 2A and 2D**) in treated green peppers are also likely attributed to these effects. The relationship between respiratory rate and MeJA treatment appears to be dependent on the species in question. For instance, González-Aguilar et al.[66] observed that MeJA had no effect on the respiration rate of mango, whereas Pérez et al.[67] noted the opposite to be true in strawberries. Firmness is associated with cell turgidity and the thickness of the skin, and it is a crucial factor in determining commercial acceptance. The major cell wall components are pectins, hemicelluloses and cellulose. The process of fruit softening is directly related to the depolymerization of these components by cell wall hydrolytic enzymes, which include polygalacturonases, pectin methyl esterases and cellulases. Therefore, the higher firmness levels at harvest and the lower softening of MeJA-treated green pepper fruit may be attributed to reduced activity of these cell wall-degrading enzymes, particularly pectinases, as previously observed in MeJA postharvest-treated longkong and mango fruit.[68,69] Other authors have similarly observed a reduction in weight loss and maintenance of firmness in lemon, peach and tomato fruit as a result of preharvest MeJA treatments. This phenomenon has been linked with the preservation of cellular integrity and a reduction in fruit respiration rates.[52,62,70,71] Furthermore, elevated phenylalanine ammonia-lyase (PAL) activity regulates lignin deposition in the cell wall, contributing to enhanced fruit firmness during ripening and low-temperature storage.[72] Previous studies have demonstrated that the application of exogenous MeJA upregulated phenolic metabolism by increasing PAL activity during postharvest storage of strawberries,[73] tomatoes,[74] sweet cherries[75] and raspberries.[63] In addition, the application of MeJA in peach fruit has been demonstrated to enhance the activity of the POD enzyme, which plays a role in lignin biosynthesis.[76] This, in turn, may contribute to an increase in fruit firmness. Indeed, Zhang et al.[77] have recently reported that preharvest MeJA treatment had an activating effect on the secondary metabolic pathway, upregulating the most differentially expressed genes in this pathway in postharvest berries. This will be discussed in further detail below. Therefore, the preservation of fruit firmness through the restriction of weight loss and respiration rate represents an additional mechanism by which the fumigation of MeJA treatment prolongs the postharvest storability of bell pepper fruits. Further studies focused on the effect on the activity of cell wall hydrolytic enzymes could be conducted.

The quality of pepper fruit is contingent upon a multitude of intrinsic characteristics, encompassing aspects of visual presentation, flavour, chemical composition, and nutritional value. Among these quality traits, the colour of pepper fruit is one of the most intuitive and essential quality traits, influencing consumer and breeder preferences during the purchase and cultivation process. The light and dark green colouration of immature peppers is associated with the chlorophyll content of the fruits. The green colour of pepper fruit, as indicated by the hue angle, was maintained during postharvest storage at 7 °C in those fruits that had been fumigated with MeJA (**Figures 3A and 3D**). This could be related to a delay in chlorophyll degradation. Similarly, Bron et al.[78] also reported a slight retention of skin colour in papaya fruits dipped in 0.01 mM MeJA solutions. Other research has investigated the impact of varying concentrations of MeJA postharvest treatment on chlorophyll degradation in apple fruit. The findings indicate that exogenous 0.01 mM MeJA delayed degreening and ripening in apple fruit, whereas a 1.5 mM MeJA treatment had the opposite effect.[79] In a similar vein, other reports have indicated that MeJA treatments have been observed to increase the red peel colour development of apples in comparison to the control treatment.[46,80] Prior research has demonstrated that plum, mango, and raspberry fruit treated with MeJA display elevated hue angle values.[36,63,81,82] This may be attributed to delayed colour development and anthocyanin degradation, as well as higher levels of flavonoids. This phenomenon is associated with the oxidation of

flavonoids and anthocyanins by free radicals during storage, which results in premature ageing and alterations in fruit colour attributes.

The green pepper fruits harvested from plants fumigated with MeJA exhibited a higher content of TSS (**Figures 3B and 3E**) and TA (**Figures 3C and 3F**) at harvest in both growing seasons. Furthermore, these higher contents were maintained throughout the postharvest period, in comparison to the untreated fruits. Therefore, preharvest MeJA fumigation resulted in both an enhancement of green pepper organoleptic quality parameters, including firmness, green colour, TSS and TA and a delay of quality losses during postharvest. Consequently, the application of MeJA at 0.01 or 0.1 mM in blackberry cultivars has been observed to increase the content of TSS, with the effect being proportional to the applied concentration.[83] In mango, preharvest MeJA treatment resulted in fruit with increased concentrations of glucose, fructose and sucrose.[82] The impact of MeJA treatments on elevating fruit TSS and sugar content can be attributed to an enhancement in the net photosynthetic rate of 'Lamuyo' pepper plants, in addition to an increase in starch degradation and the production of fructose and glucose. In this regard, it has been demonstrated that MeJA at 1.0 mM stimulates dry matter accumulation in cauliflower seedlings by promoting chlorophyll synthesis and increasing the net photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration.[84] Therefore, the application of MeJA would result in an increase in available photoassimilates, thereby supporting the growth of pepper fruit. The increase in TSS of green pepper fruits treated with MeJA is consistent with previous findings in studies involving plums,[81] lemon,[62] and yellow pitahaya.[85] Furthermore, sugar accumulation is a crucial indicator of quality and is closely linked to the expression of genes involved in defence responses.[86] Therefore, the elevated TSS observed in the treated groups may also be attributed to the crop's response to the stress induced by MeJA.[71] Nevertheless, the mechanism of jasmonate-associated sugar accumulation in pepper fruit remains to be elucidated.

With respect to the TA, the observed decrease can be attributed to the utilization of organic acids during the respiration process or their conversion into sugars. The preharvest application of MeJA resulted in lemon fruit exhibiting elevated concentrations of individual sugars and organic acids in the flavedo and juice, both at harvest and following a 35-day storage period.[62] Consequently, the utilization of reduced doses of MeJA (0.1 and 0.01 mM) has been observed to result in elevated TSS and TA content in table grapes.[37] In a previous study, the application of MeJA at concentrations of 0.1, 0.3, 0.5 and 0.7 mM to Kinnow mandarin prior to harvest resulted in elevated fruit TA values and a reduced TSS/TA ratio (indicative of the ripening index). Of these concentrations, 0.5 mM was identified as the most effective in delaying the ripening process.[87] Similarly, Martínez-Esplá et al.[36] observed that acidity losses were delayed by 0.5 mM MeJA preharvest treatments, while 1.0- and 2.0-mM doses had no significant effect. Their findings also indicated that MeJA can differentially impact each of the parameters involved in fruit ripening, a hypothesis that has been previously proposed by Rudell et al.[46] in apple fruit. From an agronomic and commercial standpoint, the results would be of significant importance, as green pepper fruit with enhanced firmness, TSS and TA would be more highly valued by consumers. The findings of past and present studies collectively indicate that a preharvest treatment with MeJA represents a promising strategy for enhancing the retention of quality traits in a range of horticultural crops after harvest. Further research is required to establish detailed relationships between jasmonates and the inhibition of senescence, and the effect of the plant growth regulator should be carefully considered on a species- and cultivar-specific basis.

3.3. The application of MeJA foliar spraying positively modulated the secondary metabolism and reduced the oxidative stress in green pepper fruit

The parameters studied from the non-enzymatic antioxidants system for controlling the free radicals, TPC and TAA, both in the H-TAA and L-TAA fractions, demonstrated a markedly increasing trend from harvest until 28 days of storage at 7 °C for all treatments tested (**Figure 4**). This increase was statistically significant ($p < 0.001$; **Table 2**). The preharvest application of MeJA resulted in a statistically significant ($p < 0.001$) enhancement in TPC compared to the control fruits at the harvest, with the phenolic content maintained at a higher level throughout the postharvest period (**Figure 4A and Table 2**), particularly for the 0.1 mM concentration. In the 2021 season, comparable outcomes were documented, with MeJA demonstrating a statistically significant ($p < 0.001$) promotion of phenolic accumulation in green pepper fruits harvested from plants treated with 0.1 mM

MeJA (**Figure 4D and Table 2**). Furthermore, a notable ($p < 0.001$) stimulation of both H-TAA and L-TAA was evident in green pepper fruits harvested from MeJA-treated plants at harvest (**Figures 4B and 4C, and Table 2**). Following prolonged storage at optimal temperatures, the TAA of both the hydrophilic and lipophilic fractions in the MeJA-treated pepper fruits was maintained at higher levels than in the untreated fruits, with significant ($p < 0.001$) differences being reported (**Figures 4B and 4C, and Table 2**). These favourable outcomes pertaining to the stimulation of H-TAA and L-TAA by MeJA foliar spraying were replicated in the 2021 growing season, with the same statistically significant differences being observed (**Figures 4E and 4F, and Table 2**).

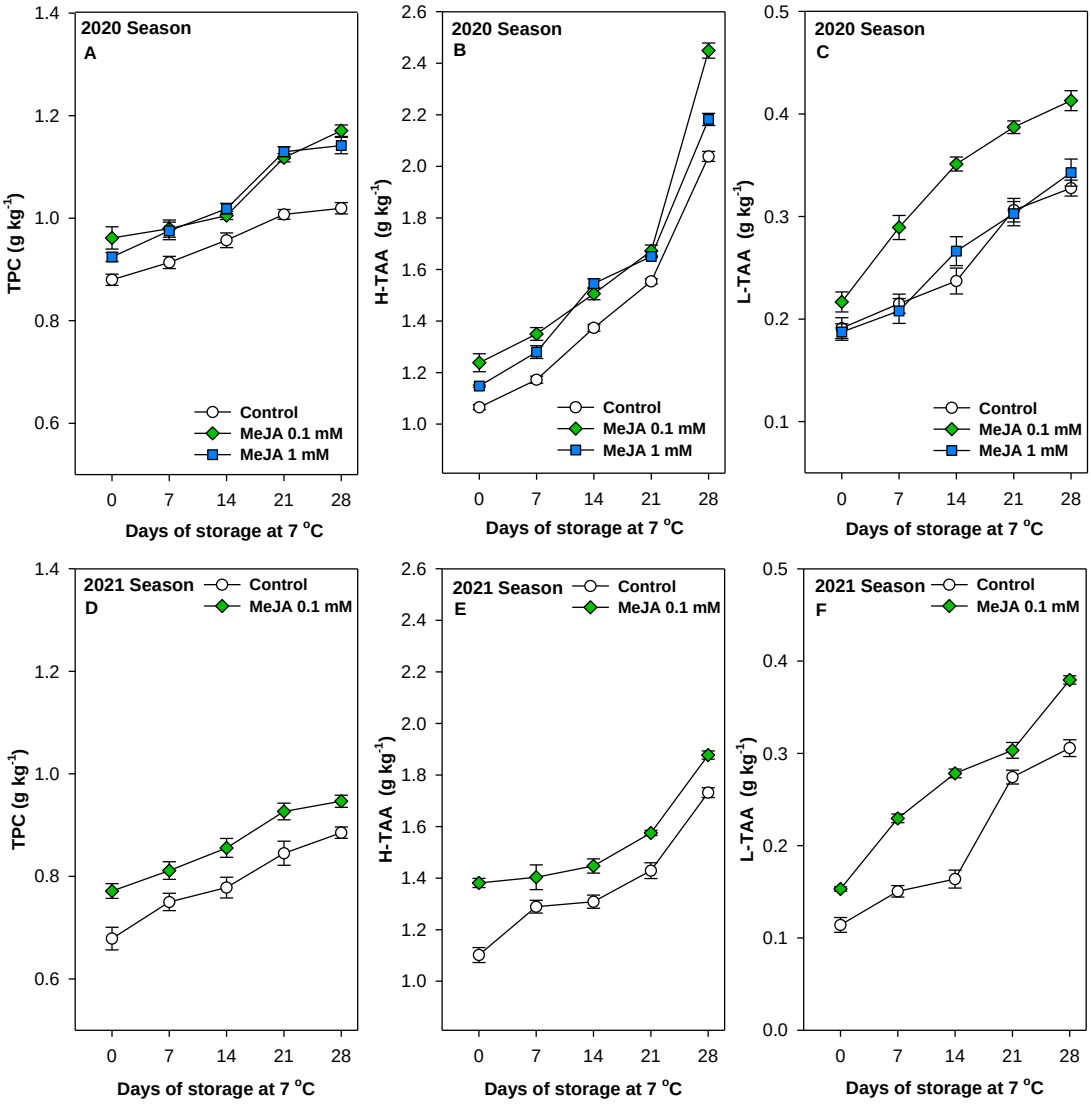


Figure 4. Effects of preharvest methyl jasmonate (MeJA) treatments at 0.1 and 1 mM in the 2020 (**A, B, and C**) and at 0.1 mM in the 2021 (**D, E, and F**) seasons on total phenolic content [TPC (g kg^{-1}; **A and D**)], and hydrophilic-total antioxidant activity [H-TAA (g kg^{-1}; **B and E**)] and lipophilic-total antioxidant activity [L-TAA (g kg^{-1}; **C and F**)] of 'Lamuyo' green pepper fruit during 28 days of cold storage at 7 °C. Data are the mean ± SE.

Total antioxidants play an essential role in increasing storability and maintaining fruit quality during the postharvest period. TPC contributes to the total non-enzymatic antioxidant levels in fresh horticultural produce.[89] It can be hypothesized that elevated levels of TPC in MeJA-treated green pepper fruits may contribute to augmented total antioxidant levels in treated fruit compared to the control (**Figure 4**). Phenolics represent one of the most significant secondary metabolites, playing a pivotal role in enhancing fruit quality and contributing to antioxidant reactions that initiate stress-mediated defence mechanisms in plants.[89] This study demonstrates, for the first time, that the applied dose of 0.1 mM of MeJA is also an important factor affecting the quality

development and secondary metabolism of 'Lamuyo' green bell pepper. Higher phenolic concentrations contribute to the maintenance of membrane integrity by mitigating the propagation of reactive oxygen species (ROS), which in turn reduces lipid peroxidation. This results in a delayed onset of oxidative stress and improved fruit quality.[89] It has been demonstrated that the application of MeJA prior to harvesting can result in elevated levels of phenolic compounds in a range of fruit crops. This phenomenon has been observed in plums,[36,90] apples,[81] mangoes,[82] table grapes,[32] lemons,[39] pomegranates,[37,40] sweet cherries,[38,75] strawberries,[73] tomatoes[74] and raspberries.[63] The stimulation of the enzymatic activities and gene expressions involved in the phenylpropanoid pathway has been attributed as a potential mechanism underlying these observations.[74]

During the postharvest storage period of green lilies, MeJA treatment was observed to significantly activate the expression of key genes involved in phenylpropane metabolism, including phenylalanine ammonia-lyase (PAL), 4-coumarate-CoA ligase (C4H) and 4-hydroxy-3-methylbutylCoA:CoA transferase (4CL), and to increase their enzyme activity.[91] Additionally, the TPC was found to be correlated with an enhancement of TAA.[62] In this regard, Baek et al.[71] observed that MeJA and SA treatments enhanced antioxidant activities at two harvesting stages and throughout the storage period, without affecting the sensory qualities of tomato. Conversely, postharvest MeJA treatments resulted in a reduction in total phenolics and antioxidant activity in carambola.[92] Additionally, the application of MeJA has been documented to enhance the synthesis of specific flavonoids. However, Wang et al.[93] observed that the changes in phenolic content were typically greater than those observed in flavonoid content. For example, the content of phenolics was increased by 25 %, while the content of flavonoids was only increased by 8 % in pomegranates treated with 0.1 mM.[94] It can therefore be concluded that the fumigation of bell pepper plants with MeJA could be an effective strategy for significantly activating the non-enzymatic antioxidant system. However, the effect on the underlying regulatory mechanism has yet to be reported. It would be beneficial for future studies to address the regulation of the relative expression of the majority of genes involved in the phenylpropane metabolism following the application of MeJA foliar spraying.

With regard to the antioxidant enzymatic system, the activities of APX, CAT, and POD were examined (**Figure 5**). The three enzymes exhibited a statistically significant ($p < 0.001$) variation in their values throughout the postharvest storage period at 7 °C for all treatments tested, with a notable decline observed after 14 days (**Figure 5 and Table 2**). The application of MeJA resulted in a notable improvement in the activities of APX (**Figures 5A and 5D**), CAT (**Figures 5B and 5E**), and POD (**Figures 5C and 5F**) at harvest. Furthermore, the activities of these enzymes remained significantly elevated ($p < 0.001$) in comparison to the control in both the 2020 and 2021 seasons (**Table 2**). The 0.1 mM dose proved to be the most efficacious in stimulating the activity of these antioxidant enzymes (**Figure 5 and Table 2**). It is well established that the production of ROS in plant cells is increased during the postharvest ripening process as a consequence of normal metabolic processes. These ROS are then eliminated by enzymatic systems. Antioxidant enzymes are involved in the radical scavenging of ROS species, thereby acting as a mechanism for repairing cell oxidative damage.[1] In eggplant, MeJA has been demonstrated to promote antioxidant enzyme activity and an increase in the relative expression of their corresponding genes, thereby enhancing the plant's protective effect against oxidative stress.[95] Similar outcomes were corroborated in other fruit species, including plums,[90] table grapes,[32] pomegranates,[37] and lemons.[62] These findings have led to an extension of the fruit shelf-life. The increase of these enzymes may enhance the tissue capacity to eliminate ROS, thereby delaying the ripening and senescence processes discussed in the previous section. Ultimately, the foliar application of MeJA to bell pepper plants demonstrated a favourable modulation of the secondary metabolism of green pepper fruits, which could be associated with the postponement of physiochemical quality deterioration during the postharvest ripening and senescence process at 7 °C.

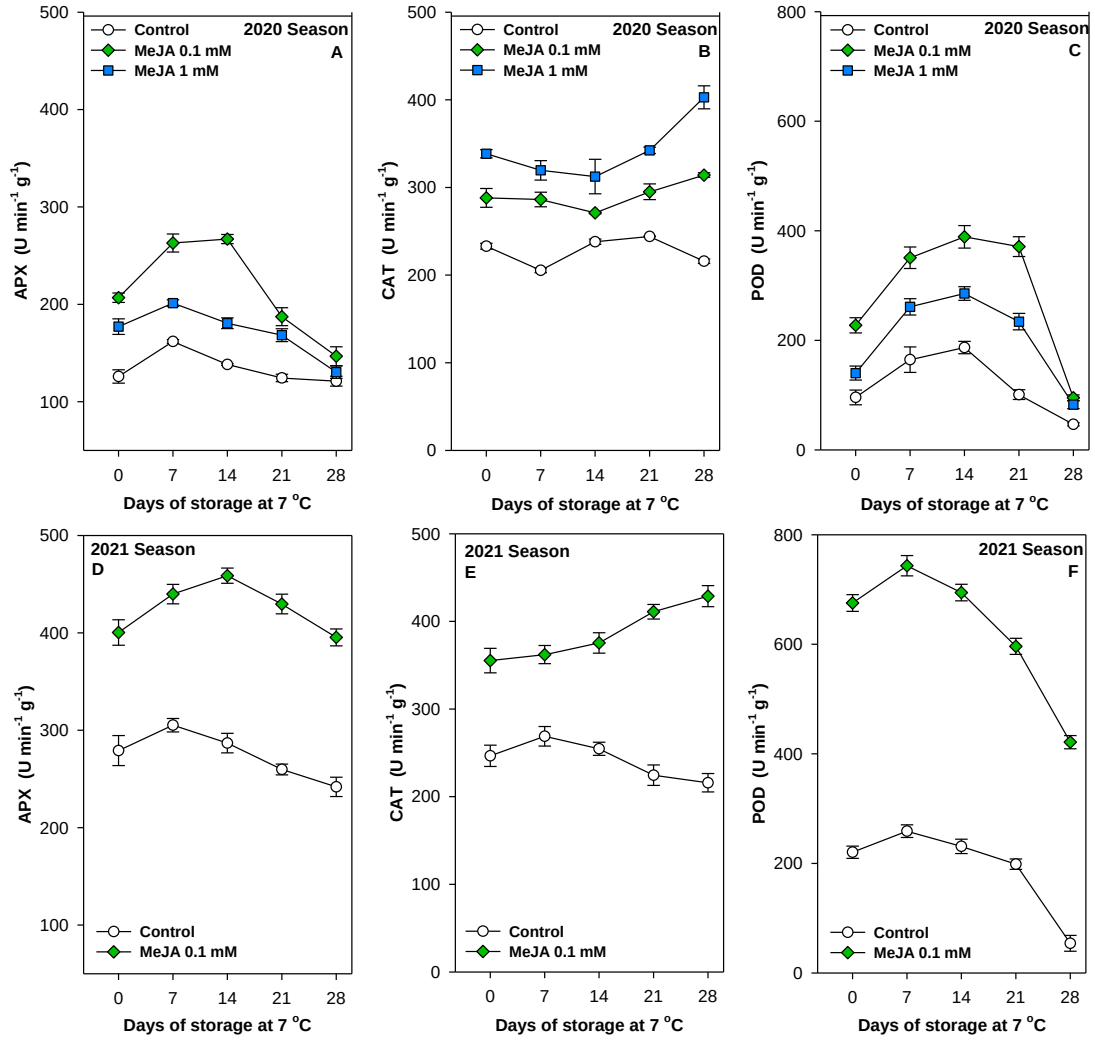


Figure 5. Effects of preharvest methyl jasmonate (MeJA) treatments at 0.1 and 1 mM in the 2020 (**A, B, and C**) and at 0.1 mM in the 2021 (**D, E, and F**) seasons on the activity of ascorbate peroxidase [APX (U min^{-1} g^{-1}; **A and D**)], catalase [CAT (U min^{-1} g^{-1}; **B and E**)] and peroxidase [POD (U min^{-1} g^{-1}; **C and F**)] of 'Lamuyo' green pepper fruit during 28 days of cold storage at 7 ºC. Data are the mean ± SE.

4. Conclusion

The preharvest application of MeJA via foliar spraying, conducted over the course of two growing seasons, resulted in a notable enhancement in crop yield for the 'Lamuyo' green pepper fruit. This enhancement was observed in two consecutive growing seasons. Moreover, the MeJA treatment mitigated losses of weight, firmness, green colour and acidity during prolonged postharvest storage at an optimal temperature of 7 °C. This was achieved by reducing the respiration rate, increasing the total soluble solids content and stimulating the antioxidant system. These effects may be related to a reduction in oxidative stress levels in the treated pepper fruits. The modulation of the secondary metabolism of green pepper fruit by MeJA foliar spraying, both at the enzymatic and non-enzymatic antioxidant level, may also result in enhanced health benefits following the consumption of these treated peppers. The results demonstrated that the most efficacious concentration for enhancing yield and quality parameters was 0.1 mM, a finding that was corroborated in the second season of 2021. The effects of MeJA on green pepper fruit may have significant commercial implications, as it has the potential to enhance quality at harvest and maintain it during postharvest storage. This may result in a delay in the onset of quality losses and the oxidative stress associated with fruit senescence, thereby extending the shelf-life of the fruit after prolonged storage at optimal temperatures. Although this is the first investigation to highlight the importance of preharvest MeJA application in regulating physiochemical quality changes during postharvest

storage at 7 °C, further molecular insight is required to fully understand the mechanisms involved. This knowledge would be beneficial for future studies on different pepper cultivars and types.

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4.5. Publication 5 — Research article

PUBLICATION 5 (Original manuscript)

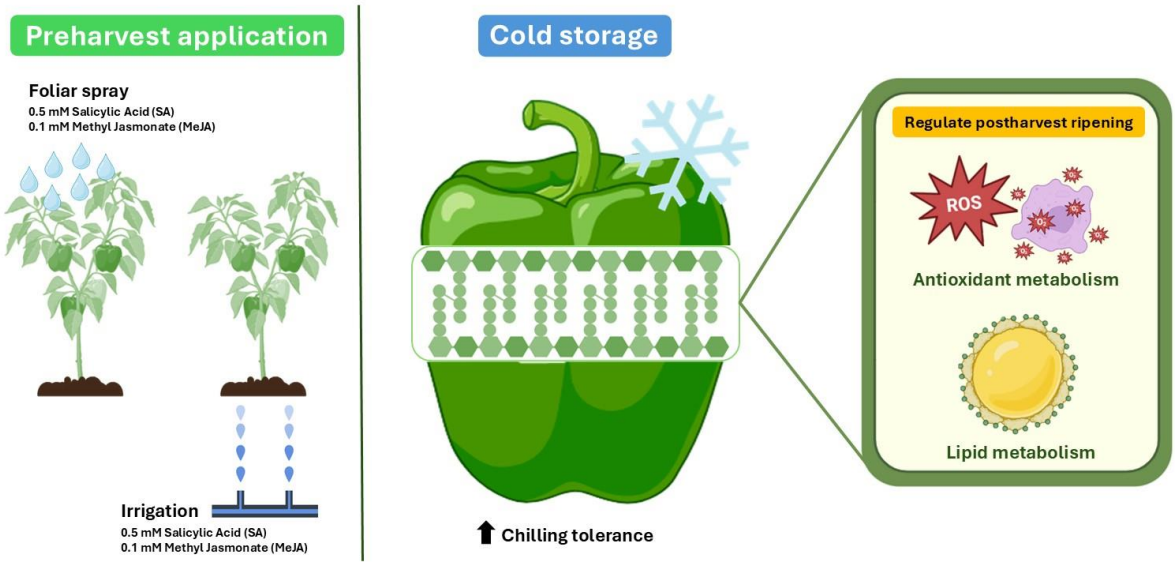
Foliar or irrigation application of salicylic acid and methyl jasmonate regulate postharvest ripening and chilling tolerance of green pepper fruit by modulating both antioxidant and lipid metabolism

Dobón-Suárez, A., Giménez, M.J., García-Pastor, M.E., Zapata, P.J.

Postharvest Biology and Technology

-Under review-

Graphical abstract:



Foliar or irrigation application of salicylic acid and methyl jasmonate regulate postharvest ripening and chilling tolerance of green pepper fruit by modulating both antioxidant and lipid metabolism

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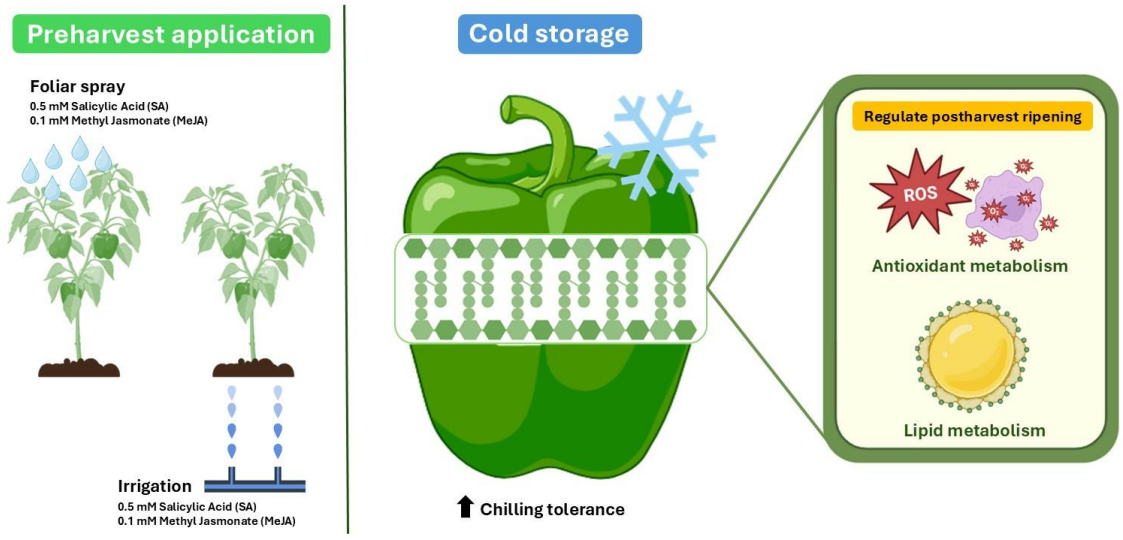
Abstract

Green pepper quality deteriorates during cold storage due to membrane lipid damage and oxidative stress, leading to chilling injury (CI). The main aim of this study is to investigate the effects of preharvest applications of 0.5 mM salicylic acid (SA) and 0.1 mM methyl jasmonate (MeJA) on 'Herminio' green peppers, applied via foliar spraying and irrigation, to enhance chilling tolerance. Therefore, the physiochemical traits, CI incidence, antioxidant metabolism, and fatty acid (FA) composition after 28 days at 2°C followed by 2 days at 20°C (shelf-life) were analyzed. Both SA and MeJA treatments, irrespective of application method, reduced weight, firmness, and colour losses compared to the control. Treated fruits also showed better colour retention, with irrigation showing superior hue angle values. The phytohormones modulated postharvest ripening, increasing total acidity after 28 days. Notably, preharvest applications, especially SA applied via irrigation, significantly mitigated the CI incidence. This protection was linked to enhanced non-enzymatic antioxidant systems, including increased total phenolic content (TPC), hydrophilic-total antioxidant activity (H-TAA), lipophilic-total antioxidant activity (L-TAA) and total carotenoid content (TCC). The study also revealed that the irrigation application of SA reduced saturated fatty acid (SFA) content, while MeJA irrigation decreased specific SFA and increased unsaturated fatty acid (UFA) content. Thus, SA irrigation resulted in the highest UFA/SFA ratio. In conclusion, preharvest applications of SA and MeJA modulated antioxidant and lipid metabolism, improving postharvest quality and enhancing chilling tolerance. Irrigation with 0.5 mM SA proved most effective, demonstrating its potential for commercial application to enhance green pepper storability and health benefits. Finally, this research provides valuable insights into the physiological effects of these phytohormones on green pepper fruit.

Keywords: Antioxidant capacity, *Capsicum annuum* L., chilling injury, membrane lipid, phytohormones, storage quality.



Graphical abstract. The preharvest application of 0.5 mM salicylic acid (SA) and 0.1 mM methyl jasmonate (MeJA), administered via foliar spraying or irrigation, to green bell pepper (*Capsicum annuum* L.) plants, induce chilling tolerance and regulate postharvest ripening in treated pepper fruits stored under cold conditions by modulating both antioxidant and lipid metabolism.

1. Introduction

The application of low temperatures storage has been demonstrated to markedly retard the senescence of fruits and vegetables (Aghadam et al., 2019; Xu et al., 2023; Zhao et al., 2024). Sweet bell pepper (*Capsicum annuu*m L.) is highly perishable vegetable that require appropriate handling and adequate care to maintain postharvest quality (Wang et al., 2019). The main issues that negatively impact the postharvest quality of peppers are water loss, softening, mechanical damage, accelerated ripening and pathological deterioration (Cheema et al., 2018; Wang et al., 2019; Gil and Tudela, 2020; Hanaei et al., 2022), as consequence of high rates of dehydration and elevated metabolic levels (Ge et al., 2019). As a cold-sensitive fruit, green pepper is vulnerable to chilling injury (CI) when the ambient temperature drops below 7 ºC. The incidence of CI is influenced by multiple factors such as the cultivar, maturity stage and period of storage at low temperatures (Wang et al., 2016). Immature green bell peppers are more sensitive to storage at chilling temperatures than coloured ripening fruits (Gil and Tudela, 2020). This damage is characterized by seed browning, peel pitting, and pulp decay (Kong et al., 2020). Apart from the external manifestation of CI, several biochemical and physiological processes are also affected as consequence of the direct impact of low temperature on cellular constituents. CI significantly affects the quality and commodity value of green peppers, leading to substantial economic losses in the industry (Huang et al., 2024).

The mechanism of CI of horticultural crops has been well investigated. It is widely believed that CI is primarily caused by membrane lipid imbalance, which is induced by decreasing fatty acids (FAs) desaturation (Sun et al., 2020). However, other studies have been demonstrated a relation between CI index and higher content of phospholipids, reduction of lipid oxidation by modifying glycerophospholipids and glycerolipids in lipid metabolism, and a stimulation of the antioxidant enzyme activity in peppers (Ma et al., 2020; Wang et al., 2022; 2024). Phospholipids are major cell components essential for maintaining cell structure, protein function, and signal transduction (Wang et al., 2020). Therefore, CI is closely related to reactive oxygen species (ROS) and membrane lipid metabolism. ROS are metabolites generated by organisms during regular aerobic respiration (Albornoz et al., 2022). They are crucial in controlling the responses of the organism to antioxidant deficiency resulting from biotic or abiotic factors and maintaining active equilibrium through enzymatic and nonenzymatic antioxidant systems (Ma et al., 2020). ROS accumulation in fruits leads to membrane lipid peroxidation, which disrupts membrane integrity and, thus, increases membrane permeability (Nukuntornprakit et al., 2020). The relationship between quality deterioration and membrane lipid content in horticultural products has attracted significant attention. There is an increasing need to develop effective preservatives to improve the cold tolerance of green peppers and to maintain their postharvest quality during storage. However, research focusing on the occurrence of postharvest CI in sweet peppers, as recently postulated by Huang et al. (2024), has been scarce.

Several technologies have been investigated as methods to mitigate CI (Wang et al., 2021). These include physical methods, such as UV-C (Vicente et al., 2005), heat treatment combined with individual shrink packaging (Ilić et al., 2012), intermittent warming cycles (Liu et al., 2015), and modified atmosphere packaging (MAP) (Serrano et al., 1997). Also, a combination of 1-methylcyclopropene (1-MCP) treatment with MAP is a promising treatment for reducing CI of bell peppers (Li et al., 2011). Other treatments to alleviate CI of fruit include the application of plant hormones such as brassinolide (BR) (Wang et al., 2012), acetyl salicylic acid (ASA) (Sayyari and Ghanbari, 2013), methyl salicylate (MeSA) alone or combined with hot water (Fung et al., 2004; Rehman et al., 2021) and methyl jasmonate (MeJA) (Meir et al., 1996; Fung et al., 2004; Wang et al., 2019; Ma et al., 2020). Recently, some studies found that MeJA can mitigate CI of bell pepper fruit by regulating membrane lipid composition, and potentially through inhibiting the MYC2-JA signalling pathway, enhancing the ASA-GSH cycle, reducing membrane lipid damage, suppressing cell wall disassembly, and activating the CMAT-CBF-ICE pathway (Ma et al., 2020; Fu et al., 2022). Similarly, salicylic acid (SA), often in combination with other compounds, like trisodium phosphate (TSP) and caraway oil coating, or as a preharvest spray, has demonstrated potential in

alleviating chilling injury throughout the enhancing of fatty acid desaturation efficiency of bell pepper fruit (Ge et al., 2020; Hanaei et al., 2022; Chen et al., 2023). Recently, melatonin postharvest treatment, an indole tryptophan derivative, ameliorated chilling injury by modulating lipid metabolism and antioxidant capacity in green bell pepper fruits (Kong et al., 2020; Huang et al., 2024). More recently, treatments involving cold shock with oxalic acid (OA) have shown promise in enhancing antioxidant enzyme activity and regulating proline metabolism to reduce chilling injury and sodium hexametaphosphate has been reported to delay chilling injury by modifying glycerophospholipids and glycerolipids (Wang et al., 2022; Huang et al., 2024). These findings collectively underscore the complex interplay of hormonal and chemical signalling in modulating the physiological responses of bell peppers to cold stress, offering avenues for developing effective postharvest preservation techniques.

A review of the extant literature reveals a conspicuous absence of detailed investigation into the preharvest applications of SA and MeJA in relation to enhancing cold tolerance in bell peppers (*Capsicum annuum* L.). While Hasan et al. (2025) comprehensively examines the effects of MeJA preharvest treatment on various horticultural crops, with a focus on aspects such as fruit ripening, colour development, yield, and chilling injury reduction in fruits like apples and pomegranates, it lacks specific details or studies on bell peppers. Similarly, Chen et al. (2023) emphasises the benefits of SA preharvest spraying for enhancing the quality of fruit and vegetable storage, while also addressing its role in abiotic stress responses. However, the paper lacks specific examples or research focused on the impact of SA preharvest spraying on chilling injury in bell peppers. Recently, Hanaei et al. (2022) studied the preharvest impact of SA foliar spraying, alone and with the incorporation of caraway oil coating, on postharvest chilling injury in sweet pepper under cold storage, although the effect on non-enzymatic antioxidant system and lipid metabolism of bell pepper was not addressed. The studies discussed by Fu et al. (2022) and Huang et al. (2024) primarily investigate postharvest treatments with MeJA and melatonin, respectively, for alleviating chilling injury in bell peppers, further underscoring the relative lack of attention given to preharvest hormonal interventions, particularly SA and MeJA, for this specific horticultural commodity and chilling stress. In this sense, Seo et al. (2020) investigated the contrasting effects of MeJA and methyl salicylate (MeSA) vapor treatments on seed browning in pepper fruit during cold storage at 2 ºC and demonstrated that MeJA treatment effectively inhibited seed browning, while MeSA application exacerbated this chilling injury symptom. Therefore, the main aim of this study is to evaluate, for the first time, the effects of SA and MeJA preharvest treatments, applied individually by foliar spraying or irrigation, on the quality losses and CI incidence of green peppers fruits stored at low-temperatures conditions and its relationship with changes in the non-enzymatic antioxidant metabolism and the FAs composition of cell membranes. To the best of our knowledge, there is a paucity of information regarding the effect of the preharvest application of both studied phytohormones by irrigation on the quality of green bell peppers at harvest or during postharvest. The results of this study could provide a theoretical background for the development of continuous storage control systems and technologies for the preservation of green peppers, and for the extrapolation of the most effective preharvest application method for commercial use.

2. Materials and methods

2.1. Plant material and experiment design

The experiment was performed in a commercial plot growing under plastic-roofed greenhouse located in El Raal (Murcia, Spain) in 2022 by using plants of 'Lamuyo' pepper type (*Capsicum annuum* L.), 'Herminio' cultivar, which were planted in January. Thus, 225 pepper plants were selected and distributed randomized complete block design containing fifteen blocks in total. Each treatment was carried out in three blocks (n = 3), with 15 plants (45 treated plants per treatment). Treatments were control (plants treated with distilled water), 0.5 mM SA (plants treated with the reagent purchased from Sigma, Sigma-Aldrich, Madrid, Spain; CAS Number: 69-72-7) and 0.1 mM MeJA (reagent from Sigma, Sigma-Aldrich, Madrid, Spain CAS Number: 39924-52-2). The concentrations of both SA and MeJA were selected based on the outcomes of prior experiments conducted during the 2020-2021 seasons to assess the impact on postharvest quality during storage at an optimal temperature of 7 ºC, as recently reported (Dobón-Suárez et al., 2021a; Dobón-Suárez et al., 2025). Both 0.5 mM SA and 0.1 mM MeJA treatments were applied in the present study in accordance with two different commercial practices: *1)* Foliar spray [Foliar SA and Foliar MeJA] and *2)* Irrigation [Irrigation SA and Irrigation MeJA], while the control plants were treated only

with distilled water by foliar spraying [Control]. During the crop cycle, exogenous SA and MeJA applications were made via foliar spraying or irrigation, at an early morning time of 8:00 a.m. The first treatment was applied before onset of the flowering stage. The treatments were applied on four consecutive times at 21-day intervals until the date of harvest. The equidistance among application dates was due to a staggered flowering cycle, except for the final application, which was conducted three days before the final commercial harvest. This application was selected based on the crop cycle duration of this specific pepper cultivar and according with our previous studies (Dobón-Suárez et al., 2021a; Dobón-Suárez et al., 2025).

All treatments were supplemented with Tween 20 at a concentration of 0.05 % (v/*v*). The foliar spray was conducted manually using a pump, while the drip application was performed automatically via the automatic irrigation system. The four application dates of all treatments tested (Control, Foliar SA, Foliar MeJA, Irrigation SA and Irrigation MeJA), which were carried out throughout the developmental and growth cycle of 'Herminio' green pepper fruit in the 2022 season are described in **Table 1**. Plants were provided with irrigation and fertilisation in accordance with the standard agricultural practices for the short-term crop cycle of the 'Lamuyo' pepper cultivar. The soil texture was determined to be sandy loam, with a pH value of 7.50. The irrigation system employed was a drip system, ensuring optimal nutrient levels were applied to the plants.

Table 1. Application dates of treatments [Control, Foliar SA, Foliar MeJA, Irrigation SA and Irrigation MeJA] carried out throughout the developmental and growth cycle of 'Herminio' green pepper fruit in the 2022 season.

Treatment	T1	T2	T3	T4
Treatment dates	15th February	08th March	22th March	12th April

The pepper fruits were harvested at the commercial stage, coinciding with the phenological stage of green pepper that is considered suitable for its consumption (Dobón-Suárez et al., 2021b). The study was conducted over a period of three months, from February to April (**Table 1**). The samples of green pepper fruits used in the present study were harvested on 15th April, a period of three days after the final application of treatments (**Table 1**). These samples were selected at random from each treatment block, ensuring uniformity in shape and colour, and the absence of any visible defects. The selected fruits were immediately transferred to the research laboratory of Postharvest Group of Fruit and Vegetables to carry out a storage experiment under chilling stress conditions.

For each treatment, 90 peppers fruits with similar shape, size and colour were selected and weighted individually and stored at 2 ºC and 85-90 % of relative humidity (RH) plus 2 days at 20 ºC (55-60 % RH). This condition of shelf-life was chosen according with a previous study in sweet pepper fruit, cv. 'Toronto' (Hanaei et al., 2022). Consequently, six peppers were selected for three replicates (n = 3; 18 pepper fruits in total) for each treatment and sampling date. Pepper fruits were analysed at harvest (day 0) and those stored for 28 days under cold conditions were evaluated every 7-day intervals. Thus, the postharvest storage experiment entailed the analysis of green pepper fruits at four distinct times: 7, 14, 21 and 28 days. In total, 72 pepper fruits per treatment were used for the analyses of the four sampling dates. Following the quality parameters measured for each sampling date of all whole fruits, weight losses, fruit firmness, colour (hue^{o}), total soluble solids (TSS), total acidity (TA) and external CI index were assayed both at harvest and during postharvest storage.

Furthermore, total phenolic content (TPC), hydrophilic-total antioxidant activity (H-TAA), lipophilic-total antioxidant activity (L-TAA), total carotenoid content (TCC) and FAs composition were quantitatively analysed both at harvest and after 28 days of storage at 2 °C. These analyses related to antioxidant and lipid metabolism were carried out on freeze-dried samples composed of both flesh and skin tissues mixed, and the results were expressed as g kg^{-1} of fresh weight (FW). Pepper fruits were meticulously sectioned into longitudinal strips prior to being divided into 1 x 0.5 cm squares, thereby ensuring a homogeneous sample from both flesh and skin tissues of six green peppers from each replicate (n = 3), with the peduncle and seeds removed. This approach yielded a total of 18 green pepper fruits, which was deemed to be a sufficient sample size for the experimental conditions and the designated sampling date. These samples were then frozen in liquid N_2 and freeze-dried in an Alpha 2–4 freeze

drier (Christ Alpha 2–4; Braum Biotech) for 1 d under reduced pressure, 2.2 MPa. The temperature in the drying chamber was set at −25 ºC, while the heating plate reached 15 ºC. Finally, the samples were milled until they reached a fine powder, after which they were vacuum-packed for use in measuring the aforementioned parameters.

2.2. Physiochemical parameters at harvest and during cold storage

Green pepper fruits were weighted at harvest and at each sampling date during postharvest storage. Weight losses (WL) of pepper fruits were determined by the individual fruit weight at harvest (day 0) and after 7,14, 21 and 28 storage days. WL were expressed as a percentage (%) of the fruit weight at each sampling date with respect to the initial pepper fruit weight at day 0. The results were the mean ± SE. Level of firmness of each pepper fruit was assessed individually by measuring the deformation force with a digital TX-XT2i Texturometer (Stable Microsystems, Godalming, UK). The instrument employed a flat steel plate for the measurement of the equatorial fruit diameter applying force that achieved a 5 % deformation of this diameter, in accordance with the protocol described by Dobón-Suárez et al. (2025). The results were the relation between the applied force and the distance travelled expressed as a force-deformation ratio (N mm^{-1}), and were expressed as the mean ± SE. Colour was measured on three points of the equatorial pepper fruit diameter by using a Minolta colourimeter (CFRC400, Minolta Camera Co., Kanto, Tokyo, Japan) with the CIELab coordinates expressed as the hue angle (h^o) parameter. A homogeneous sample was prepared from each replicate and treatment by blending the fruit in a blender. The content of TSS were determined in duplicate in the juice obtained from one sample of each replicate by using a digital refractometer Atago PR101 (Atago Co. Ltd., Japan) at 20 ºC, and results were the mean ± SE, expressed as g kg^{-1} FW. On the other hand, the TA was determined in duplicate in 1 mL of the same juice diluted in 25 mL distilled H_2O by potentiometric titration with 0.1 N NaOH up to pH 8.10, and results were the mean ± SE expressed as g of malic acid equivalent kg^{-1} FW.

2.3. Chilling injury (CI) incidence during cold storage

The external CI index was individually evaluated in each fruit according to a 6-point hedonic scale (**Fig. 1**) based on the percentage of surface affected by CI symptoms (dehydration, browning and pitting): 0 (no symptoms), 1 (1–20 %), 2 (21–40 %), 3 (41–60 %), 4 (61–80 %) and 5 (> 81 %). Photographs of green pepper fruits, captured by the authors from previous studies, served as the visual reference for the evaluation of CI symptoms. The degree of CI symptoms was subsequently assessed based on these photographs. The CI index was calculated as follows: Σ (value of hedonic scale) x (number of fruit with the corresponding score) / (total number of fruit in the sample). The results were expressed as the mean value ± SE of three replicates of six green pepper fruits, according to the protocol previously described by García-Pastor et al. (2020).

Fig. 1. Hedonic scale to evaluate external chilling injury (CI) index of 'Lamuyo' green pepper fruit during 28 days of storage at 2 ºC + 2 days at 20 ºC.

2.4. Total phenolics, total antioxidant activity and total carotenoids at harvest and after 28 days of cold storage

Total phenolics content (TPC), total antioxidant activity (TAA) and total carotenoid content (TCC) were extracted and measured at harvest (day 0) and after 28 days of storage at 2 ºC + 2 days at 20 ºC, according to the protocol recently described in green pepper fruit (Dobón-Suárez et al., 2021a; Dobón-Suárez et al., 2025). Briefly, 5 g of green pepper fruits were homogenized with 10 mL of 50 mM phosphate buffer pH = 7.8 and 5 mL of ethyl acetate using a homogenizer (Ultraturrax, T18 basic, IKA, Berlin, Germany) for 30 s. The extracts were centrifuged at 10,000 *g* for 15 min at 4 ºC and the upper and lower fractions were used to quantify total lipophilic (L-TAA) and hydrophilic (H-TAA) total antioxidant activity, respectively.

The TPC quantification was determined from the hydrophilic phase (H-TAA) obtained from the total antioxidant activity extraction and its quantification was performed in duplicate on the lower fraction for each extract using the Folin–Ciocalteu reagent. Results were expressed as g gallic acid equivalent (GAE) kg^{-1} of FW and were the mean ± SE of three replicates (n = 3). In addition, TAA was measured according to Sayyari et al. (2011a), which enables to determine hydrophilic (H-TAA) and lipophilic (L-TAA) antioxidant activity in the same extraction. In both cases, TAA was determined in a reaction mixture comprising 2,20-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horse radish peroxidase enzyme and its oxidant substrate (hydrogen peroxide), in which $ABTS^{+}$ radicals are generated and monitored at 730 nm. Subsequently, green pepper fruit extract was added, and the decrease in absorbance after 90 s was calculated, which was proportional to the TAA of the sample. A calibration curve was constructed using trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0–20 nmol) from Sigma Aldrich (Madrid, Spain). The results were expressed as g of trolox equivalent (TE) kg^{-1} of FW and were the mean ± SE of three replicates (n = 3).

The total carotenoid content (TCC) was extracted according to a previously described method (Knee, 1972) with some modifications. Approximately 0.20 g of fine freeze-dried powder for the three biological replicates (n = 3) were manually grounded in a mortar and pestle and mixed with 5 mL of acetone extract solution containing 0.1 % butylated hydroxytoluene (BHT) to prevent the pigment from oxidizing. Then, the mixed extraction was ultrasonically extracted for 15 min and then centrifuged at 10,000 *g* for 10 min at 4 ºC to obtain the supernatant. The sample was repeatedly extracted until the residue was colourless. Acetone solution containing 0.1% BHT was used for a constant volume of collected supernatants (25 mL) to the subsequent estimation of TCC. Based on the methods reported by Lichtenthaler and Wellburn (1983), the absorbance of the extracts was detected at 662 nm by spectrophotometric absorbance (UV-1900i-UV-VIS Spectrophotometer, Shimadzu Corporation, Germany) to quantify the total carotenoid contents, which were calculated from the equation: TCC = (1000A470 - 2.27Ca − 81.4Cb)/227. Ca and Cb indicate the content of chlorophyll a and chlorophyll b, respectively, and A470 represent the absorbance for TCC at 662 nm. The results of TCC were expressed as g kg^{-1} FW.

2.5. Fatty acids (FAs) profile and quantification at harvest and after 28 days of cold storage

Samples of freeze-dried green bell pepper flesh and skin tissues, previously ground into powder, were directly methylated according to Trigueros and Sendra (2015). The profile and quantification of FAs were ascertained through high-resolution gas chromatography, analysing the fatty acid methyl esters obtained by trans-esterification of 20 mg of sample with 2 mL of 0.5 M sodium methoxide, following the protocol described by García-Pastor et al. (2020) with slight modifications. Methyl esters were separated on a gas chromatograph (GC) Shimadzu GC-2030 coupled with a flame ionisation detector (FID) an automatic injector AOC-20i. The same conditions reported by García-Pastor et al. (2020) were applied, as follows: Helium was used as carrier gas, injector and FID-detector temperatures were 220 ºC and 250 ºC, respectively, and oven temperatures were 140 ºC for 2 min, which increased to 165 ºC at 6 ºC min^{-1} and thereafter from 165 ºC to 225 ºC at 2.8 ºC min^{-1} and was held at 225 ºC for 25 min. Volume of injected sample was 1 µL with split 1:20. Fatty acid methyl esters were identified by comparison of their retention times with Supelco 37-component FAME Mix reference standard (Sigma-Aldrich Co., St. Louis, MO, USA). Quantification was carried out in duplicate in each sample based on peak areas using nonadecanoic acid (19:0 c-19) as internal standard and results (mean ± SE) were expressed as mg nonadecanoic acid equivalent kg^{-1} FW. As the fatty acid results of the samples did not show substantial differences in terms of application method, these results are presented only for the irrigation SA and MeJA treatments, as this is the simpler

method and represents a lower cost of application for the agri-food industry compared to foliar application (Dobón-Suárez et al., 2025).

2.6. Statistical analysis

Results are expressed as mean ± SE of three replicates (n = 3). Data for the statistical analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatment. Mean comparisons were performed using HSD Tukey's test to examine if differences among the storage time, treatment and their interactions (storage time*treatment) were statistically significant at $p < 0.05$. The resulting differences were represented as *, **, and *** symbols when $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. No statistically significant differences were identified when the probability value (p) was equal to or greater than 0.05 and, thus, represented as NS. All analyses were conducted using the SPSS software package, version 17.0 for Windows (SPSS, 2001, IBM Corporation, Armonk, NY, USA). The PCA model was also constructed with normalized data using the version 17.0 of SPSS software package.

3. Results

3.1. Impact of foliar or irrigation application of salicylic acid and methyl jasmonate on postharvest ripening, chilling injury incidence and antioxidant metabolism of green bell pepper

The weight losses of the green pepper fruits significantly ($p < 0.001$) increased during postharvest storage for all treatments tested, reaching the highest values of 8.44 ± 0.45 % in untreated fruits at 28 storage days (**Fig. 2A and Table 2**).

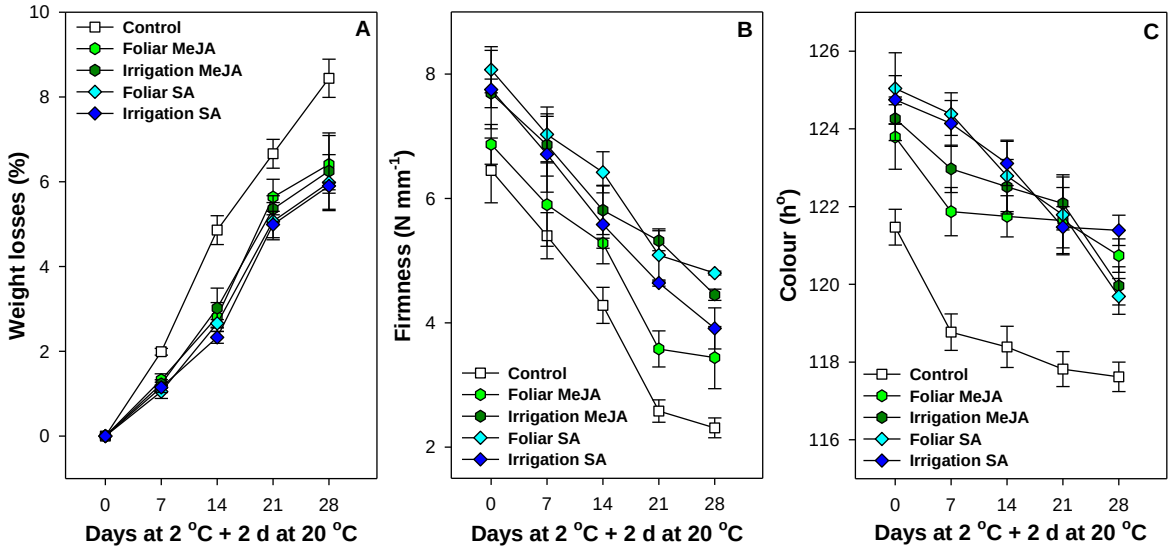


Fig. 2. Effect of salicylic acid (SA) and methyl jasmonate (MeJA) preharvest treatments, applied via foliar spraying and irrigation, on weight loss (%) **[A]**, firmness (N mm^{-1}) **[B]**, and colour (hº) **[C]** of 'Lamuyo' green bell pepper during 28 days of storage at 2 ºC + 2 days at 20 ºC.

Table 2. Analyses of variance (ANOVA) for weight losses (%), physiochemical parameters [firmness (N mm^{-1}), colour (hue°), total soluble solids (TSS; g kg^{-1}) and total acidity (TA; g kg^{-1})], chilling injury (CI) incidence (hedonic scale), and the antioxidant system [total phenolic content (TPC; g kg^{-1} FW), hydrophilic-total antioxidant activity (H-TAA; g kg^{-1} FW), total carotenoids content (TCC; g kg^{-1} FW) and lipophilic-total antioxidant activity (L-TAA; g kg^{-1} FW)] using the storage time (days) and the treatment as factors for the 2022 growing season.[Y]

Parameter	Storage time (d)	Treatment	Storage time (d)* Treatment
Weight losses (%)	549.80*** 7 = a 14 = b 21 = c 28 = d	28.32*** Control = B Foliar MeJA = A Irrigation MeJA = A Foliar SA = A Irrigation SA = A	3.52***
Firmness (N mm^{-1})	101.15*** 0 = d 7 = c 14 = b 21 = a 28 = a	34.11*** Control = A Foliar MeJA = B Irrigation MeJA = C Foliar SA = C Irrigation SA = C	0.72 NS
Colour (h^o)	104.43*** 0 = d 7 = c 14 = c 21 = b 28 = a	127.40*** Control = A Foliar MeJA = B Irrigation MeJA = CD Foliar SA = C Irrigation SA = D	7.15***
TSS (g kg^{-1})	198.50*** 0 = a 7 = b 14 = c 21 = d 28 = e	1.97 NS Control = A Foliar MeJA = A Irrigation MeJA = A Foliar SA = A Irrigation SA = A	9.79***
TA (g kg^{-1})	838.31*** 0 = d 7 = d 14 = c 21 = b 28 = a	1131.40*** Control = A Foliar MeJA = E Irrigation MeJA = B Foliar SA = D Irrigation SA = C	60.21***
CI (hedonic scale)	4772.30*** 0 = a 7 = b 14 = c 21 = d 28 = e	267.94*** Control = D Foliar MeJA = C Irrigation MeJA = B Foliar SA = C Irrigation SA = A	28.61***
TPC (g kg^{-1} FW)	22.60***	120.64*** Control = A Foliar MeJA = B Irrigation MeJA = C Foliar SA = C Irrigation SA = C	3.78**
H-TAA (g kg^{-1} FW)	247.95***	97.47*** Control = A Foliar MeJA = B Irrigation MeJA = BC Foliar SA = D Irrigation SA = C	20.58***
TCC (g kg^{-1} FW)	6.20*	24.35*** Control = A Foliar MeJA = B Irrigation MeJA = B Foliar SA = C Irrigation SA = B	33.33***
L-TAA (g kg^{-1} FW)	176.02***	45.70*** Control = A Foliar MeJA = B Irrigation MeJA = B Foliar SA = B Irrigation SA = B	8.71***

[Y] NS = not significant; *, ** and *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; data were previously tested for normality test. Different lowercase letters indicate significant differences among storage time (days) for each parameter tested. Capital letters show significant differences among treatments for each parameter evaluated.

However, those green pepper fruits treated with SA or MeJA showed a significantly ($p < 0.001$) lower percentage of weight losses (≈ 6 %) compared with control at the end of the cold storage; although no significant differences were observed between both methods applied (foliar or irrigation) for none of the applied

phytohormones (**Fig. 2A and Table 2**). Therefore, the interaction between storage time (d) and treatment for weight losses was significant with a value of $p < 0.001$, as can be observed in **Table 2**.

The firmness of SA- and MeJA-treated bell peppers showed a 1.06- and 1.19-fold increase for foliar and irrigation MeJA treatment, respectively, at harvest, while those fruits treated with foliar and irrigation SA had an increase of 1.25- and 1.20-fold, respectively, compared to control **(Fig. 2B)**. Therefore, significant ($p < 0.001$) differences on firmness were observed among treatments, since SA and MeJA enhanced these values compared to untreated fruits, although the irrigation MeJA, foliar SA, and irrigation SA were the most effective in this improvement (**Fig. 2B and Table 2**). During 28 days of postharvest storage, the firmness values significantly ($p < 0.001$) decreased from harvest for all treatments tested, although these values were significantly ($p < 0.001$) higher in all SA and MeJA-treated fruits compared to control (**Fig. 2B and Table 2**). At the end of the postharvest storage, MeJA applied via irrigation and SA applied both via foliar and irrigation caused an increase of firmness of 1.92-, 2.08-, and 1.69-fold than untreated fruits, respectively (**Fig. 2B**).

External green colour of bell peppers was expressed in terms of hue angle parameter (hº) and significant ($p < 0.001$) differences were detected for the interaction storage time (d) and treatment in the statistical analysis of this parameter (**Table 2**). The hue angle values significantly ($p < 0.001$) decreased from harvest until 28 days of storage at 2 ºC + 2 days at 20 ºC for all treatments (**Fig. 2C and Table 2**). Untreated green pepper fruits had the lowest hº values both at harvest (121.47 ± 0.46 hº) and after 28 storage days (117.62 ± 0.38 hº), showing significant ($p < 0.001$) differences among treatments (**Fig. 2C and Table 2**). These differences in terms of colour were reflected in the visual appearance of the 'Lamuyo' green pepper fruits, since a lower hº value shows a lighter green colour and does not reflect the dark green hue that was observed both at harvest and after 28 days of cold storage in the SA and MeJA-treated peppers (**Supplementary Fig. 1**). Thus, both MeJA and SA applied via irrigation showed a higher average value of ≈ 122 hº during cold storage than the foliar pulverization of both phytohormones (**Fig. 2C**). However, MeJA and SA showed the same effectiveness delaying colour losses (**Fig. 2C**).

Both the storage time (d) factor and the interaction storage time (d) * treatment were found to be significant at a value of $p < 0.001$ on TSS (**Table 2**). However, the treatment factor did not demonstrate significant ($p \geq 0.05$) differences during postharvest storage (**Table 2**). The content of TSS significantly increased from an average of ≈ 46 g kg^{-1} in all treatments until reach a mean value of ≈ 54 g kg^{-1} at the end of the storage; except for MeJA and SA treatments applied by foliar spraying, which showed an increase of 11 % on TSS after 28 days of cold storage (**Fig. 3A**). On the other hand, the TA content was significantly ($p < 0.001$) influenced by both factors studied; storage time (d) and treatments, and, thus, their interaction was also significant ($p < 0.001$) (**Table 2**). The average values of TA were 1.19 ± 0.01 g kg^{-1} and 1.30 ± 0.01 g kg^{-1} for control and MeJA- and SA-based treatments at harvest, respectively (**Fig. 3B**). The days of storage significantly ($p < 0.001$) decreased the TA values from harvest until the end of storage by 17 % and 15 % for control and phytohormones treatments, respectively (**Fig. 3B and Table 2**). However, the significant ($p < 0.001$) differences appreciated among treatments at harvest were magnified during postharvest storage, since the highest levels of TA after 28 storage days were found in those green peppers that were foliar sprayed in preharvest with MeJA (1.19 ± 0.02 g kg^{-1}), followed by the foliar pulverization of SA (1.13 ± 0.01 g kg^{-1}) (**Fig. 3B and Table 2**).

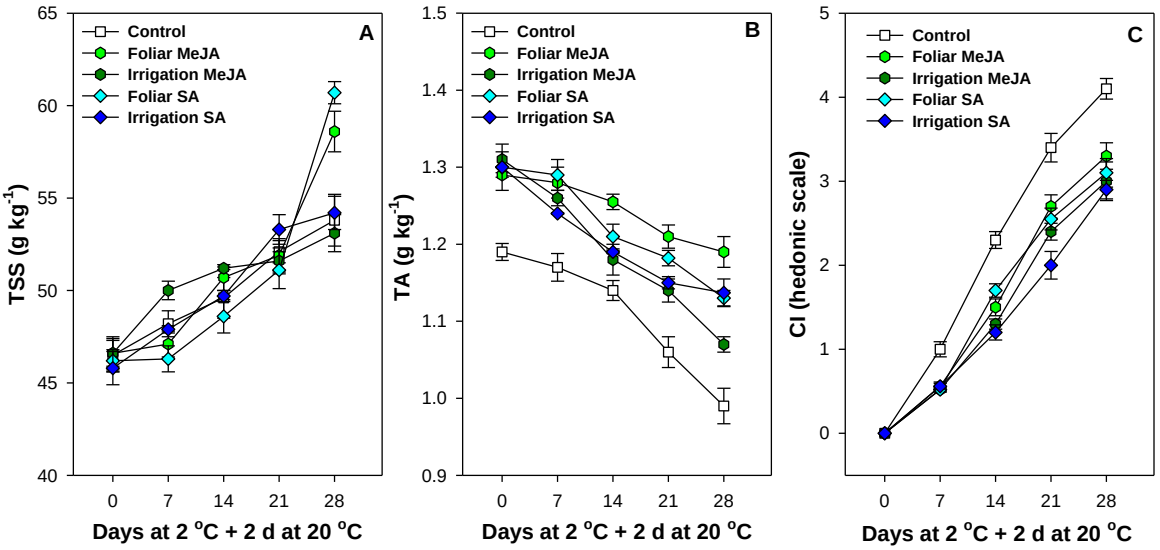


Fig. 3. Effect of salicylic acid (SA) and methyl jasmonate (MeJA) preharvest treatments, applied via foliar spraying and irrigation, on total soluble solids (TSS) (g kg^{-1}) **[A]**, total acidity (TA) (g kg^{-1}) **[B]**, and chilling injury (CI) incidence (hedonic scale) **[C]** of 'Lamuyo' green bell pepper during 28 days of storage at 2 ºC + 2 days at 20 ºC.

The CI incidence was evaluated with a 5-point hedonic scale (**Fig. 1**) and this parameter was significantly ($p < 0.001$) affected by both storage time (d) and treatment (**Table 2**). Therefore, the statistical interaction between both factors was also significant ($p < 0.001$) (**Table 2**). The incidence of CI showed a pronounced and significant ($p < 0.001$) increase as the time of prolonged cold storage of the bell pepper increased, although a significant ($p < 0.001$) reduction of the incidence was observed with phytohormone treatments (**Fig. 3C and Table 2**). Both MeJA and SA treatments applied via foliar spray showed a decrease of 1.24- and 1.32-fold on CI incidence than control after 28 days of cold storage, although the highest reduction was with the irrigation of both treatments (≈ 1.40-fold decrease) (**Fig. 3C**). Generally, SA applications via both foliar and irrigation were more effective on reducing CI during cold storage than foliar and irrigation MeJA treatments (**Fig. 3C and Table 2**). This significant reduction on the CI symptoms of 'Lamuyo' green pepper fruit was reflected in an improvement in the visual appearance of the fruit after 28 days of cold storage, as can be observed in the **Supplementary Fig. 1**.

The TPC was significantly ($p < 0.001$) affected by both factors (**Table 2**); storage time (d), in which only the content was quantified at harvest (day 0) and after 28 storage days, and treatments, and their interaction was also significant ($p < 0.01$). All treatments tested significantly ($p < 0.001$) enhanced the TPC (≈ 0.80-0.96 g kg^{-1} FW) compared with control (≈ 0.68 g kg^{-1} FW) both at harvest and after 28 days of storage at 2 ºC + 2 days at 20 ºC, although the application of SA by both methods and MeJA via irrigation were the most effective (**Fig. 4A and 4B and Table 2**).

With regard to H-TAA, this functional parameter changed significantly ($p < 0.001$) from harvest to 28 days of storage under refrigeration and was also dependent on the treatment studied ($p < 0.001$), leading to a significant ($p < 0.001$) interaction between both factors (**Table 2**). All treatments based on the preharvest application of SA and MeJA significantly ($p < 0.001$) improved the H-TAA (≈ 0.39-0.53 g kg^{-1} FW) compared to control (0.30 ± 0.01 g kg^{-1} FW) at harvest (**Fig. 4C and Table 2**). However, the highest H-TAA value was achieved in those green pepper fruits treated with SA via foliar (0.53 ± 0.05 g kg^{-1} FW) (**Fig. 4C**). After 28 days of storage at 2 ºC + 2 days at 20 ºC, the H-TAA significantly ($p < 0.001$) increased for all treatments tested, including the control (an increase of 1.30-fold); although the highest enhancement (≈ 1.74-fold increase) was observed for those peppers harvested from SA-treated plants via foliar spraying, followed by peppers also treated with SA via irrigation and, finally, by MeJA-treated peppers (**Fig. 4D and Table 2**).

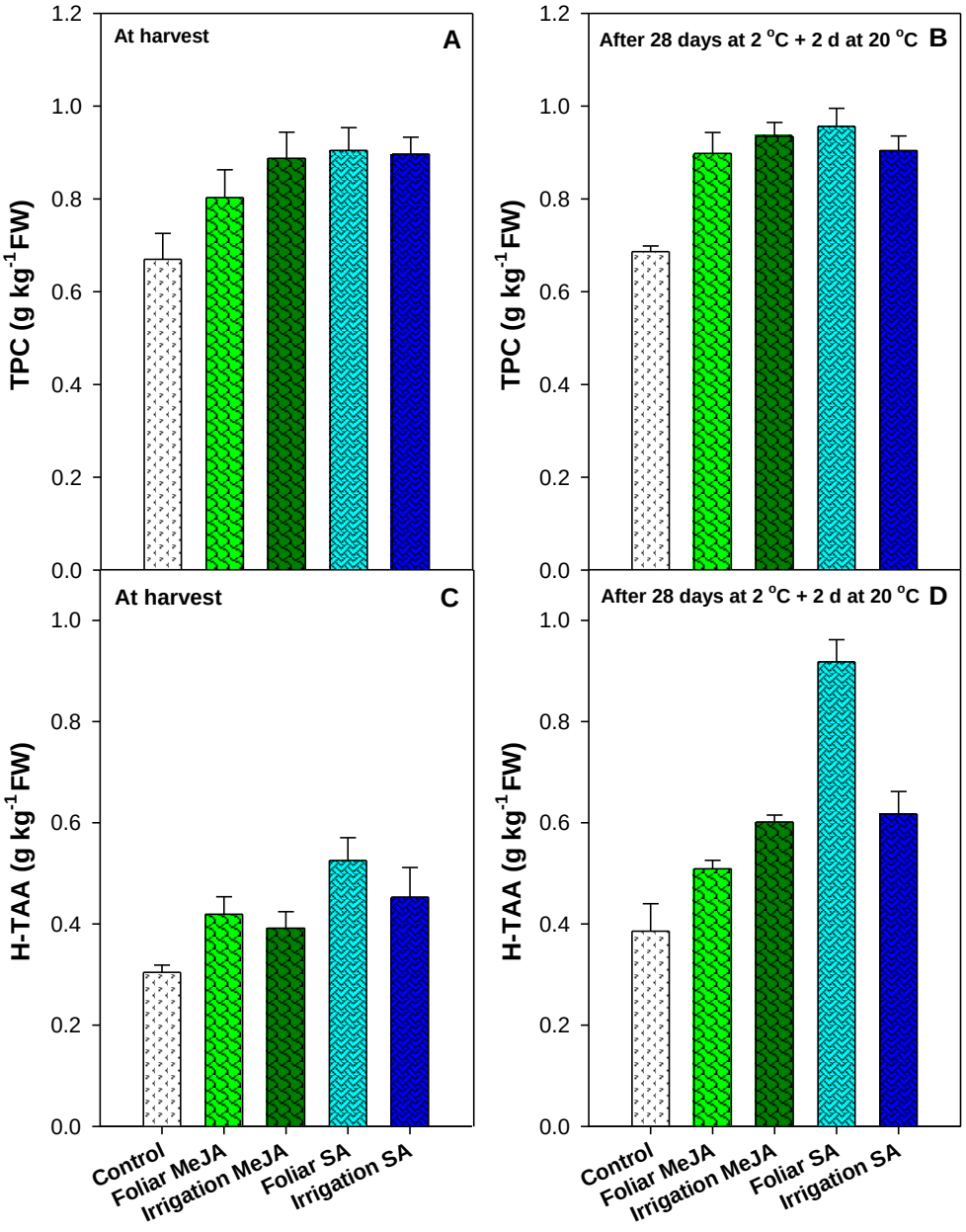


Fig. 4. Effect of salicylic acid (SA) and methyl jasmonate (MeJA) preharvest treatments, applied via foliar spraying and irrigation, on total phenolic content (TPC) (g kg^{-1} FW) at harvest **[A]** and after 28 days of storage at 2 ºC + 2 days at 20 ºC **[B]**, and on hydrophilic-total antioxidant activity (H-TAA) (g kg^{-1} FW) at harvest **[C]** and after 28 days of storage at 2 ºC + 2 days at 20 ºC **[D]** of 'Lamuyo' green bell pepper.

Similarly, the TCC was significantly ($p < 0.05$) influenced by storage time (d) and this impact was more intensely ($p < 0.001$) when the influence of the treatment was statistical analysed, leading into a significant ($p < 0.001$) interaction between both factors (**Table 2**). The treatments studied did not show any differences in TCC (≈ 0.23 g kg^{-1} FW) at harvest (**Fig. 5A**), although this content was significantly ($p < 0.001$) higher in green pepper fruits treated with SA and MeJA than in untreated fruits after 28 days of cold storage, the SA treatment applied by foliar spraying being the most effective in increasing these lipophilic bioactive compounds (0.31 ± 0.02 g kg^{-1} FW) (**Fig. 5B and Table 2**).

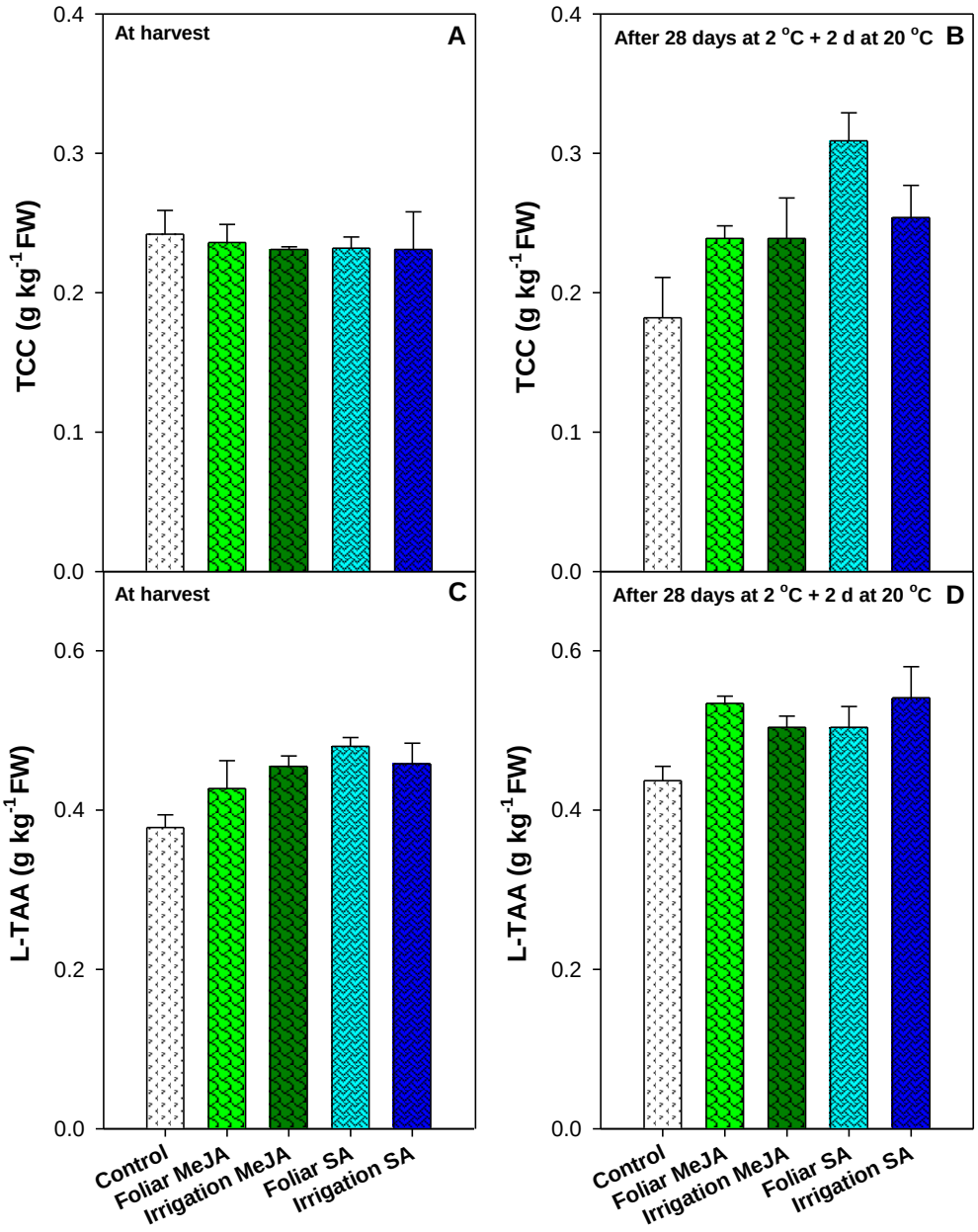


Fig. 5. Effect of salicylic acid (SA) and methyl jasmonate (MeJA) preharvest treatments, applied via foliar spraying and irrigation, on total carotenoid content (TCC) (g kg^{-1} FW) at harvest **[A]** and after 28 days of storage at 2 ºC + 2 days at 20 ºC **[B]**, and on lipophilic-total antioxidant activity (L-TAA) (g kg^{-1} FW) at harvest **[C]** and after 28 days of storage at 2 ºC + 2 days at 20 ºC **[D]** of 'Lamuyo' green bell pepper.

The **Table 2** indicates that the storage time (d), the treatment and the interaction of storage time (d) * treatment were significant ($p < 0.001$) on the L-TAA analysed. At harvest, both SA and MeJA significantly ($p < 0.001$) improved the L-TAA compared with the control, irrespective of the method applied (**Fig. 5C and Table 2**). The storage time of 28 days at 2 ºC + 2 days at 20 ºC significantly ($p < 0.001$) enhanced the L-TAA for all treatments (0.50-0.54 g kg^{-1} FW) than control (0.44 ± 0.02 g kg^{-1} FW) (**Fig. 5D and Table 2**). Nevertheless, no differences can be observed neither among treatment nor the method of application studied at the end of the experiment (**Fig. 5D**).

3.2. Impact of irrigation application of salicylic acid and methyl jasmonate on lipid metabolism of green bell pepper

Sixteen fatty acids were identified and quantified in 'Lamuyo' green bell pepper fruit, the major ones being C18:2n6c (linoleic), C18:3n3 alpha [alpha-linolenic acid (ALA)], C14:0 (myristic), C16:0 (palmitic) and C18:0

(stearic) acids followed by C18:1t9 (trans-oleic acid). In addition, other eleven minor fatty acids were also identified in the samples of green pepper fruit: C15:1 (pentadecenoic acid), C16:1 (palmitoleic acid), C17:1 (margaroleic acid), C18:1c9 (oleic acid), C18:3n6 gamma [gamma-linolenic acid (GLA)], C20:2 (eicosadienoic acid), C20:3n3 (omega-3 eicosatrienoic acid), C20:3n6 [dihomo-gamma-linolenic acid (DGLA)], C8:0 (caprylic acid) and C24:0 (lignoceric acid). The profile of these fatty acids was similar in Control and MeJA and SA treated fruit, either at harvest or after 28 days of cold storage. The peaks of the five major FAs (linoleic, linolenic, palmitic, myristic and steric acids) identified in the chromatograms from the untreated green pepper fruits at harvest are shown in **Supplementary Fig. 2.**

The analysis of saturated fatty acid content (SFA) in the samples revealed significant variations across different storage time (at harvest and after 28 storage days) and treatments (**Fig. 6A and 6B and Table 3**). Nevertheless, the magnitude of the significance of each factor was dependent on each individual SFA analysed, as can be observed in **Table 3**. At harvest, the control group exhibited the highest levels of C14:0 and C16:0 fatty acids (9.21 ± 0.39 and 11.03 ± 0.85 mg kg^{-1} FW, respectively), while the irrigation with SA resulted in a substantial reduction in these SFA (4.27 ± 0.62 and 7.43 ± 1.20 mg kg^{-1} FW, respectively) (**Fig. 6A**). Moreover, the application of MeJA via irrigation also showed a ≈ 1.20-fold decrease on the content of both C16:0 and C18:0 compared to control at harvest (**Fig. 6A**). Notably, the C18:0, followed by C8:0 and C24:0 fatty acids, showed relatively low concentrations across all treatments tested at harvest (**Fig. 6A**). However, no differences among treatments were observed on the C8:0 and C24:0 content at harvest (**Fig. 6A**).

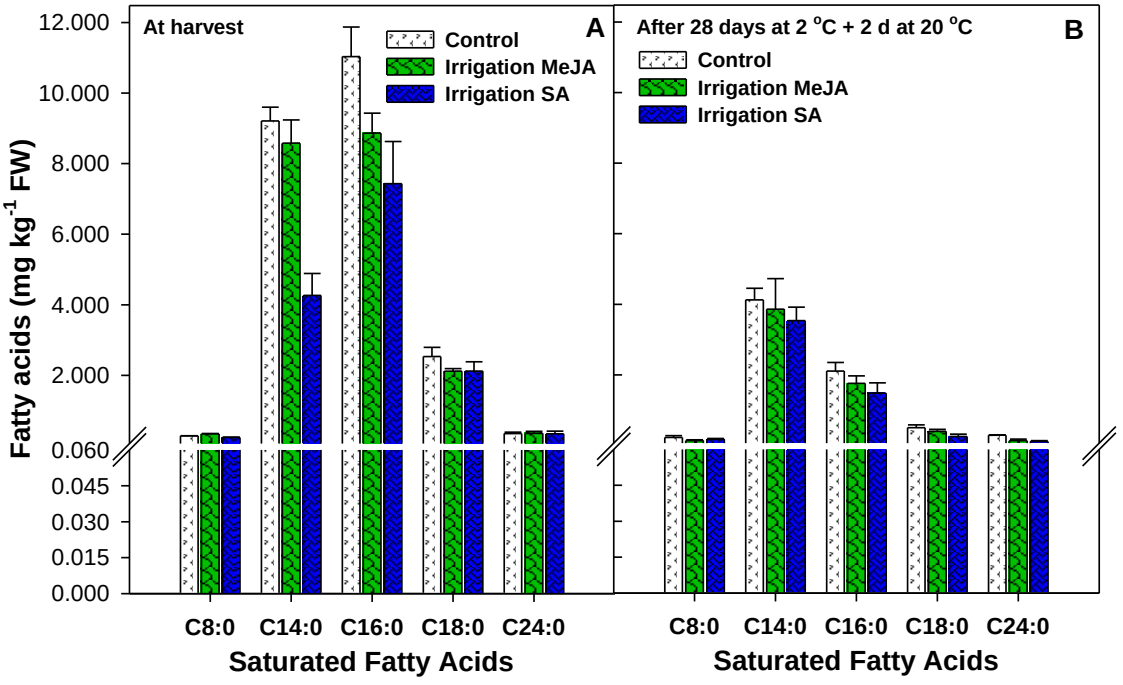


Fig. 6. Effect of salicylic acid (SA) and methyl jasmonate (MeJA) preharvest treatments, applied via irrigation, on the content of saturated fatty acids (SFA; mg kg^{-1} FW) at harvest **[A]** and after 28 days of storage at 2 ºC + 2 days at 20 ºC **[B]** of 'Lamuyo' green bell pepper.

Table 3. Analyses of variance (ANOVA) for the fatty acids (FAs) composition [saturated fatty acid (SFA) (C8:0, C14:0, C16:0, C18:0 and C24:0) and unsaturated fatty acids (UFA) (C15:1, C16:1, C17:1, C18:1t9, C18:1c9, C18:2n6c, C18:3n6 gamma, C18:3n3 alfa, C20:2, C20:3n3 and C20:3n6)] using the storage time (days) and the treatment as factors for the 2022 growing season.[Y]

Parameter	Storage time (d)	Treatment	Storage time (d)* Treatment
C8:0 (mg kg^{-1} FW)	60.39***	1.32 NS Control = A Irrigation MeJA = A Irrigation SA = A	19.34***
C14:0 (mg kg^{-1} FW)	185.39***	84.82*** Control = B Irrigation MeJA = B Irrigation SA = A	33.80***
C16:0 (mg kg^{-1} FW)	312.83***	6.37** Control = B Irrigation MeJA = AB Irrigation SA = A	6.43**
C18:0 (mg kg^{-1} FW)	407.79***	4.10* Control = B Irrigation MeJA = A Irrigation SA = AB	1.17 NS
C24:0 (mg kg^{-1} FW)	10.88**	3.44* Control = B Irrigation MeJA = AB Irrigation SA = A	1.93 NS
C15:1 (mg kg^{-1} FW)	181.36***	3.24 NS Control = A Irrigation MeJA = A Irrigation SA = A	6.37**
C16:1 (mg kg^{-1} FW)	0.09 NS	0.49 NS Control = A Irrigation MeJA = A Irrigation SA = A	0.21 NS
C17:1 (mg kg^{-1} FW)	107.45***	1.82 NS Control = A Irrigation MeJA = A Irrigation SA = A	5.24*
C18:1t9 (mg kg^{-1} FW)	621.28***	11.02*** Control = A Irrigation MeJA = B Irrigation SA = A	23.58***
C18:1c9 (mg kg^{-1} FW)	446.00***	11.72*** Control = A Irrigation MeJA = B Irrigation SA = A	29.60***
C18:2n6c (mg kg^{-1} FW)	116.93***	2.63 NS Control = A Irrigation MeJA = A Irrigation SA = A	1.50 NS
C18:3n6 gamma (mg kg^{-1} FW)	44.49***	0.139 NS Control = A Irrigation MeJA = A	2.76 NS

		Irrigation SA = A	
C18:3n3 alpha (mg kg^{-1} FW)	121.62***	0.03 NS Control = A Irrigation MeJA = A Irrigation SA = A	0.47 NS
C20:2 (mg kg^{-1} FW)	19.44***	4.85* Control = A Irrigation MeJA = B Irrigation SA = AB	3.78*
C20:3n3 (mg kg^{-1} FW)	16.05**	4.35* Control = A Irrigation MeJA = AB Irrigation SA = B	2.10 NS
C20:3n6 (mg kg^{-1} FW)	13.23**	1.60 NS Control = A Irrigation MeJA = A Irrigation SA = A	0.60 NS
Ratio UFA/SFA	53.82***	18.64*** Control = A Irrigation MeJA = A Irrigation SA = B	3.44*

[Y] NS = not significant; *, ** and *** significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; data were previously tested for normality test. Capital letters show significant differences among treatments for each parameter evaluated.

After 28 days of storage at 2 ºC + 2 days at 20 ºC, a general significant ($p < 0.001$) decrease in C14:0, C16:0 and C18:0 was observed in all treatments with respect to the content at harvest, although the content of C8:0 and C24:0 showed only a slightly fluctuation (≈ 1.20-fold decrease) (**Fig. 6A and 6B and Table 3**). Thus, a 2.23-fold decrease was registered for the C14:0 fatty acid from harvest to 28 storage days in untreated green pepper fruits, while an approximately 5.10-fold decrease was observed for both C16:0 and C18:0 fatty acids (**Fig. 6A and 6B**). However, the samples treated with SA via irrigation decreased the content of C16:0, C18:0 and C24:0 by 30 %, 48 % and 59 %, respectively, compared to the content quantified of these SFA in the untreated green pepper fruits (**Fig. 6B**). In addition, MeJA via irrigation also led a decrease of 20 % on the content of C18:0 and around the 52 % on the C24:0 fatty acid (**Fig. 6B**). These results suggest that MeJA and SA irrigation effectively modulate the SFA content in the samples compared to the control, both at harvest and after 28 days of cold storage, potentially highlighting the role of SA via irrigation rather than MeJA on decreasing the content of C14:0 ($p < 0.001$), C16:0 ($p < 0.01$) and C24:0 ($p < 0.05$) fatty acids (**Fig. 6A and 6B and Table 3**).

Regarding the unsaturated fatty acids (UFA), the content of C16:1 was not statistically influenced neither the storage time (days) or treatment (**Fig. 7A and 7B and Table 3**). As can be observed both in **Fig. 7A and 7B**, as well as in **Table 3**, the UFA that were significantly ($p < 0.001$) affected only by the storage time (days) but not by the treatment were as follows: C15:1, C17:1, C18:2n6c, C18:3n6 gamma, and C18:3n3 alpha. The content quantified of all these UFA significantly decreased from harvest to 28 storage days at 2 ºC + 2 days at 20 ºC (**Fig. 7A and 7B**). In addition, the content of C20:3n6 was significantly ($p < 0.01$) influenced by the storage time (**Fig. 7A and 7B and Table 3**). Nevertheless, the SA and MeJA treatments showed an increase on the C18:2n6c content (an average of 42.66 mg kg^{-1} FW) compared with control (31.72 ± 2.51 mg kg^{-1} FW) at harvest (**Fig. 7A**). Similarly, the content of C17:1 was higher in green pepper fruits-treated SA after 28 days of storage at 2 ºC + 2 days at 20 ºC (0.31 ± 0.02 mg kg^{-1} FW), followed by those samples treated with MeJA (0.29 ± 0.02 mg kg^{-1} FW), compared with

untreated green pepper fruits (0.23 ± 0.02 mg kg^{-1} FW) (**Fig. 7B**). Specially, MeJA applied via irrigation showed a 3.8-fold increase on the content of C18:3n3 gamma after 28 storage days compared to the other treatments (**Fig. 7B**).

Both the storage time (days) and treatment, as well as their interaction, were significant ($p < 0.001$) on the content of C18:1t9 and C18:1c9 (**Fig. 7A and 7B and Table 3**). However, the content of both C20:2 and C20:3n3 was also significantly influenced by the storage time ($p < 0.001$ and $p < 0.01$, respectively) and treatment ($p < 0.05$), being their interaction only significant ($p < 0.05$) for the C20:2 fatty acid (**Fig. 7A and 7B and Table 3**). In general, the application of MeJA via irrigation increased the content of C18:1t9, C18:1c9 and C20:2 compared to the other treatments tested, although the SA treatment enhanced the C20:3n3 content (**Fig. 7A and 7B and Table 3**). However, both C18:1t9 and C18:1c9 contents of irrigation MeJA-treated green pepper fruits showed an approximately 1.10-fold increase at harvest compared to control (**Fig. 7A**). At this point, SA applied via irrigation enhanced the content of both C20:2 (0.37 ± 0.06 mg kg^{-1} FW) and C20:3n3 (0.56 ± 0.10 mg kg^{-1} FW) than untreated samples (0.29 ± 0.04 mg kg^{-1} FW and 0.39 ± 0.05 mg kg^{-1} FW, respectively) (**Fig. 7A**).

The percentage of decline of both C18:1t9, C18:1c9 and C20:3n3 fatty acids from harvest until 28 days of cold storage plus shelf-life was 72.49 %, 78.00 % and 79.67 %, respectively (**Fig. 7A and 7B**). However, the irrigation of MeJA (decline of 62.03 %, 69.51 % and 45.17 %, respectively) and SA (48.96 %, 46.46 % and 60.59 %, respectively) lead to green pepper fruits with a lower decline of those UFA after 28 storage days (**Fig. 7A and B**). Therefore, after 28 days of cold storage, an enhancement on the content of C18:1t9 (≈ 0.43 mg kg^{-1} FW), C18:1c9 (≈ 0.29 mg kg^{-1} FW) and C20:3n3 (≈ 0.21 mg kg^{-1} FW) was observed in those samples treated with MeJA and SA compared to control (0.27 ± 0.01 01 mg kg^{-1} FW; 0.17 ± 0.01 mg kg^{-1} FW; and 0.08 ± 0.01 mg kg^{-1} FW, respectively), although no differences were appreciated among both phytohormones (**Fig. 7B**).

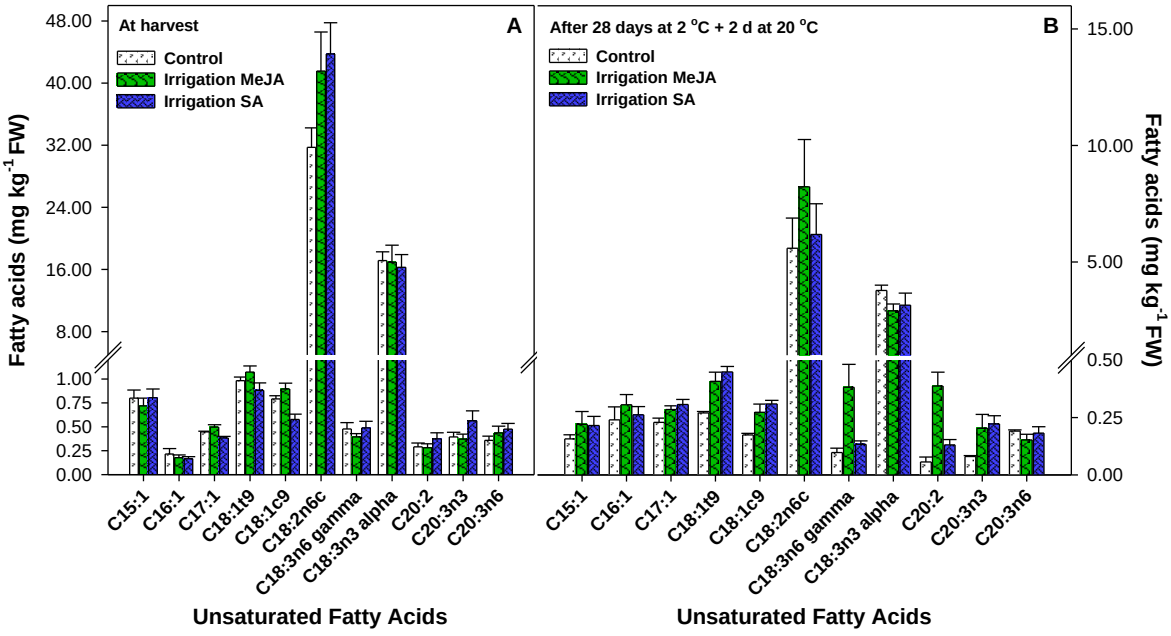


Fig. 7. Effect of salicylic acid (SA) and methyl jasmonate (MeJA) preharvest treatments, applied via irrigation, on the content of unsaturated fatty acids (UFA; mg kg^{-1} FW) at harvest **[A]** and after 28 days of storage at 2 ºC + 2 days at 20 ºC **[B]** of 'Lamuyo' green bell pepper.

In terms of C20:2 fatty acid, the percentage of decline in untreated green bell peppers was 80.62 % compared to the values at harvest. Although the application of SA via irrigation showed a lower percentage of decrease (64.99 %) compared to control, the MeJA treatment led to an increase of 27.46 % on the content of this UFA after 28 storage days **Fig. 7A and B**). Thus, an increase on the content of C20:2 fatty acid was detected by both phytohormones compared to control (0.06 ± 0.02 mg kg^{-1} FW), although the highest content was achieved in those samples treated with MeJA (0.39 ± 0.06 mg kg^{-1} FW) followed by SA (0.13 ± 0.02 mg kg^{-1} FW) (**Fig. 7B**). Finally,

the UFA/SFA ratio was significantly ($p < 0.001$) influenced by both storage time (days) and treatment factors, as well as their interaction ($p < 0.05$) (**Table 3**). This ratio was significantly ($p < 0.001$) lower in all treatments tested after 28 days of storage at 2 ºC plus 2 days at 20 ºC (shelf-life) (**Supplementary Fig. 3**). At harvest, MeJA and SA treatments enhanced the UFA/SFA ratio with values of 3.36 ± 0.48 and 4.68 ± 0.41, respectively, compared with control (2.31 ± 0.16), although the highest increase was observed in 0.5 mM SA-treated green bell peppers (**Supplementary Fig. 3**). In fact, those pepper fruits treated with SA were the samples with a significantly ($p < 0.001$) higher ratio of UFA/SFA after 28 days of storage (2.43 ± 0.12) than the other treatments (≈ 1.45 ± 0.10) (**Supplementary Fig. 3 and Table 3**).

4. Discussion

Salicylic acid (SA) and methyl jasmonate (MeJA) are crucial endogenous signaling molecules that regulate stress responses and developmental processes in plants (Fung et al., 2004). Their application has been explored to modulate postharvest quality and extend the shelf-life of various horticultural crops, including peppers (*Capsicum annuum* L.). Some studies have investigated the impact of preharvest SA application on weight loss in sweet peppers during cold storage. Thus, Hanaei et al. (2022) observed that foliar spraying of SA at concentrations of 1.5 mM and 3 mM, both alone and in combination with caraway oil coating, generally led to reduced weight losses compared to the control during 60 days of storage at 4 ± 2 °C. This suggests that SA treatment can play a role in maintaining the water content of pepper fruits during storage, potentially by influencing transpiration rates or cuticle integrity. Similarly, preharvest treatment with SA at 0.5 mM concentration, applied through foliar spraying or irrigation, was found to improve fruit quality of green peppers, resulting in a slowing of the ripening and senescence processes during postharvest storage at non-chilling temperatures (Dobón-Suárez et al., 2025). While the specific effect on weight loss is not explicitly detailed beyond improved quality and slowed senescence, reduced weight loss is often associated with these phenomena.

Firmness is a critical textural attribute affecting consumer acceptance in pepper fruit. The effect of SA on pepper firmness appears to be positive in maintaining this quality. Hanaei et al. (2022) reported that foliar application of 1.5 mM and 3 mM SA, especially when combined with caraway oil, generally helped in retaining firmness in sweet peppers during cold storage compared to the control. This could be attributed to SA's potential role in inhibiting cell wall degradation enzymes or maintaining cell turgor. The study by Dobón-Suárez et al. (2025) also indicated that SA preharvest treatment, by slowing ripening and senescence, indirectly contributes to maintaining fruit firmness during postharvest at 7 ºC.

As far as we know, this is the first report that has studied the preharvest impact of MeJA on the delay of losses of weight and firmness during cold storage of green pepper fruit. In a general term, both MeJA and SA phytohormones were effective delaying weight losses regardless of the application method studied (**Fig. 2A and Table 2**). Nevertheless, the application of both phytohormones via irrigation showed a greater reduction of firmness losses than foliar application in the case of MeJA, with no differences observed between the two application methods for SA (**Fig. 2B and Table 2**). Although there is no study today comparing the effectiveness of both application methods on bell pepper quality, a recent report published that the application of SA via irrigation can be more effective than foliar application for specific quality parameters in green pepper fruit, as evidenced by the study of Dobón-Suárez et al. (2025). The research indicated that salicylic acid (SA) application through irrigation was the most effective treatment in delaying postharvest ripening, demonstrated by a maturation index of 37.28 during storage. In contrast, the foliar SA application showed the lowest maturation index at harvest (25.98), suggesting a more sustained effect of irrigation on delaying ripening during postharvest. Furthermore, irrigation-applied SA was the most efficient method for stimulating the activity of the antioxidant enzyme catalase (CAT) at harvest. The observed differences in effectiveness for distinct parameters are hypothesized to be related to the differential absorption, translocation, and persistence of SA and MeJA within the plant depending on the application method. Root uptake through irrigation may facilitate a more sustained and systemic distribution of the phytohormone (Huntenburg et al., 2022). This sustained absorption could be particularly advantageous for long-term processes such as the delay of postharvest ripening. Additionally, the distribution of the phytohormone to

various plant tissues and organs may vary according to the application pathway, consequently influencing the activity of specific antioxidant enzymes within the fruit (Dobón-Suárez et al., 2025).

Colour development and retention are vital for the visual appeal and marketability of 'Lamuyo' green pepper fruits. Our results suggest that MeJA applied via irrigation and SA by both foliar and irrigation showed a higher hue angle (h°) values at harvest and during 28 days of cold storage plus 2 days at room temperature (shelf-life) than control (**Fig. 2C and Table 2**). However, the highest values for both phytohormones were achieved with the application via irrigation (**Fig. 2C and Table 2**). These results of hue angle colour means that those green pepper fruits treated with MeJA and SA by irrigation showed an intense dark green hue, as can be observed in **Supplementary Fig. 1**. In this sense, MeJA has been widely reported to influence fruit colour, often enhancing the accumulation of pigments. Preharvest MeJA treatment has shown impressive results for improving fruit colour in different fruit crops (García-Pastor et al., 2024; Hasan et al., 2025). For instance, Saniewski and Czapski (1983) reported that MeJA affects lycopene and β-carotene accumulation in ripening red tomatoes (Saavedra et al., 2017). Although this study focused on tomatoes, it highlights the potential of MeJA to modulate carotenoid biosynthesis, which are key pigments in pepper colouration. Other recent studies have highlighted the overall role of oxylipins, including JAs, for colour improvement and their association with other phytohormones (Kondo, 2022). Thus, preharvest application of MeJA has been shown to improve fruit colour in various harvested produces (Rehman et al., 2021). In bell peppers, Ma et al. (2020) found that 1 μmol L^{-1} MeJA postharvest treatment delayed chilling injury and influenced membrane lipid composition, which can indirectly affect chlorophyll degradation and, thus, the colour retention in green bell peppers. Similarly, Fu et al. (2022) hypothesized that MeJA maintained the original colour of green peppers by preventing chlorophyll degradation associated with cold stress throughout the mitigation of cold damage. These effects on the colour contribute to a better visual appearance of green pepper fruit at harvest and implies better retention of visual quality, as it was reported previously (Meir et al., 1996; Wang et al., 2019).

The role of SA in colour modulation is also evident by different studies. Chen et al. (2023) reviewed the effects of preharvest and postharvest application of SA and its derivatives on fruit and vegetable storage, noting that they can suppress colour change. Since colour change in peppers is associated with ripening, Dobón-Suárez et al. (2025) recently found that SA preharvest treatment can help in maintaining the initial colour for a longer-term of storage at non-chilling temperatures in green pepper fruits. Specifically, that study found that SA treatment stimulated the antioxidant system in green peppers -and hypotheses that these antioxidant mechanisms can help in preserving cellular integrity, delaying senescence and, thus, maintaining firmness and colour while minimizing weight loss.

Cold storage is a widely used method for preserving the quality of pepper fruits; however, it can induce detrimental physiological changes, notably affecting total soluble solids (TSS), titratable acidity (TA), and leading to chilling injury (CI). Furthermore, pre- or postharvest treatments with MeJA and SA have been explored for their potential to mitigate these adverse effects. The refrigeration can have varying effects on TSS content in peppers. Some studies indicate that TSS might remain relatively stable or even slightly increase during cold storage due to water loss and concentration of solutes (Dobón-Suárez et al., 2025). However, other factors like the ripening stage and storage duration can influence this. In terms of TA, cold storage generally leads to a decrease in organic acids due to their utilization in respiration processes (Dobón-Suárez et al., 2025). This decline in TA can impact on the overall flavour profile of the pepper. A significant challenge in cold storage of peppers is CI, which manifests as symptoms like surface pitting, seed browning, and decay (Fung et al., 2004; Kong et al., 2020; Ge et al., 2020; Fu et al., 2022). Green peppers are particularly susceptible to CI when stored below 7 °C (Huang et al., 2024), restricting the development of CI the shelf-life and marketability of pepper fruits.

The findings of TSS demonstrated that the treatments did not have a significant impact on the accumulation of TSS at either the point of harvest or during the cold storage period (**Fig. 3A and Table 2**). Regarding the TA content (**Fig. 3B and Table 2**), the impact of both MeJA and SA during the cold storage can be indirect. By delaying ripening and senescence, MeJA and SA, specially applied via foliar, might help in maintaining a more stable TA content from harvest over a longer storage period compared to untreated fruits (Dobón-Suárez et al., 2025). Our results showed, for the first time, that the exogenous application of MeJA and SA via irrigation delayed the CI

incidence in 'Lamuyo' green pepper fruit, highlighting the effectiveness of SA than MeJA (**Fig. 3C and Table 2**). In this sense, both phytohormones applied via irrigation that effectively reduced CI in green pepper fruit can be linked to a delay in losses of weight, firmness, colour and TA through its impact on fundamental physiological and biochemical processes affected by low temperatures of 2 ºC. We hypothesized that MeJA and SA alleviated CI and induced cold tolerance to 'Lamuyo' green pepper fruit, 'Herminio' cv., through the maintenance of membrane stability and the reduction of lipid peroxidation after the enhancement of the antioxidant metabolism, as will be discussed subsequently (Ge et al., 2020; Ma et al., 2020; Fu et al., 2022).

Briefly, MeJA has shown promising effects in alleviating CI in bell peppers. In the past, Meir et al. (1996) investigated the application of MeJA to three chilling-sensitive fruits: avocado, grapefruit, and red bell pepper. The results showed that MeJA dipping or gassing significantly reduced both the severity of CI symptoms and the percentage of injured fruits in red bell peppers. Moreover, Fung et al. (2004) demonstrated that MeJA vapor treatment (10^{-4} M) applied to green bell pepper fruit for one day prior to cold storage (0 °C for 14 days) significantly reduced CI symptoms. The research suggests that MeJA treatments can activate the reactive oxygen species (ROS) defense system in bell peppers, potentially through the involvement of ROS scavenging and avoidance genes like alternative oxidase (AOX) (Wang and Baker, 1979; Considine et al., 2002). Other research explored the combined effects of low-temperature conditioning (LTC) and MeJA applied at 10 µmol L^{-1} on bell pepper fruit by dipping for 10 min (Wang et al., 2019). The findings indicated that MeJA treatment, alone and in combination with LTC, alleviated CI, as evidenced by measurements of malondialdehyde (MDA) concentration, CI index, chlorophyll content, Vitamin C content, and antioxidant enzyme activities (APX, POD, CAT) and their encoding gene expression levels (Wang et al., 2019).

Additionally, Fu et al. (2022) examined the effects of a preharvest treatment of 30 µmol L^{-1} MeJA on green bell peppers stored at 4 °C for 6 days, using transcriptome, metabolome, and proteome analyses. The findings suggest that MeJA treatment mitigates CI through multiple mechanisms, including inhibiting the MYC2-JA signaling pathway, enhancing the ASA-GSH cycle (antioxidant system), reducing membrane lipid damage, suppressing cell wall disassembly, and activating the CMAT-CBF-ICE pathway (related to cold response). However, the direct effects of MeJA on these parameters might vary depending on the concentration, application method, and storage conditions. Recently, other authors investigated the effects of MeJA and MeSA on CI in pepper fruit during postharvest cold storage at 2 °C (Seo et al., 2020). The study found that MeJA treatment significantly reduced the CI index and seed browning. The analysis of endogenous hormone content, hormone-related gene expression, and metabolites aimed to clarify the cellular mechanisms involved. The study provides a model pathway for the effect of MeJA on seed browning, suggesting the involvement of jasmonic acid (JA) biosynthesis and signaling. Finally, Ma et al. (2020) reported the role of MeJA in regulating membrane lipid composition to alleviate CI in green bell peppers, delaying the appearance of CI symptoms during storage, which is crucial for maintaining cell membrane integrity under low temperatures.

On the other hand, SA treatment, as another phytohormone known for its role in stress responses, has been shown to alleviate CI in pepper fruits by enhancing antioxidant metabolism, fatty-acid desaturation efficiency, and water retention (Ge et al., 2020; Hanaei et al., 2022). Foliar spraying of SA incorporated with caraway oil coating also showed a reduction in CI percentage in sweet pepper during cold storage (Hanaei et al., 2022). Some studies suggest that SA's effects on CI can be concentration-dependent and might vary with the specific crop (Seo et al., 2020). Collectively, these studies provide substantial scientific evidence that MeJA and SA treatments, applied both pre- and postharvest, can effectively alleviate CI in bell peppers. The mechanisms of action appear to be multifaceted, involving the activation of antioxidant defence systems, the maintenance of cell membrane integrity through regulation of lipid composition, and the modulation of gene expression related to stress responses and hormone signaling pathways. Therefore, to contribute to the improvement of our knowledge, this article studies for the first time the preharvest impact of the application of MeJA and SA, both via foliar and irrigation, on the non-enzymatic antioxidant system of green bell pepper fruit under cold storage, as well as on the lipid metabolism to hypotheses the action mechanism of the induction of chilling tolerance.

The results obtained on the mitigation of CI by both phytohormones, especially with the application of SA could be related with the effect on the enhancement of the non-enzymatic antioxidant metabolism. The exogenous application of MeJA and SA has been shown to have significant effects on the biochemical composition of fruits and vegetables, including TPC, H-TAA, TCC and L-TAA, which is often associated with mitigation of CI (Fung et al., 2004; Seo et al., 2020; Chen et al., 2023; Hasan et al., 2025). This study has shown that the preharvest application of SA, both via foliar and irrigation, and MeJA only applied by irrigation enhanced the TPC than untreated green pepper fruits both at harvest and after 28 days of storage at 2 ºC plus 2 days at 20 ºC (**Fig. 4A and 4B and Table 2**). In this sense, the H-TAA was improved with the same treatments cited above, although the foliar spray of SA showed the highest enhancement at both sampling dates studied (**Fig. 4C and 4D and Table 2**). A similar effect was observed regarding the lipophilic bioactive compounds, since the TCC was increased in those samples of green pepper fruits treated with SA or MeJA after 28 days of cold storage plus shelf-life, although the highest achievement was obtained with the pulverization of SA (**Fig. 5B and Table 2**). Related with that, the L-TAA was improved with both the foliar and the irrigation of SA and MeJA, and no statistical differences between the two methods applied or the two phytohormones studied were appreciated (**Fig. 5C and 5D and Table 2**).

The preharvest application of MeJA can significantly increase the levels of TPC in a wide range of fruits such as apples (Shafiq et al., 2011; 2013), blackberries (Shah et al., 2024), raspberries (Shah et al., 2025), black currents (Flores and Ruiz del Castillo, 2016), table grapes (García-Pastor et al., 2019), pomegranates (García-Pastor et al., 2020) and blood oranges (Vithana et al., 2024). This increase is attributed to the activation of phenylpropanoid metabolism, where key enzymes such as phenylalanine ammonium-lyase (PAL) and shikimate dehydrogenase (SKDH) can increase their activity during fruit ripening (Hasan et al., 2024). For example, in MeJA-treated mangoes, a significant increase in TPC was observed, correlated with increased PAL enzyme activity (Muengkaew et al., 2016). In 'Yoho' and 'Jiro' persimmons, MeJA also influenced phenolic metabolism, showing higher levels of total phenols and SKDH and PAL activities (Hasan et al., 2024). In general, MeJA improves the total antioxidant capacity of fruits, which can include both hydrophilic (H-TAA) and lipophilic (L-TAA) activity. The preharvest application of MeJA has been associated with increased anthocyanin levels in various fruits, which are phenolic compounds that contribute significantly to H-TAA. Furthermore, MeJA spray application has shown great potential for increasing ascorbic acid or vitamin C levels and the activities of antioxidant enzymes, which also contributes to antioxidant defence (Karaman et al., 2013; Serna-Escolano et al., 2019; 2021; Rehman et al., 2021). Similarly, TCC, which are important lipophilic antioxidants, were significantly increased in response to preharvest MeJA spray application to 'M7 navel' orange, and 'Mahachanok' mango, and 'Yoho' and 'Jiro' persimmon fruit, which displayed a bright yellow-orange colour (Muengkaew et al., 2016; Rehman et al., 2021; Hasan et al., 2024).

On the other hand, the pre- and postharvest application of SA and its derivatives such as MeSA and acetylsalicylate (ASA) can also influence phenolic compounds. Preharvest SA has been shown to improve phenolic compounds in 'Niagara Rosada' table grapes (Gomes et al., 2021). In general, the application of SA and its derivatives can activate the antioxidant system, which is often linked to the metabolism of phenolic compounds (Chen et al., 2023). Application of SA can increase both hydrophilic and lipophilic antioxidant activity (Kang et al., 2012; Schippers et al., 2016; Shanbehpour et al., 2020; Serna-Escolano et al., 2021; González-Villagra et al., 2022). For example, SA treatment in navel orange, cv. 'Cara cara', has been shown to affect the antioxidant system in the pulp at different storage temperatures (Huang et al., 2008). SA can also induce the expression of the enzyme alternative oxidase (AOX), which is involved in the stress response and may be related to the regulation of oxidative stress (Rhoads and McIntosh, 1992; 1993). Furthermore, Kang et al. (2012) reported that SA affects growth and water stress tolerance in wheat, and its proteomics revealed changes, although effects on carotenoids in other fruits and vegetables are not directly specified in the literature. However, given the role of SA in regulating metabolism and stress response, it is plausible that it may influence carotenoid content under certain conditions.

Scientifically discussing the effects of MeJA and SA treatments on FAs profiles and content, and their relation to CI mitigation in fruits and vegetables, reveals that both phytohormones play a role in maintaining membrane integrity under cold stress by influencing FAs composition. The destructive process of cellular membranes during fruit storage at chilling temperatures has been ascribed to phospholipids hydrolysis into free

fatty acids and peroxidation of UFA due to the coordinated action of lipid metabolizing enzymes, such as phospholipase, lipase and lipoxygenase (Lin et al., 2017; Wang et al., 2018; Zhang et al., 2018). As demonstrated in the relevant literature, an increase in the degree of unsaturation of cellular membranes has been shown to result from an increase in the levels of unsaturated fatty acids, including linolenic acid (C18:3). This, in turn, has been shown to enhance membrane fluidity and cellular function by increasing membrane integrity (Aghdam and Bodbodak, 2013). Research indicates that MeJA treatments, whether applied preharvest, postharvest, or in combination, can effectively reduce CI in various horticultural crops, including bell pepper, avocado, grapefruit and pomegranate fruit, among others (Meir et al., 1996; Ma et al., 2020; García-Pastor et al., 2020). This mitigation is closely linked to the effects of MeJA on the FAs composition of cell membranes (García-Pastor et al., 2020; Ma et al., 2020). In pomegranate, preharvest (Pre) or combined pre- and postharvest (Pre + Post) MeJA treatments led to a higher concentration of UFA at harvest, which was sustained at higher levels during 90 days of storage at 2 °C, while the concentration of SFA was lower in treated fruit compared to controls (García-Pastor et al., 2020). Thus, MeJA treatments are shown to increase the ratio of unsaturated to saturated FAs (UFA/SFA). Moreover, Cao et al. (2009) showed that postharvest treatment with MeJA of loquat fruit significantly reduced CI and maintained higher UFA/SFA ratio during storage, suggesting that MeJA induced CI tolerance in fruit tissues by reducing losses in UFA and maintaining a high UFA/SFA ratio. In green bell pepper, MeJA treatment also alleviates CI by regulating membrane lipid composition (Ma et al., 2020). CI is widely believed to be primarily caused by membrane lipid imbalance due to decreased FAs desaturation (Sun et al., 2020). MeJA treatment is involved in maintaining physiological and biochemical functions under cold stress, potentially by affecting the JA signaling pathway (Chen et al., 2019).

SA and its derivatives have also been shown to influence FAs profiles and mitigate CI (Aghdam and Bodbodak, 2013). In bell pepper, a combined treatment of trisodium phosphate (TSP) and SA enhanced FAs desaturation efficiency, as indicated by increased expression of key FAs desaturase genes and a higher content of UFA (Ge et al., 2020). This combined treatment also inhibited CI-induced membrane damage, manifested as lower electrolyte leakage and malondialdehyde content (Ge et al., 2020). This suggests that SA, particularly in combination with other compounds, can contribute to maintaining membrane integrity under chilling stress by promoting the synthesis of UFAs. This effect is considered a mechanism of acclimation to low temperatures, helping to maintain membrane semi-permeability and leading to lower ion leakage (IL) and reduced CI symptoms (García-Pastor et al., 2020). The maintenance of a high UFA/SFA ratio by both MeJA and SA suggests a preservation of the liquid-crystalline state of membrane lipids at chilling temperatures, thus preventing membrane peroxidation and damage, which are known to accelerate CI. Finally, other treatments, such as glycine betaine, reduced CI in peach fruit, throughout reduction of lipoxygenase, phospholipase D and lipase activities and their gene expression and increasing the expression of genes related to FAs biosynthesis and desaturation (Wang et al., 2019).

Although some studies are focused on the mitigation of CI in peppers with the exogenous application of MeJA and SA and thereby discuss the effects on the UFA/SFA ratio, as far as we know, there are no studies to date that indicate the individualized effect on each FAs identified in the profile that composes the lipid membrane. The most important FAs in relation to CI appear to be the UFAs, such as oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), as was previously highlighted (García-Pastor et al., 2020). Their double bonds introduce kinks in the acyl chains of phospholipids, preventing tight packing and maintaining membrane fluidity at low temperatures. A decrease in the proportion of these UFAs and a consequent reduction in the UFA/SFA ratio are key indicators of membrane damage and increased susceptibility to CI (Wongsheree et al., 2009). Treatments with MeJA and SA that help preserve or increase the levels of UFAs and maintain a higher UFA/SFA ratio are thus crucial in mitigating CI in fruits and vegetables.

After identified and quantified the peaks of the five major FAs (linoleic, linolenic, palmitic, myristic and steric acids; **Supplementary Fig. 2**), as well as the minor ones, our results suggest that the irrigation of both MeJA and SA regulates the lipid metabolism in ‘Lamuyo’ green pepper fruit. Therefore, the SFA content, especially for the C14:0 (myristic), C16:0 (palmitic) and C24:0 (lignoceric) fatty acids, was significantly reduced with the application of SA via irrigation in green pepper fruits both at harvest and after 28 storage days (**Fig. 6A and 6B**

and Table 3). In addition, the content of C18:0 (stearic) fatty acid was significantly decreased with the irrigation of MeJA compared to the other treatments tested (**Fig. 6A and 6B and Table 3**). Regarding the UFA content, the levels of C18:1t9 (trans-oleic), C18:1c9 (oleic) and C20:2 (eicosadienoic) fatty acids were significantly enhanced in those green pepper fruits harvested from irrigation MeJA-treated plants compared to control (**Fig. 7A and 7B and Table 3**). Nevertheless, the highest content of C20:3n3 (omega-3 eicosatrienoic acid) was observed in those samples from irrigation SA treatment (**Fig. 7A and 7B and Table 3**). These results of both SFA and UFA led to calculate the UFA/SFA ratio in all treatments tested, as can be seen in **Supplementary Fig. 3**. The results of this ratio showed that the most effective treatment on the CI mitigation was SA applied via irrigation to 'Lamuyo' green pepper plants and, accordingly, this treatment was the most effective reducing the CI incidence during 28 days of cold storage (**Fig 3C, Table 2 and Table 3**).

A principal component analysis (PCA) was applied to the results about the postharvest ripening traits and those parameters related with the antioxidant and lipid metabolism of green pepper fruits harvested control, MeJA and SA-treated plants via irrigation at harvest and after 28 days of cold storage (**Supplementary Fig. 4A and 4B**). The PC-1 and PC-2 accounted for the 56 % and 14 % of the total variance of the X and Y variables, respectively, the accumulative variance contribution being 70 %. The green arrows originating from the origin represent the loadings of the original variables on the PCA. The plot A (**Supplementary Fig. 4A**) provides insights into the relationships between the measured variables. In the positive side of the PC-1, the firmness, TA, L-TAA, UFA/SFA ratio and some individual UFA fatty acid, such as C17:1, C18:1t9, C18:1c9, C18:2n6c, C18:3n6 gamma, C18:3n3 alpha, C20:2, C20:3n3 and C20:3n6, were the most relevant parameter and the SFA content of C8:0, C14:0, C16:0, C18:0 and C24:0, combined with the C15:1 fatty acid contributed to the negative side (**Supplementary Fig. 4A**). The most important parameters in the positive side of the PC-2 were TSS, TPC, TCC and H-TAA, and weight loss, colour, CI and C16:1 fatty acid in the negative side (**Supplementary Fig. 4A**). Based on the plot, potential positive correlations between variables that have vectors pointing in similar directions, such as TPC and H-TAA, has been observed (**Supplementary Fig. 4A**). Additionally, potential negative correlations between variables with vectors pointing in opposite directions are also appreciated, like TPC and CI (**Supplementary Fig. 4A**).

On the other hand, the plot B (**Supplementary Fig. 4B**) illustrates how different experimental groups are positioned relative to each other based on underlying factors derived from the data. Both plots (**Supplementary Fig. 4A and 4B**) help in understanding the complex relationships within the dataset and the differences among the studied treatments. The **Supplementary Fig. 4B** showed the distribution of samples belonging to the control, irrigation MeJA and irrigation SA groups in the space defined by the two REGR factor scores. Therefore, samples from SA and MeJA treatments are clustered in a different region of the plot and were well-separated, showing an effectively discrimination compared to control samples (**Supplementary Fig. 4B**). This fact suggests that these phytohormones have different characteristics as captured by the underlying variables and the extracted factors (**Supplementary Fig. 4B**). Specifically, SA and MeJA were closely and positively correlated to firmness, TA, L-TAA, UFA/SFA ratio and some individual UFA (C19:1t9, C19:1c9, C20:2 or C20:3n3, among others), as was observed in the **Supplementary Fig. 4A**. The same effect was observed for these treatments with the TSS, TPC, TCC and H-TAA (**Supplementary Fig. 4A and 4B**). The results of the present study showed that the application of SA via irrigation was most effective to mitigate the CI incidence in green pepper fruit than MeJA, and this fact is also corroborated in the **Supplementary Fig. 4B**.

Nevertheless, the control samples showed an opposite effect and a negative correlation with SA and MeJA treatments was observed. These samples were closely to weight loss, colour, CI and C16:1 fatty acid in the negative side (**Supplementary Fig. 4A**). Furthermore, control samples showed a substantial correlation with the SFA content of C8:0, C14:0, C16:0, C18:0 and C24:0, as well as with the C15:1 fatty acid (**Supplementary Fig. 4A and 4B**). Those results of PCA are in line with the previous results discussed and reinforced that the irrigation application of SA and MeJA regulate postharvest ripening and CI of 'Lamuyo' green pepper fruit by modulating both antioxidant and lipid metabolism. A regulatory model to describe a possible regulatory mechanism of the effects of both SA and MeJA treatments applied via irrigation in reducing CI of 'Lamuyo' green bell pepper fruit has been developed (**Fig. 8**). This mechanism of action is mainly associated with the activation of non-enzymatic antioxidant systems

and the reduction of membrane lipid damage throughout the increase in both the fatty acid unsaturation and the UFA/SFA ratio. However, information on the molecular mechanisms related to chilling response of both phytohormones could provide a basis for the practical application of SA and MeJA in order to enhance the chilling tolerance of green pepper fruit and for a deeper understanding of CI mechanism with respect to hormone actions after its absorption by the plant's root system.

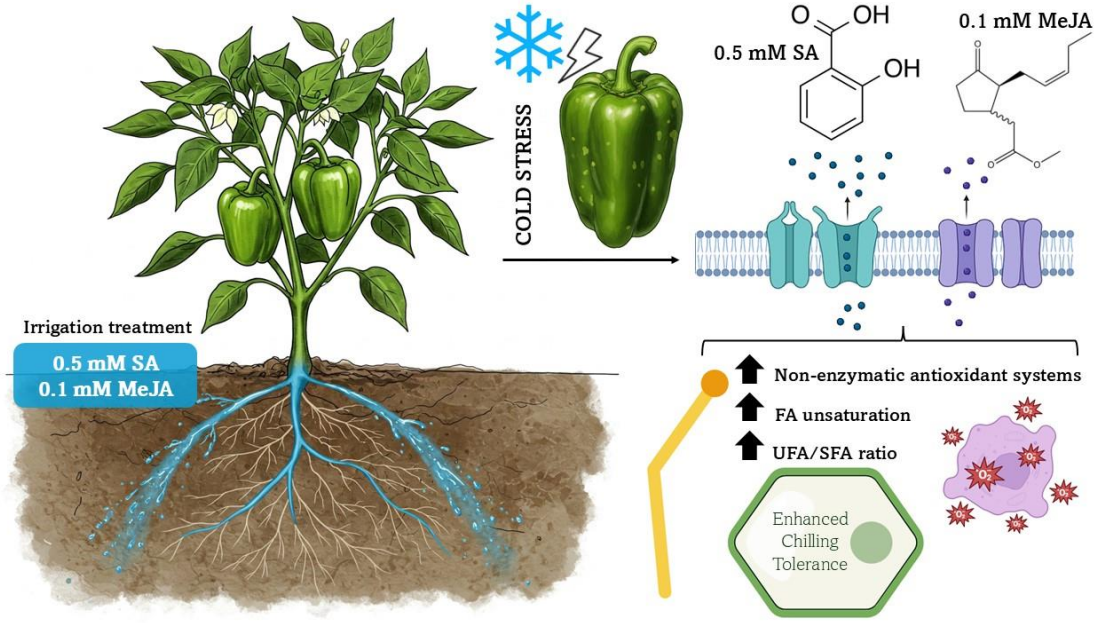


Fig. 8. A hypothetical working model illustrates the regulatory mechanism of both salicylic acid (SA) and methyl jasmonate (MeJA) treatments applied via irrigation to plants in reducing chilling injury (CI) of 'Lamuyo' green bell pepper fruit. Some abbreviations are as follows: fatty acid (FA), unsaturated fatty acid (UFA) and saturated fatty acid (SFA).

5. Conclusions

The findings of this study demonstrate that both SA and MeJA treatments applied by foliar or irrigation methods delayed the deterioration of quality and reduced the CI symptoms in 'Lamuyo' green pepper fruit over a 28-day period of cold storage, followed by a further 2 days of shelf-life. This is likely to be a consequence of the modulation of postharvest ripening, the stimulation of non-enzymatic antioxidant systems, and the maintenance of cell membrane structure through the reduction of UFA losses and the enhancement of the UFA/SFA ratio. For most of the analysed parameters, no significant differences were observed between the SA and MeJA treatments or between the two application methods. However, the exogenous application of SA via irrigation demonstrated an optimal efficiency in inducing CI tolerance, concomitant with enhanced the UFA/SFA ratio. Consequently, the utilisation of a preharvest SA treatment at a concentration of 0.5 mM, administered via irrigation, is recommended for pepper plants to enhance the storability of 'Lamuyo' green bell pepper fruit during cold storage. This approach has been shown to reduce CI symptoms, augment the quality traits and bioactive compounds with antioxidant potential, and consequently promote beneficial health effects.

Declaration of Competing Interest

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4.6. Publication 6 — Review article

PUBLICATION 6 (Original manuscript)

A Comprehensive Review on Characterization of Pepper Seeds: Unveiling Potential Value and Sustainable Agrifood Applications

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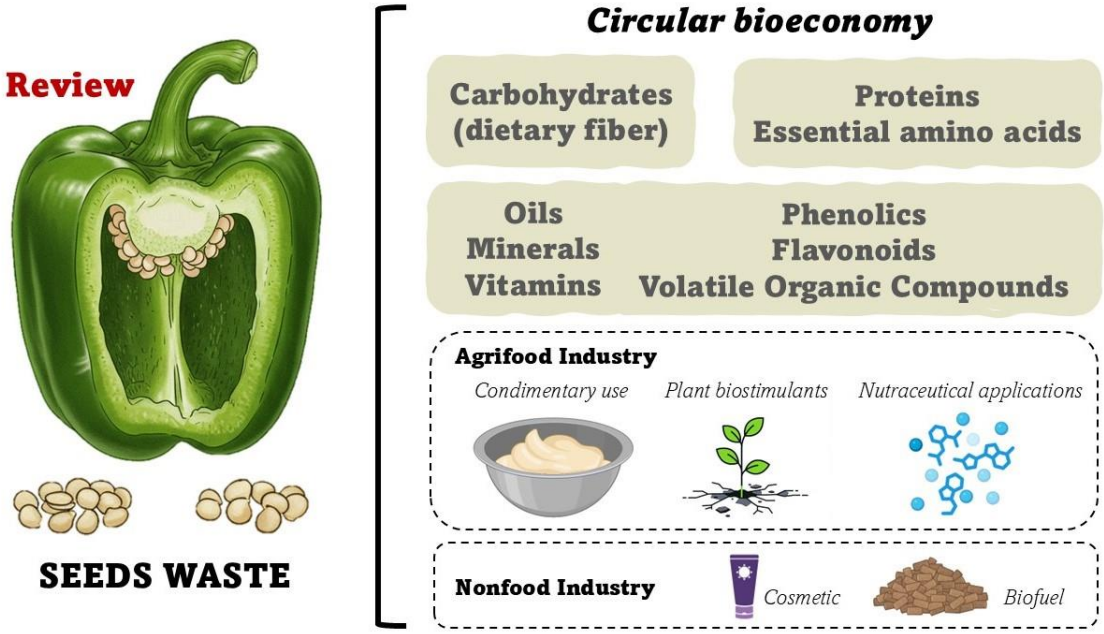
Foods

-Under review-

Graphical abstract:



PUBLICATIONS

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Review

A Comprehensive Review on Characterization of Pepper Seeds: Unveiling Potential Value and Sustainable Agrifood Applications

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Abstract: Pepper (*Capsicum annuum* L.) processing generates significant by-products, with seeds emerging as a promising resource due to their rich content of oils, proteins, phenolic compounds and minerals. This comprehensive review critically evaluates the existing literature on the characterization of pepper seeds, highlighting their significant nutritional value and diverse bioactive constituents. The substantial oil content, characterized by a high proportion of unsaturated fatty acids such as linoleic and oleic acids, positions pepper seeds as a valuable source for edible oil and potential biofuel production. In addition, the presence of significant amounts of proteins, carbohydrates, dietary fiber and essential amino acids underlines their potential for the development of functional foods and dietary supplements. The current review also summarizes the findings on the phenolic profile and antioxidant activities of pepper seeds, indicating their relevance for nutraceutical and cosmetic applications. Finally, the potential utilization of pepper seeds in various agri-food industrial applications, such as food condiments, biostimulants, and biomass for energy, is discussed, promoting sustainability and a circular bioeconomy approach to valorize this underutilized resource. This systematic review summarizes current knowledge, identifies knowledge gaps, and highlights the potential of pepper seeds as a sustainable and economically viable alternative in multiple sectors.

Keywords: by-product valorization; *Capsicum annuum* L.; functional ingredient; nutritional value; waste

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1. Introduction

Peppers (*Capsicum annuum* L.) are considered to be one of the most widely consumed vegetable crops on the global stage, cultivated in substantial quantities across diverse regions of the world [1,2]. The primary producers of both sweet and hot pepper fruit are China, Mexico, Indonesia, Turkey, Spain, and the USA. For instance, the annual Spanish production of sweet and chilli peppers was up to 1,416 million tons in 2023 [3]. Peppers are classified into three primary categories based on their colour: red, green, or yellow, being used as a vegetable or a spice, whether fresh or dried [4]. Furthermore, they undergo various processes, including dehydration, pickling, freezing after slicing or dicing, and transformation into final products, such as sauce, puree, flakes, powder, and oleoresin [5]. Processing techniques significantly alter the biochemical composition of pepper and its

by-products, influencing the concentration and bioaccessibility of valuable compounds, such as capsaicinoids, phenolics, and carotenoids [5].

The consumption of fresh peppers has been shown to provide significant health benefits due to their high nutritional value and antioxidant content [6]. They are a source of vitamin C, provitamin A, E, carotenoids, chlorophylls, phenolic acids, and flavonoids [7,8]. Peppers represent an exceptional source of antioxidants, whose antioxidant profile plays a key role in reducing oxidative stress. In this sense, studies have indicated that antioxidant compounds are effective in scavenging reactive oxygen species (ROS) and protecting cellular structures against free radical damage caused by oxidative processes [9]. Scientific literature supports the correlation between free radicals and the development of various pathologies, including diabetes mellitus, cardiovascular disease, cancer and neurodegenerative diseases [10,11]. However, bioactive compounds present in *Capsicum annuum* species have been shown to reduce the production of free radicals and possess anti-inflammatory properties, stimulate the immune system and reduce the likelihood of developing chronic disorders and diseases [12-14].

The substantial antioxidant content of peppers represents a significant opportunity for the valorization of by-products within the agri-food sector. Residues generated during the processing of peppers, including peels, seeds, and pulp remnants, have been found to be abundant in bioactive compounds, such as phenolics, carotenoids, vitamins, minerals, and dietary [15-17]. These by-products have the potential to be repurposed for the extraction of natural antioxidants, food colourants, and nutritional supplements, thereby fostering sustainability and mitigating waste [18]. In addition, the fiber present in these residues can be incorporated into the formulation of functional foods, enhancing their nutritional value and promoting digestive health [19]. Consequently, there is a necessity for research into extraction and processing technologies for pepper by-products, with the objective being to maximize the utilization of these resources and to develop innovative products that confer benefits to both human health and the environment.

2. Addressing Pepper Waste: A Focus on the Revalorization of Seeds

In recent years, the food industry has increasingly focused on the use of by-products and waste from food processing and underutilized agricultural products. This approach has been demonstrated to optimize available resources whilst concomitantly fostering the development of new food products, thus contributing to environmental sustainability and economic growth [20,21]. The reduction of food loss and waste has been demonstrated to engender substantial environmental benefits, whilst concomitantly enhancing global food security, a pivotal element in the overarching pursuit of sustainable development. In response to these challenges, the industry has intensified research into more environmentally friendly methods of food and nutritional supplement production [22].

A particularly encouraging approach involves the revalorization of food waste, a process which entails the extraction of bioactive compounds for the purpose of creating functional foods and nutraceuticals. This strategy is expected to yield a number of significant benefits, including both economic and health-related advantages [23]. This approach is consistent with the tenets of the circular economy, an emerging economic model that advocates the reuse of organic waste as raw materials, thereby reducing waste generation, optimizing resource efficiency and improving the safety and security of global food supply [24]. In accordance with the three core elements of the framework of food industry 5.0 (**Figure 1**), the food industry is adopting a sustainable approach and the principles of the circular economy with a view to reducing food waste from farm to fork [25]. This approach conceptualizes a future in which technological advancement serves as a catalyst for positive social and economic impacts, thereby aligning industrial progress with the well-being and active participation of humanity.

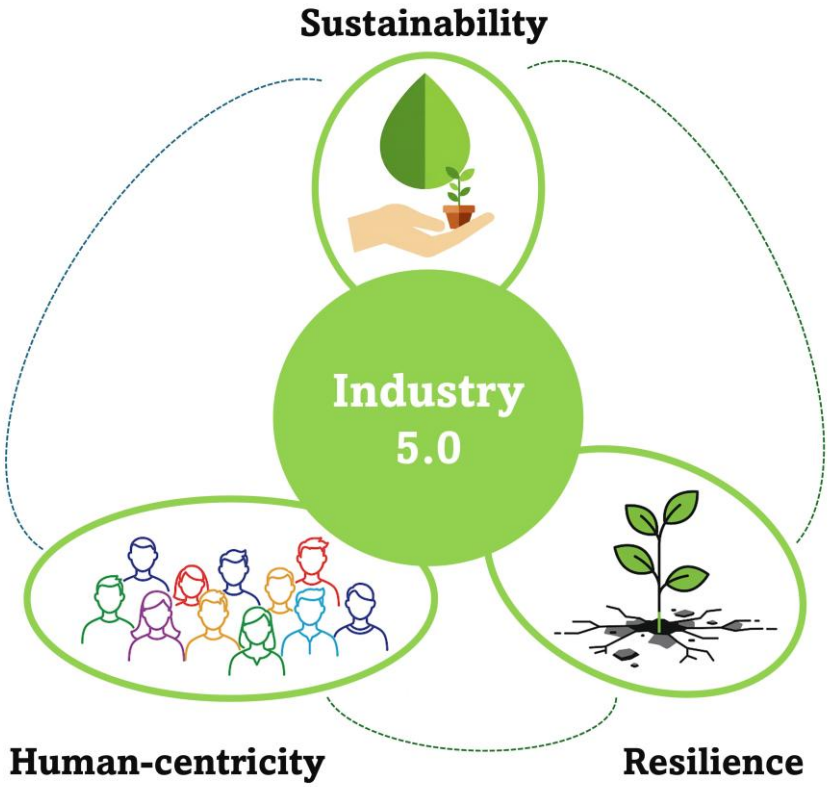

Figure 1. Core principles of the framework of food industry 5.0: Sustainability, human-centricity and resilience. The dashed line establishes a connection among them.

The traditional linear economic model, known as the 'make-use-dispose' paradigm, has been superseded by a circular economy model that is based on the '5Rs'—reduce, recycle, reuse, recover, and restore — creating a more sustainable and regenerative system [26]. The agro-industrial sector has undergone significant transformation due to modernization and industrialization, leading to an increase in agri-food waste [27]. The organic waste from fruits and vegetables has been identified as a significant source of bioactive compounds, including carbohydrates, dietary fiber, proteins, lipids, organic acids, minerals, polyphenols, and phytosterols [28]. These valuable components open new possibilities for high-value applications, such as the production of biofuels, animal feed, and food supplements. Postharvest losses in peppers present a substantial challenge to the achievement of the Sustainable Development Goals (SDGs). These losses have a direct impact on SDG 2 (Zero Hunger) by reducing the availability of nutritious food, and on SDG 12 (Responsible Consumption and Production) by contributing to food waste along the supply chain. Furthermore, the economic repercussions of these losses have a profound effect on SDG 1 (Poverty Eradication) for farmers and associated stakeholders, particularly in developing economies where postharvest infrastructure may be deficient [29].

Numerous studies have demonstrated that pepper losses can be significantly attributed to several factors, including inadequate storage conditions, pathogen infections and mechanical damage [30,31]. In addition, external factors occurring outside the food supply chain have been shown to induce substantial levels of postharvest losses. These factors can be categorized into two primary classifications: environmental factors and socio-economic patterns or trends [32]. Consequently, there is an imperative for the implementation of improved postharvest handling and storage technologies to minimize such losses and advance the SDGs related to food security and sustainable development [33]. Sweet peppers, for instance, experience approximately 40 % postharvest loss each year due to their perishable nature, limited shelf-life, and susceptibility to many diseases [34].

On the other hand, the cultivation and processing of pepper fruit for minimally processed plant-based food products generate a significant amount of waste, estimated at 5–30 %. This includes fruit waste (e.g. seeds, peduncle, peel, placenta, and unused fruits) as well as plant waste (e.g. stems and leaves). The potential for valorization of these by-products is increasingly recognized, due to their bioactive richness, and the presence of valuable compounds such as carotenoids, phenolics, dietary fiber, essential fatty acids, and vitamins [5,35]. Pepper seeds have attracted significant attention due to their substantial oil content, which is characterized by a high proportion of unsaturated fatty acids, such as linoleic and oleic acids. In addition to this, they contain significant amounts of phenolic compounds, proteins, and minerals that offer health benefits [5,36-39]. Recent studies have indicated that pepper seeds could be repurposed into valuable products such as edible oil, carbohydrate-rich flour, and protein-based ingredients [4,20,40-42]. Notwithstanding their nutritional and functional potential, pepper seeds constitute approximately 45 % of the dry weight of peppers and are frequently discarded or utilized for low-value applications such as animal feed [43]. This represents a significant opportunity for optimizing resources and promoting sustainability within the agri-food sector. The development of innovative applications for pepper seed-derived products has the potential to reduce food waste, while simultaneously unlocking new economic and nutritional benefits.

A preliminary evaluation revealed a paucity of exhaustive scientific review literature specifically addressing pepper seeds as by-products, particularly with regard to their sustainable agrifood applications in the industry. A number of authors have conducted reviews of the nutritional components and bioactivities of pepper seeds, as well as the details of their extractions from pepper seeds, further highlighting the relative scarcity of review literature dedicated to their derived by-products [5]. Consequently, there is a necessity for dedicated scientific reviews that consolidate and analyze the potential applications and valorization strategies for specific pepper seed by-products, within the food and other relevant sectors.

3. Potential Value of Pepper Seeds: Nutritional Profile and Phytochemical Compounds

The increase in the production of agri-food by-products has stimulated research into the valorization of resources that are traditionally considered waste, but which have considerable nutritional and economic potential. In this context, pepper seeds emerge as a promising by-product due to their rich composition of macronutrients, minerals and amino acids. These components not only provide high energy and functionality but also suggest applications in the development of functional foods, nutraceuticals and other innovative products [16,44,45]. This review examines the nutritional and phytochemical composition of pepper seeds, analyses the variability derived from the cultivar, and highlights its relevance for improving the sustainability and diversification of the agri-food industry.

3.1. Nutritional Composition of Pepper Seeds: Carbohydrates, Dietary Fiber, Proteins, Fats, Moisture and Ash

Pepper seeds show a considerable variability in their constituents, reflecting both the diversity of varieties and growing conditions, and the different processing and analytical methods used in the studies. In terms of macronutrients, the range of carbohydrates is from 43.60 to 80.89 g 100 g^{-1} [17,39,46], suggesting that these seeds can be an important source of energy (**Table 1**). Chouaibi et al. [39] found the lowest carbohydrate content in pepper seeds (43.60 %). In the carbohydrate content of pepper seeds, the most dominant component is dietary fiber, with significant levels ranging from 26 % [4] to 61 % [40]

(**Table 1**). The ratio of insoluble and soluble dietary fiber was around 10:1, according to Azabou et al. [40]. The high content of insoluble fiber in pepper seeds could represent a new ingredient in the food industry, such as pepper seed flour, which could be used to enrich various products (e.g. jams, sauces or soups) by increasing the levels of indigestible insoluble compounds [16].

Pepper seed proteins have not been extensively studied, as evidenced by the literature. As demonstrated in **Table 1**, pepper seeds exhibit high concentrations of crude protein (6.30-28.30 g 100 g^{-1}), with the highest levels recorded by Chouaibi et al [39]. It is acknowledged that variations in protein yield can be attributed to several factors, including cultivar, cultivation methods, climatic conditions, the ripening stage of the seeds, and the timing of the harvest. For instance, the protein content of pepper seeds from Croatian cultivars was found to be 16.5 % for the Slavonka cultivar and 16.7 % for the Podravka cultivar [20]. The quality of the protein is contingent upon its amino acid composition. For example, the study of Embaby and Mokhtar [47] revealed that the proteins from the sweet pepper seeds, lanta seeds and nabak seed kernels had a low biological value. Consequently, these types of proteins are classified as incomplete proteins. Consequently, these seeds necessitate supplementation with complementary proteins if they are to be utilized as a food source [47]. Pepper seeds contain most of the essential amino acids, as discussed below, and can be used as a good protein source in a variety of food applications [44,48]. Pepper seed flour, which is characterized by its elevated lysine content, has the potential to serve as a functional ingredient, with the capacity to enhance the protein quality of wheat flour, which is known to be deficient in lysine [5]. These findings suggest the possibility of using pepper seed protein as an alternative, inexpensive source of protein [5], but the dose will determine whether it will dominate the taste of food products. Hence, pepper seed flour has the potential to be used in some bakery products, as a meat replacer and as a thickening agent in soups [36]. Red pepper placenta and defatted seeds rich in protein and fiber were also used to fortify pasta, which improved the amino acid composition and total dietary fiber content of the fortified pasta [49].

The presence of crude fat, ranging from 11.00 to 23.65 g 100 g^{-1} [17,39,40,46-48] is notable (**Table 1**), as it provides nutritionally significant lipids and essential fatty acids. The fat content of the pepper placenta was recently determined to be 3.15 % dry weight (DW), and 10.40 % in the defatted pepper seeds [49]. Prior to the defatting process, the seeds exhibited a fat content of 26.01 % DW [49], which is congruent with the findings reported by Cvetković et al. [16]. Finally, 100 g of pepper seeds contain from 4.48 to 5.96 g of moisture [46,47] and ≈ 4.94 g ash [47] (**Table 1**). However, the total ash content has been reported to vary from 1.81 to 12.54 g per 100 g of seeds [17,46,47,49] (**Table 1**), suggesting that it is both indicative of the stability to storage and total mineral content of these by-products.

Additionally, bell peppers contain some nutritionally important compounds such as vitamins (A, C, E, K, B3 and B6) and minerals (sodium, potassium, phosphorus, magnesium, calcium, iron, zinc, manganese and copper) (**Table 1**). In this sense, the frequent consumption of bell peppers provides essential nutrients for human health [50-52]. For example, fresh bell pepper consumption (100 g) provides the total ascorbic acid recommended daily intake [53]. However, the nutritional content of bell peppers depends directly on the colour of the fruit, cultivar, growing conditions, and postharvest processing, among other factors [50]. Therefore, vitamin C contents ranged from 93 mg 100 g^{-1} FW for creasing pepper type (green; *Capsicum annuum* L.) to 393 mg 100 g^{-1} FW for long-point pepper type (red; *Capsicum annuum* L.), depicting a 4-fold variation between cultivars [53]. In addition, Zhuang et al. [53] found that fully red mature long-point pepper type (red; *Capsicum annuum* L.) had significantly more vitamin C than fully developed long-point pepper fruit (green; *Capsicum annuum* L.). In this sense, the total vitamin C content, as the

sum of both forms of ascorbic acid (AA) and dehydroascorbic acid (DHA), was also significantly influenced by the phenological stage of the green pepper fruit, and a 3-fold increase in total content was observed between the first and last stage (S1-S12) [54]. Thus, pepper waste and byproducts are rich sources of nutrients and bioactive compounds, such as vitamins among others, which exhibited anti-inflammatory, antidiabetic, antimicrobial, and immunomodulatory effects, among others [5,50]. It is evident that peppers contain a high concentration of bioactive phytocompounds. Consequently, pepper fruit waste and its processing byproducts may provide a cost-effective source for the extraction of bioactive molecules. Similar to fruits, pepper fruit waste contains several bioactive compounds such as capsaicin, dihydrocapsaicin, vitamins, carotenoids, flavonoids, and phenolic compounds (19,40,46,47,49). The other pepper plant waste, including seeds, stems, and leaves, also contains many bioactive phytocompounds [5].

Table 1. Nutritional profile and content of macronutrients, vitamins, minerals, and essential and non-essential amino acids of pepper seeds.

Macronutrients	**Content by 100 g**	**References**
Carbohydrate (g)	43.60-80.89	[17,39,46]
Dietary fiber (g)	26.00- 61.00	[4,16,20,40,47]
Crude protein (g)	6.30-28.30	[5,17,20,36,39,40,44,46-49]
Crude fat (g)	11.00-23.65	[17,39,40,46-49]
Moisture (g)	4.48-5.96	[46,47]
Ash (g)	1.81-12.54	[17,46,47,49]
Vitamins	**Content by 100 g**	**References**
Vitamin A (IU)	3131.00	[50-52]
Vitamin C (mg)	127.70	[50-54]
Vitamin E (mg)	1.58	[50-52]
Vitamin K (Âµg)	4.90	[50-52]
Vitamin B3 (mg)	0.98	[50-52]
Vitamin B6 (mg)	0.29	[50-52]
Minerals	**Content by 100 g**	**References**
Sodium (mg)	2.35-2546.28	[17,40,46,47,49]
Potassium (mg)	306.89-921.33	[17,46,47,49]
Phosphorus (mg)	69.21-707.00	[17,46,49]
Magnesium (mg)	141.80-279.00	[17,47,49]
Calcium (mg)	38.80-174.71	[17,40,47,49]
Iron (mg)	3.01-17.49	[17,46,47]
Zinc (mg)	1.05-7.97	[17,46,47]
Manganese (mg)	0.38-4.05	[17,46,47]
Copper (mg)	0.72-3.33	[17,46]
Essential amino acids	**Content by 100 g**	**References**
Leucine (Leu; mg)	830-4006	[17,46,47]
Cysteine (Cys; mg)	413-3463	[17,46,47]
Histidine (His; mg)	192-1620	[17,46,47]
Lysine (Lys; mg)	537-1476	[17,46,47]
Phenylalanine (Phe; mg)	655-1344	[17,46,47]

Threonine (Thr; mg)	478-1188	[17,46,47]
Isoleucine (Ile; mg)	399-869	[17,46,47]
Tyrosine (Tyr; mg)	200-831	[17,46,47]
Methionine (Met; mg)	67-820	[17,46,47]
Valine (Val; mg)	85-760	[17,46,47]
Non-essential amino acids	**Content by 100 g**	**References**
Glutamic acid (Glu; mg)	1188-3668	[17,46,47]
Aspartic acid (Asp; mg)	1188-2030	[17,46,47]
Arginine (Arg; mg)	894-1731	[17,46,47]
Serine (Ser; mg)	558-1088	[17,46,47]
Glycine (Gly; mg)	617-894	[17,46,47]
Alanine (Ala; mg)	578-706	[17,46,47]
Proline (Pro; mg)	100-680	[17,46,47]

Mineral elements are important components of blood and bone in maintaining osmotic pressure and acid-base balance, although some factors such as cultivar, growth environment or the production and processing conditions could affect the mineral composition. In the mineral analysis conducted (**Table 1**), a broad spectrum of sodium concentrations of 2.35-2546.28 mg 100 g^{-1} was quantified [17,40,46,47,49], which might be attributable to brine processes or distinct environmental conditions. Conversely, potassium levels, 306.89-921.33 mg 100 g^{-1} [17,46,47,49] are maintained at moderate levels [17,46,47,49]. Phosphorus, with concentrations ranging from 69.21 to 707.00 mg 100 g^{-1} [17,46,49], highlights the potential of these seeds to provide nutrients of high biological value. On the other hand, magnesium and calcium with levels of 141.80-279.00 mg 100 g^{-1} [17,46,49] and 38.80-174.71 mg 100 g^{-1} [17,40,47,49], respectively, which play a pivotal role in vital functions such as metabolic regulation, energy production, and bone health. Finally, micro-minerals, such as iron, zinc, manganese and copper, are found in lower concentrations [17,46,47], yet are essential for various enzymatic functions, as well as for a wide range of biochemical processes that are vital for maintaining health, growth, and overall well-being.

In **Table 1**, the amino acid profile analysis reveals an interesting nutritional capacity, with the presence of essential amino acids such as leucine, cysteine, histidine, lysine, phenylalanine, threonine, isoleucine, tyrosine, methionine, and valine [17,46,47], whose presence varies significantly according to the cultivar of pepper seeds, suggesting that some types of seed could offer a better protein quality than others. Leucine had the highest content among the essential amino acids, which ranged from 830 mg 100 g^{-1} to 4006 mg 100 g^{-1} [17,46,47], as shown in **Table 1**. Seven kinds of non-essential amino acids were identified, namely, glutamic acid, aspartic acid, arginine, serine, glycine, alanine, and proline [17,46,47]. In **Table 1**, glutamic acid had the highest content among the non-essential amino acids in the pepper seeds, and it ranged from 1188 mg 100 g^{-1} to 3668 mg 100 g^{-1} [17,46,47]. In the domain of food nutrition, essential amino acids have historically been a subject of interest. These amino acids are of particular significance for humans, as the body is incapable of synthesizing them. The present review demonstrates that pepper seeds are rich in amino acids, particularly the ten essential amino acids necessary for the human body. Conversely, the seven non-essential amino acids have been shown to enhance sensory attributes, including taste and texture, in various food products, rendering them components of considerable interest to the food industry.

The extant literature suggests that pepper seeds have the potential to be a valuable by-product within the agri-food chain. This is due to their composition, which is rich in macronutrients, vitamins, minerals and both essential and non-essential amino acids. It is proposed that, through the implementation of suitable processing methodologies, these seeds could be integrated into the development of functional foods, nutritional supplements and other innovative products. This approach would contribute to the reduction of waste and the enhancement of value in the agri-food industry, thereby promoting sustainability and the diversification of nutritional sources.

3.2. Functional Composition of Pepper Seeds: Total Phenolics and Flavonoids

Phenolic compounds are a vital category of secondary metabolites in plants. Peppers are considered to be a valuable source of bioactive compounds, including phenolic compounds, carotenoids, ascorbic acid (vitamin C), tocopherols (vitamin E), and capsaicinoids [55]. Analyses employing high-performance liquid chromatography coupled with tandem mass spectrometry and electrospray ionization (HPLC-ITMS) techniques have facilitated the identification of a number of polyphenolic compounds in *Capsicum annuum* fruits, including caffeic acid, coumaric acid, coumaroylquinic acid, 3-*O*-caffeoylquinic acid, ferulic acid, sinapic acid, apigenin-*O*-hexoside, and quercetin-*O*-rhamnosyl-*O*-hexoside [56]. Recent studies have highlighted the high quality of pepper seeds by-product, indicating its potential to direct the food industry towards the creation of new opportunities within the context of a circular bioeconomy. It is imperative to acknowledge the significance of recognizing food losses and waste as a potential source of biologically active components. These include antioxidants, complex soluble polysaccharides, vitamins, enzymes and fatty acids, among other bio-reagents that can be employed in a range of industries, such as the food, health, medicine and pharmaceutical industries [57].

The extant literature addresses research on various species of pepper seeds. Nevertheless, as indicated in **Table 2**, there is a paucity of available data regarding total phenolic and flavonoid contents in pepper seeds. The growing popularity of natural antioxidants can be attributed to the toxic and anticarcinogenic properties of synthetic antioxidants [57,58]. Consequently, the potential of natural antioxidants, such as by-products from pepper processing, is being investigated, as are the phytochemicals polyphenols, which are present in such plant materials. For instance, the total phenolic content of raw and scalded Jalapeño pepper industrial seeds (*Capsicum annuum* L.) had a range of values from 10.01 mg GAE g^{-1} to 13.09 mg GAE g^{-1} in dry weight (DW) base [15]. In that article, the major phenolic compounds, rutin, epicatechin and catechin comprised 90 % of the total compounds detected by HPLC of each Jalapeño pepper byproducts [15]. Other studies have shown that *Podravka* pepper seeds contained 1.58 mg GAE g^{-1} DW of polyphenols, while *Slavonka* pepper seeds contained lower quantities (1.50 mg GAE g^{-1} DW) [20]. This can be explained by the fact that polyphenol content depends on the pepper cultivar.

Red pepper seed extracts exhibited a polyphenol content of 21.50 mg GAE g^{-1} DW [40], while Sim and Sil [59] reported a content of 29.10 mg GAE g^{-1} DW in red pepper seeds. In addition, Sim and Sil [59] quantified the total flavonoid content of red pepper seed extracts and was found to be a catechin equivalent (CAE) value of 21.27 mg g^{-1} DW. The crude extracts of red chilli (*Capsicum frutescens* L.) seeds had total phenolic content and the total flavonoid content in the ranges of 7.95-26.15 mg GAE g^{-1} and 4.64-12.84 mg of rutin equivalents (RU) g^{-1} DW of extract, respectively [60]. It is evident that hot peppers exhibited a divergent polyphenol content in comparison to sweet peppers. On the other hand, the extraction with 100 mL of ethanol: acetone (50:50, *v/v*) of phenolics form seeds showed an extracted yield of 22.30 mg 100 g^{-1} DW and 88.60 mg 100 g^{-1} DW in seeds from red and green pepper fruit (*Capsicum annuum* L.), respectively [61]. In addition, Ahmad et al. [61] identified the phenolic profile and quantified the content of gallic acid (GA),

scopoletin (SC), rosmarinic acid (RA) and resveratrol (RV), and they concluded that the seeds for the green pepper fruits exhibited more phenolics amounts, as can be observed in **Table 2**. Overall, the study of the biological potential of numerous natural plant phenolic compounds still remains a hot topic among the scientific community [62]. Despite all of those advances, the available knowledge about the responsible phytochemicals for biological potential, their mechanisms of action, the establishment of therapeutic and prophylactic doses, and even the occurrence of biochemical inter-relations, is considerably scarce [62]. Finally, the observed variability in the polyphenol and flavonoid content has been attributed to the different pepper cultivars [63] and the maturity stage [64] since stage from green to red decreased the total phenolic content in peppers.

Table 2. Functional profile and content of phenolics and flavonoids of pepper seeds.

Fruit type	TPC[ϒ]	GA (ppm)	SC (ppm)	RA (ppm)	RV (ppm)	TFC[ǂ]	References
Jalapeño pepper seeds (*Capsicum annuum* L.)	10.01-13.09	-	-	-	-	-	[15]
Podravka pepper seeds (*Capsicum annuum* L.)	1.58	-	-	-	-	-	[20]
Slavonka pepper seeds (*Capsicum annuum* L.)	1.49	-	-	-	-	-	[20]
Red pepper seeds (*Capsicum annuum* L.)	21.50	-	-	-	-	0.04	[40]
Red pepper seeds (*Capsicum annuum* L.)	29.10	-	-	-	-	21.27	[59]
Red chilli seeds (*Capsicum frutescens* L.)	7.95-26.15	-	-	-	-	4.64-12.84	[60]
Red pepper seeds (*Capsicum annuum* L.)	22.30	5.53	14.52	23.87	0.00	-	[61]
Green pepper seeds (*Capsicum annuum* L.)	88.60	6.96	2.21	6.31	0.00	-	[61]

[ϒ] TPC: Total phenolic content (mg GAE g^{-1} DW). [ǂ] TFC: Total flavonoid content (mg QE, CAE or RU g^{-1} DW).
Abbreviations: GA (gallic acid), SC (scopoletin), RA (rosmarinic acid) and RV (resveratrol).

Notwithstanding the ongoing discussion surrounding the fluctuations in phytochemicals within *Capsicum spp.*, researchers posit that the presence of phenolics and flavonoids is more pronounced in the green and immature stages of these species [65-67]. The literature indicates that pepper seed byproducts possess potent antioxidant activity, including chopped pepper seeds [43], pepper seed core waste [68], seeds [69,70], pepper seed oil [42,71], pepper seed flour [20], and protein hydrolysates obtained from red pepper seeds [72], among others. Consequently, the implementation of a botanical classification scale for the purpose of differentiating between concentrations is proposed, with foods being classified as low (0.10-39.90 mg kg^{-1}), moderate (40-99.90 mg kg^{-1}) or high (> 100 mg kg^{-1}). The proposed classification system is rooted in the work of Howard et al. [63]. and Peterson and Dwyer [73]. Consequently, the red pepper seed extracts were categorized as exhibiting elevated flavonoid content.

3.3. Fatty Acid Profile of Pepper Seeds: Saturated and Unsaturated Fatty Acids

Pepper seed oil of *Capsicum annuum* L. is rich in unsaturated fatty acids, with linoleic acid (C18:2) representing the predominant fatty acid, accounting for between 67.80 % and 77.90 % of the total fatty acids (**Table 3**) [1,20,36,39,40,41,42,44,46]. According to **Table 3**, the remaining total fatty acids are oleic acid (C18:1; 4.6-14.6 %), which is classified as unsaturated, and the saturated acids are primarily palmitic acid (C16:0; 10.60-14.40 %) and stearic acid (C18:0; 2.40-4.10 %) [1,20,36,39,40,41,42,44,46]. As Konscek et al. [41] demonstrated, the fatty acid composition of pepper seed oil remained consistent irrespective of cultivar or growing season. No significant differentiation in fatty acid profile was identified between the *Podravka* and *Slavonka* species and cultivars [16,20]. Matthäus et al. [74] reached a similar conclusion, indicating that the variation in linoleic acid (C18:2) between *Capsicum annuum* cultivars was minimal (69.50-74.70 %), as was the variation in the other determined fatty acids, such as C16:0 (10.70-14.20 %), C18:0 (2.50-4.10 %) and C18:1 (8.90-12.50 %). Accordingly, the most dominant fatty acid in cold-pressed paprika seed oil was linoleic acid with a percentage of 69.60 ± 2.30 % [75].

Table 3. Fatty acid composition (%) of pepper seed oil (*Capsicum annuum* L.).

Fatty acid	Content (%)	References
Palmitic acid (C16:0)	10.60-14.40	[1,20,36,39,40,41,42,44,46]
Stearic acid (C18:0)	2.40-4.10	[1,20,36,39,40,41,42,44,46]
Oleic acid (C18:1)	4.60-14.60	[1,20,36,39,40,41,42,44,46]
Linoleic acid (C18:2)	67.80-77.90	[1,20,36,39,40,41,42,44,46]

The beneficial effect is obtained with a daily intake of 10 g of linoleic acid, and the replacement of saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels [monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) both being unsaturated fats] [16]. In a study by Konscek et al. [41], it was demonstrated that 10 g of pepper seed oil, when used as a salad oil, can provide 7.0-7.4 g of linoleic acid, which is equivalent to 70-74 % of the suggested beneficial minimum daily intake.

3.4. Characterization of Volatile Organic Compounds of Pepper Seeds

Volatile organic compounds (VOCs) in pepper seeds are critical to their aroma and flavour, which influence both their commercial and culinary value. The categorization of VOCs can be approached through the lens of their biosynthetic origins, which can be broadly classified into the following groups: fatty acid derivatives, amino acid derivatives, terpenoids, phenylpropanoids/benzenoids, and species- or genus-specific compounds not involved in the major classes [76,77]. Pepper volatile compounds that have been identified in studies are mainly aldehydes, esters, terpenes and alcohols [78,79]. These compounds vary significantly among cultivars and tissues, with higher concentrations typically observed in placental tissues. Despite the existence of several studies on pepper aroma substances, the majority of these studies concentrate on fresh and dried pepper fruit and chili pepper [78-81]. However, factors such as the maturity stage and processes such as roasting or fermenting chopped peppers have been demonstrated to influence the profiles of these substances [17,79,82], with the potential to enhance existing flavours or create new ones.

Forero et al. [79] identified 140 constituents as the steam volatile components of chile pepper (*Capsicum annuum* L. var. *glabriusculum*) at two ripening stages (green and red) using GC and GC–MS. Hexyl isopentanoate, hexyl 2-methylbutanoate, limonene, hexyl isohexanoate, (E)-2-hexenal, isopentyl isopentanoate and (Z)-3-hexenyl isopentanoate were found to be the major components. During fruit maturation, the majority of volatile

compounds decreased [79]. In general, green chile peppers have higher amounts of esters, with their fruity odour notes, than red fruits [79]. Attending to the differences in the number of total volatiles, which is higher at the green stage in comparison with the mature stage, it can be concluded that the green stage is better in terms of its flavour than the red stage [79]. On the other hand, Chen et al. [17] reported the VOCs profile of chopped pepper seeds, with aldehydes, esters, and alcohols as the predominant groups. A key finding was the presence of five common key VOCs across all three chopped pepper seeds cultivars: 2-pentylfuran, methional, ethyl 3-methylbutanoate, dimethyl disulfide, and nonanal [17]. These compounds are considered significant contributors to the aroma due to their relative odor activity values (ROAV ≥ 1) [17]. However, the study also revealed remarkable variations in the aroma profiles among the three cultivars, these differences being likely attributed to factors such as the origin and cultivar of the raw peppers, different production seasons, and the fermentation methods employed during chopped pepper manufacturing [17]. Finally, the identified VOC profile in chopped pepper seeds showed similarities to that of pepper and chili sauce products, with the presence of compounds like 1-octen-3-ol, methyl salicylate, and ethyl acetate [17]. This suggests that chopped pepper seeds have the potential to be used as a functional food ingredient and a natural flavoring agent in the food industry to enhance flavor.

Red pepper seeds were subjected to a roasting process, with constant agitation, for 6, 9, 10 and 12 minutes at a temperature of 210 °C [82]. The oils were subsequently extracted from the roasted red pepper seeds using an expeller [82]. Thirteen alkylpyrazines were identified in the roasted red pepper seed oils: 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine, 2,6-diethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, tetramethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2-isobutyl-3-methylpyrazine, and 3,5-diethyl 2-methylpyrazine [82]. The pyrazine content exhibited a marked increase with an increase in roasting time, with levels of 2.63, 5.01, 8.48, and 13.10 mg of total pyrazine being recorded per 100 g of oil from red pepper seeds roasted for 6, 8, 10 and 12 minutes at 210 °C, respectively [82]. The 2,5-dimethylpyrazine content in the roasted red pepper seed oil appeared to be the component most responsible for the pleasant nutty aroma of the oils [82]. Furthermore, an increase in the oxidative stability of the oils was observed as the roasting time was increased [82]. Johnson et al. [83] hypothesized that alkylpyrazines contributed to the roasted peanut flavour. That study proposed that alkylpyrazines, specifically 2,5-dimethylpyrazine, 2-methylpyrazine, trimethylpyrazine and 2,6-dimethylpyrazine, contribute to the roasted peanut flavour in red pepper seed oil [83]. These findings corroborate earlier studies that had previously identified these alkylpyrazines in roasted peanut volatiles [84]. This research is of paramount importance in comprehending the compounds responsible for the roasted flavour, and it may have significant ramifications for the food industry.

4. Sustainable Agrifood Applications: Integral Valorisation of Pepper Seeds

4.1. The Condimentary Use of Pepper Seeds in Food

Pepper seeds are a common by-product of the processing of pepper fruits. Their nutritional and functional characteristics have been well-documented, and they may be regarded as a potentially valuable raw material for the production of oil or the extraction of protein and fiber [20]. The extraction of oil from pepper seeds can be achieved through the utilization of a cold-pressing method, whereby the residual defatted pepper seed meal, otherwise known as press cake, undergoes additional processing to yield high-value protein and fiber [48]. An additional strategy for enhancing the viability of this by-product could be to grind the pepper seeds after drying, thereby producing a flour that can be

utilized in the creation of novel and functional products with more favourable chemical and sensory properties due to the incorporation of parts of pepper fruit [85,86]. As can be observed in the **Table 4**, some studies have focused on the valorisation of pepper seeds as by-products to develop new products [48,87,88]. Bostanci et al. [48] studied the valorisation of capia pepperseeds, which is waste product of capia pepper processing, in food applications, specifically in the development of innovative spreadable pastes. Two formulations, chocolate type and molasses type, were evaluated [48]. Both formulations included 23.7 and 30.1 % pepperseed flour, chocolate liquor or molasses as aroma source, sugar, palm oil, citric acid, potassium sorbate and lecithin as the ingredients [48]. The developed spreadable pastes were found to contain elevated levels of linoleic acid, sterols, tocopherols and dietary fiber, in comparison to commercially available products of a similar nature [48]. In the study conducted by Yilmaz et al. [87] the capia pepperseed flour possessed a characteristic peppery flavour. Notwithstanding, these overall acceptable sensory properties have permitted the successful incorporation of pepperseed flour in quantities of up to 20 % in the development of vegetable and spicy sauces [87]. Furthermore, Guo et al. [88] conducted a study on the development of new hot pot sauces based on pepper seed press cake. The results obtained from this study indicated that enriching the sauce with the 5-10 % of pepper seed press cake increased the content of palmitic and linoleic acids, enhanced storage stability, and improved the rheological behaviour and textural properties [88].

Table 4. By-product valorization through product development.

Ingredient	Dosage (%)	New Product Development	References
Capia pepperseed flour	23.7-30.1	Novel spreadable pastes	[48]
Capia pepperseed flour	20.0	Novel breakfast sauce	[87]
Pepper seed press cake	5.0-10.0	Novel hot pot dipping sauce	[88]

Conversely, bioactive compounds extracted from red and yellow peppers have been employed as natural colourants in dairy products, such as yoghurt and isotonic drinks, enhancing their visual appeal, sensory attributes and nutritional value [85,89,90]. Furthermore, in the meat industry, *Capsicum spp.* extracts have been demonstrated to be efficacious in extending the shelf-life of beef and curtailing bacterial proliferation [91,92]. In a similar manner, the seeds of the pepper plant also contain bioactive compounds that could be utilised for these purposes, thus complementing and extending the established uses of the previously mentioned pepper extracts. A substantial number of studies have identified the potential of pepper seeds as a source of valuable compounds, including fiber and antioxidants, which could be used in the development of new products [48,87,88]. Pepper seeds contain a high concentration of fiber and antioxidants, and their grinding to produce flour could be a viable method of incorporation into various food products, such as bakery items and other processed foods, with the objective of enhancing their nutritional value. The oil obtained from the seeds, characterised by its richness in unsaturated fatty acids, has significant potential for use in the food industry. The oil's high antioxidant content endows it with antimicrobial properties, rendering it an appealing ingredient for the preservation of foodstuffs. This approach may also provide a technologically feasible and economically sound solution to valorise food by-products. However, there is currently a paucity of literature on the use of *Capsicum spp.* seeds as supplements, additives or coatings in food products. Nevertheless, given its nutritional value, the by-product of *Capsicum spp.* seeds after a drying or roasting process could be another promising avenue for future agrifood applications and research.

The strategic utilisation of food industry by-products is gaining significant traction, fuelled by a plethora of scientific studies demonstrating their capacity to enhance both the antioxidant and organoleptic qualities of food products. As demonstrated by Çalişkanlar et al. [93], the incorporation of pomegranate and grape seed powder into yogurt production has been shown to enhance the phenolic and antioxidant profiles, thereby positively impacting sensory attributes and increasing consumer appeal. An analogous development has been observed with pumpkin seeds, which have been incorporated into a diverse range of food products, including bakery items, dairy, meat, confectionery, and snacks, thereby significantly enriching their nutritional value with fatty acids, proteins, fibers, minerals, and bioactive compounds [94]. In addition, cocoa hulls have been explored as functional ingredients in biscuits, emphasising the versatility of these by-products [95]. The potential of oils derived from passion fruit and grape seeds, recognised for their fatty acid content [96], further underscores this trend. Grape seed oil with its potent antioxidant and antimicrobial properties has been effectively incorporated into innovative food formulations such as yogurt, chocolate, canned fish, and sausages [97]. Beyond oils and seeds, flours from mango seed and kernel have been used in porridge [98], while powders from wine industry waste have been used as oxidative stabilisers in perishable foods like chicken pâté [99], and grape pomace flour has enriched bread [100]. The preceding studies indicate the considerable versatility of by-products from the food industry, which have the capacity to enhance nutritional profiles and sensory attributes, while concurrently promoting sustainable food production. In this respect, pepper seeds are emerging as a comparable and sustainable alternative with significant potential for by-product valorisation through product development.

4.2. The Organic Pepper Seed Extracts as Plant Biostimulants

The use of elicitors or plant growth regulators (PGRs) at the preharvest stage is not a widespread practice among companies. This technology is still in the early stages of development and is currently the subject of experimental research. Elicitors that have been tested are based on a profile of different bioactive compounds. A notable example is the application of elicitors of salicylic acid (SA), jasmonic acid (JA), oxalic acid (OA), gamma-aminobutyric acid (GABA) and their derivatives, which have been used as tools to increase yield and quality of various crops, including sweet pepper, artichoke, pomegranate and sweet cherry, lemon, plum, table grape [101-111]. However, the main drawback of this approach is that obtaining the commercial compound places an economic burden on the company. By-products of fruit and vegetable processing include seeds, skin, pomace and peels, which are not usually consumed on a regular basis, but contain bioactive compounds such as phytochemicals and secondary metabolites that are stored in the tissues [112,113].

It has been observed that these by-products often have higher concentrations of bioactive compounds compared to the edible part of the fruit [114,115]. For example, pepper seeds have been shown to have a remarkable concentration of phenolic compounds, suggesting their potential use in the formulation of biostimulants for agricultural applications aimed at promoting growth and enhancing plant defence mechanisms. The biostimulant and bioprotective properties of exogenously applied extracts and phenolic compounds from aromatic plants have recently been evaluated [116-120]. Reported properties include improvements in seed germination, rooting, sprouting and fruiting, as well as antimicrobial, insecticidal, nematocidal and herbicidal properties [116-120].

As reported by Mejri et al [121], novel resistance inducers have been identified from sugar beet by-products that have the potential to improve resistance of wheat to *Zymoseptoria tritici*. In another study by Lujan et al [122], phenolic extracts from pecan shells and hulls were shown to have the ability to reduce infection of chilli plants by *Phytophthora*

capsici. These findings provide a framework for further exploration of the use of secondary metabolite extracts as enhancers of plant defence responses against pathogens. However, there is a need to identify novel compounds that act as elicitors but are derived from a plant by-product, such as the by-product seeds from green pepper processing. The biostimulant and bioprotective properties of exogenously applied extracts and phenolic compounds from aromatic plants have recently been evaluated. Reported properties include improvements in seed germination, rooting, sprouting and fruiting, as well as antimicrobial, insecticidal, nematocidal and herbicidal properties [116-120].

4.3. Nutraceutical Applications to Benefit Human Health

With the increasing prevalence of diet-related health concerns, the need for dietary supplements has become increasingly apparent. Pepper seeds are a source of essential compounds and oils, although their concentrations are low. Nutraceuticals are defined as compounds derived from foods or food components that provide health benefits by treating or preventing disease. They are characterised by the presence of antioxidants, dietary fibers, fatty acids and polyphenols [17,20,40,46]. The presence of bioactive compounds in fruit and vegetable wastes has been shown to serve as a reservoir to produce nutraceuticals [123]. Polyphenols are a heterogeneous group of bioactive compounds that play an important role in the nutraceutical industry. These compounds, which include phenolics, flavonoids, phenylparanoids, quinones, tannins and lignin, act as antioxidants and contribute to the prevention of chronic, degenerative and cardiovascular diseases [124]. The phenolic content of pepper seeds could be identified as a useful resource in preservatives, additives or dietary supplements (post-encapsulation). In addition, it has been postulated that the use and improvement of dietary fiber intake are bulk mediators responsible for improving faecal hydration, sugar absorption and intestinal motility [125]. It consists mainly of carbohydrates, hemicellulose, lignin, cellulose and pectin [126].

Seeds represent a significant proportion of plant by-products and are a rich and valuable source of bioactive compounds of interest to the nutraceutical industry. Grape seeds, for example, are known for their high proanthocyanidin content, which has been used to improve cardiovascular health and reduce inflammation [127]. Pumpkin seeds, which are known for their high levels of phytosterols, have been shown to be beneficial for both prostate and cardiovascular health [128]. In addition, Kumar et al [129] documented that the high lycopene content of tomato seeds provides protection against oxidative damage and promotes cardiovascular health. Furthermore, cranberry seeds have been shown to have antibacterial properties due to the presence of type A proanthocyanidins [130]. On the other hand, chia seeds, which are high in fibre and omega-3, have been shown to improve digestive health and regulate blood sugar levels [131]. Finally, Mueed et al [132] documented the beneficial effects of flaxseed on cardiovascular and digestive health. These effects are thought to be due to the alpha-linolenic acid (ALA), fiber and lignan content of the seeds.

As shown in the study by Viuda-Martos et al [133], pomegranate seeds, which contain punicalagins and punicic acid, have been shown to have antioxidant and anti-inflammatory benefits. In addition, kiwifruit seeds, which are rich in fibre and antioxidants, have been shown to improve immune function and digestive health [134]. Given the growing interest in the use of by-product seeds as nutraceuticals, pepper seeds are a promising option. Previous research has shown that *Capsicum spp.* seeds contain phenolic compounds and other phytochemicals with anti-inflammatory and antimicrobial properties, suggesting potential applications in digestive health and disease prevention. However, further specific research is needed to validate these findings. Nevertheless, the nutritional composition of *Capsicum spp.* seeds suggests that they could be added to the list of seeds used by the nutraceutical industry, following the trend of using plant by-products for

human health. In this regard, the agricultural industry needs to invest in technologies that can convert by-products and waste into bioactive compounds for optimal nutrient recovery.

4.4. Pepper Seeds as Suitable Ingredient for Nonfood Industries: Cosmetic and Biofuel Source

Considering the prevailing eco-friendly consumer and industrial trends, there is a significant interest in the exploration of bioactive compounds, raw plant materials, and plant extracts as natural ingredients or excipients for cosmetic or pharmaceutical applications. Vegetable oils are defined as organic compounds that are obtained through extraction processes from seeds and other parts of plants [135]. The chemical composition of these oils is dominated by triglycerides, diglycerides and fatty acids, including stearic, linoleic, oleic and linolenic acid, along with other minor constituents such as tocopherols and sterols. Fatty acids have been demonstrated to exert pivotal functions in the hydration, softness and suppleness of the skin, in addition to their contribution to the repair of the epidermis [136]. The properties of fatty acids have resulted in their frequent utilisation in the domains of cosmetics and dermatological pharmaceuticals.

It is evident that oils derived from seeds, fruits and vegetables have experienced a marked increase in popularity. This is primarily attributable to their distinctive characteristics and their favourable effects on the skin and hair. A prime example is argan oil, extracted from the seeds of the argan tree. This oil is highly prized for its moisturising and antioxidant properties and is an essential component in anti-ageing products [137]. In addition, rosehip oil, obtained from the seeds of the rose hip, is recognised for its ability to improve the appearance of scars and stretch marks, thanks to its high content of essential fatty acids [138]. A plethora of oils obtained from seeds and pits of fruits, including but not limited to pomegranate, fenugreek, poppy, blackcurrant, chokeberry, rosehip, perilla, elderberry and carrot, have been found to possess antioxidant properties. These oils provide a valuable complement to synthetic antioxidants, offering additional benefits to the skin [139]. The oils, when considered in conjunction with other compounds, offer a multitude of benefits, including but not limited to hydration, nourishment and protection against environmental damage. These components are widely regarded as being of paramount importance in the formulation of cosmetic products.

The by-product of *Capsicum spp.* seeds has attracted increasing interest in the cosmetic industry, due to its composition, which is rich in bioactive compounds. Evidence suggests that the seeds contain oils with high levels of unsaturated fatty acids, including linoleic acid and oleic acid [1,20,36,39,40,41,42,44,46]. These acids are widely acknowledged for their emollient and moisturising properties. The incorporation of these oils into creams, lotions and body oils has the capacity to enhance skin hydration and restore its lipid barrier. In addition, *Capsicum spp.* seeds have been demonstrated to be a source of antioxidants, including carotenoids and polyphenols, which have been shown to protect the skin from damage caused by free radicals and oxidative stress. Furthermore, *Capsicum spp.* seeds have been found to contain phenolic compounds, including flavonoids, which possess both anti-inflammatory and antioxidant properties [15,20,40,59,60,61]. Consequently, these seeds could be utilised in the formulation of skin care products that aim to provide soothing and rejuvenating effects. In this regard, extracts obtained from these seeds could be incorporated into creams intended for use on the skin. The cosmetic industry has been engaged in an investigation into the potential of *Capsicum spp.* seeds as a natural and sustainable ingredient for the development of innovative and effective products, with the possibility of influencing the final product performance. Further study is required to ascertain the physical properties (colour, texture or permeability) and bioactivities (antimicrobial and antioxidant) of *Capsicum spp.* seeds, and their impact on cosmetic products.

Regarding biofuels and biomass sources, the oil obtained from pepper seeds has emerged as a potentially viable source to produce biodiesel, owing to its abundant fatty acid composition, which is comparable to that of other vegetable oils commonly utilised in the biofuel industry. The transesterification process, which involves the conversion of oil into biodiesel, can be applied to this by-product, resulting in the generation of a renewable and biodegradable fuel. The study carried out by Toghiani et al. [140] demonstrates the potential for the utilisation of pistachio by-products in the production of fungal biomass, characterised by its high protein content, through the fermentation process with edible fungi. This finding could have significant applications in the field of animal feed and biorefinery. Moreover, a recent review has investigated various valorisation techniques to produce biofuels such as biodiesel, biogas, biohydrogen and fuel pellets from fruit residues and by-products. A plethora of fruits have been documented as suitable for biofuel production, including mango, pineapple, banana, papaya, avocado and watermelon [141].

In this sense, the utilisation of pepper seeds for biodiesel production has the potential to contribute to the principles of the circular bioeconomy. This is because it could reduce dependence on fossil fuels and minimise the generation of agricultural waste. The implementation of efficient technologies for oil extraction and biodiesel production from pepper seeds could stimulate the development of a more sustainable and diversified biofuel industry. In the domain of sustainable agriculture, pepper seeds have emerged as a promising resource, demonstrating remarkable versatility. These materials could be used as biomass for the purpose of thermal energy generation. The process under discussion involves the combustion of the seeds, either in a direct manner or after a densification process, such as pellet production. The resultant energy could be utilised for heating or electricity generation. The implementation of this technology is especially pertinent in regions where pepper cultivation is prevalent, as it facilitates the effective utilisation of agricultural waste. The utilisation of pepper seed biomass as a source of energy could have the potential to contribute to the reduction of greenhouse gas emissions and to the diversification of the energy matrix. Moreover, it has the potential to result in the establishment of a more sustainable economic model by assigning value to an agricultural by-product that would otherwise be disposed of.

5. Conclusions

In conclusion, pepper seeds, often discarded as waste, are a valuable by-product with significant potential for application in sustainable applications. Their composition is characterized by a high content of oils, proteins and bioactive compounds, which can be extracted and utilized in various industrial contexts. For instance, pepper seed oil, with its favorable fatty acid profile, is suitable for use in the agrifood, pharmaceutical and cosmetic industries. This process has the dual benefits of reducing reliance on other vegetable oils and mitigating waste. Furthermore, the seeds contain high-quality proteins suitable for incorporation into animal feed or human food, thus contributing to food security and diversification of protein sources. Furthermore, the seeds contain a high concentration of antioxidants and other compounds that have significant applications in the nutraceutical and cosmetic industries. The extraction of these valuable compounds allows the creation of highly valuable-added products from waste material, and the remaining seed material can be composted and reintegrated into the pepper crop cycle as organic fertilizer, thereby effectively closing the cycle and minimizing environmental impact. The integral valorization of pepper seeds has been demonstrated to engender a reduction in waste, whilst concomitantly engendering new economic opportunities and contributing to the development of a circular bioeconomy.

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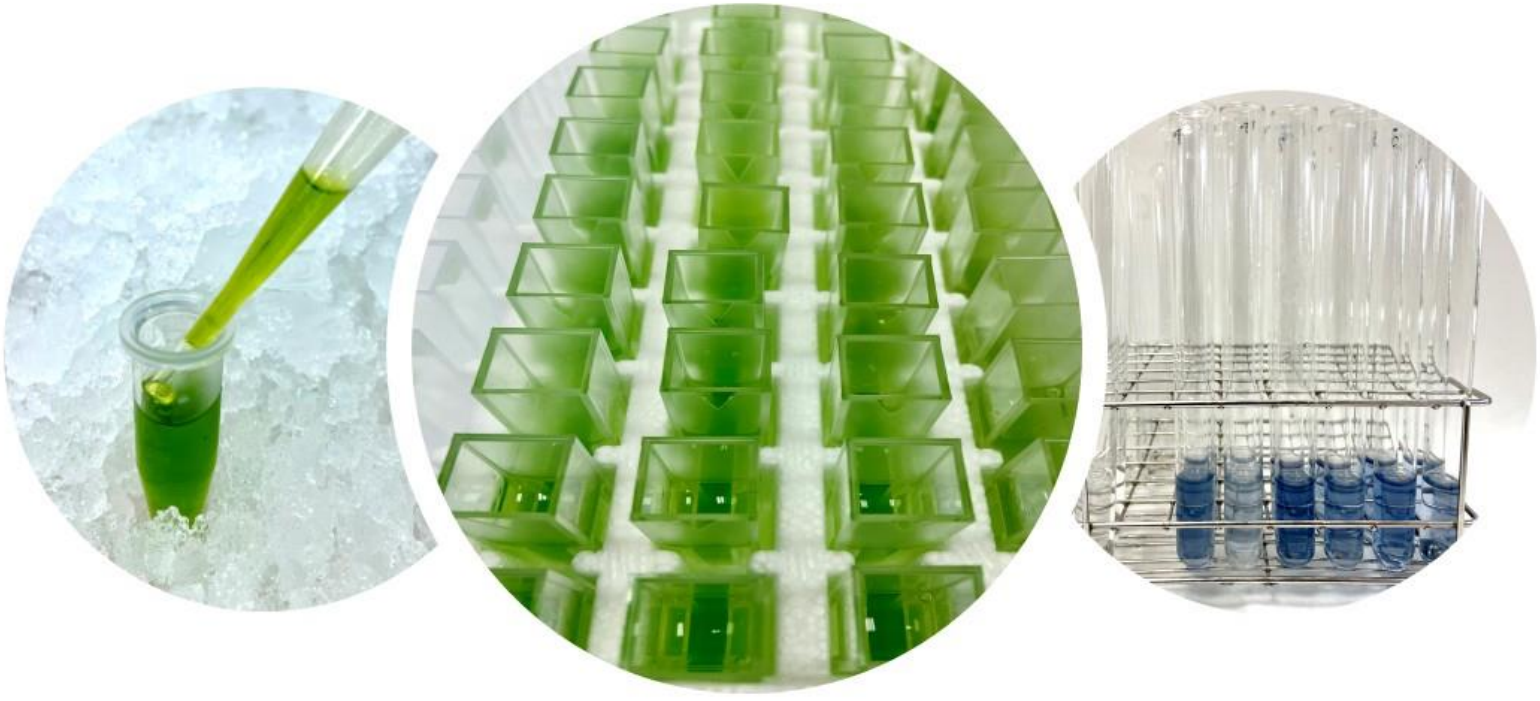
Results and Discussion



5. RESULTS AND DISCUSSION

The challenge of consistently producing high quality crops is a perennial issue in the arable industry today. In order to increase yields, it is essential that the plant undergoes optimal development and produces high quality, as well as large sized produce at harvest. The quality of the final product is influenced by several factors both before and after harvest. In addition, a few abiotic and biotic factors can affect the yield and shelf-life of harvested fruit and vegetables. Plant tissues have the physiological capacity to respond to these agents indirectly through a series of biochemical signals. However, it has been shown that the stage of the plant is directly related to the efficiency and tolerance of its natural defence system to suboptimal conditions. After harvest, fruit and vegetables undergo an energy stress triggered by the ripening and senescence of tissues. Quality at harvest has a significant impact on postharvest shelf-life, so factors such as firmness and nutritional content of the fruit are essential to support handling and marketing processes and to achieve the required acceptance by the final consumer (Valero & Serrano, 2010).

As mentioned in the introduction of the present PhD thesis, the pepper fruit is a highly perishable vegetable. About 30 % is lost during transport, storage and marketing due to its high-water content, thin cuticle and active postharvest metabolism (Airaki et al., 2012). Maintaining quality during postharvest storage requires appropriate handling. A temperature of 7 °C is optimal for maintaining the quality of pepper fruits during storage. Nevertheless, it is recognised that the quality of sweet peppers is susceptible to deterioration during postharvest storage. The main problems that occur and affect the quality parameters of peppers are mainly the incidence of rot, mainly caused by *Alternaria alternata*, water loss, colour and firmness (Rao et al., 2011). Conversely, cold storage has been shown to retard metabolic activity in peppers, delaying the onset of senescence and prolonging shelf-life during storage. However, pepper fruits are highly susceptible to CI when stored at temperatures below 5 ºC for prolonged periods. The symptoms associated with this disease manifest themselves when peppers are stored at low temperatures, including skin pitting, skin bagging and browning of the seeds and calyx. This results in a significant deterioration in the quality of the pepper (Ge et al., 2019).

The focus on the use of plant compounds as preharvest strategies is due to legal restrictions on the use of chemical treatments with artificial substances and consumer concerns about these compounds, mainly in postharvest. Therefore, the main aim of the present PhD thesis is to investigate the application of plant-occurring natural compounds, specifically SA and MeJA. To this end, this PhD thesis has evaluated the potential benefits of implementing this novel strategy in the preharvest area, particularly regarding the ability to delay ripening and maintain fruit quality during storage at optimal or suboptimal (refrigeration at 2 ºC) temperatures for green pepper fruit. This section synthesises and critically evaluates the results obtained from the interrelated studies (***publications 1, 2, 3, 4 and 5***) that investigated the importance of determine the optimal phenological stage and harvest date in order to achieve the highest functional quality, as well as the influence of elicitation strategies in preharvest with SA and MeJA on crop yield, quality parameters, postharvest ripening, bioactive compounds, antioxidant capacity, chilling injury tolerance and fruit decay of Lamuyo-type green pepper fruits (*Capsicum annuum* L., cv.

'Herminio'). Finally, the characterisation of the phytochemical composition of pepper seeds as by-products generated in the industry and their potential value for agrifood applications have been reviewed (***publication 6***). The purpose of this review article is to emphasise the significance of research lines associated with the near future within the paradigm of the circular bioeconomy.

5.1. Determination of the phenological stage, the harvest date, and the influence of elicitors on crop yield

The present PhD thesis describes the correlation between the phenological stage and harvest time of green pepper fruit and its functional compounds. The development and growth cycle of green pepper fruit exerts a substantial influence on its bioactive compound content and overall quality. In ***publication 1***, two methodological approaches were used to evaluate the effect of both phenological stage and harvest date on the bioactive compound composition of Lamuyo-type green pepper fruit. Firstly, the present study examined twelve different phenological stages of green pepper fruits. Secondly, green pepper fruits at the same phenological stage were analysed at seven different harvest dates spanning the winter and spring periods within the same growth cycle.

As outlined in ***publication 1***, the developmental and growth cycle of green pepper fruit significantly affects its bioactive compound content and overall quality. The phenological stage and harvest date are critical determinants of the accumulation of health-promoting compounds in Lamuyo-type green peppers. It is noteworthy that peppers harvested at the most advanced phenological stage (S12) in April exhibited the highest concentrations of antioxidant compounds, particularly ascorbic acid (AA), dehydroascorbic acid (DHA), and total phenolic content. Green pepper fruit, harvested before reaching full ripeness, has been observed to exhibit an increasing trend of AA content in the most advanced phenological stages, as evidenced by studies on various pepper cultivars (Navarro et al., 2006; Martí et al., 2011; Zhuang et al., 2012; Ghasemnezhad et al., 2011). The increase in TPC observed in the final phenological stages may be associated with previous findings indicating that the ripening process in fruits and vegetables is characterised by a significant accumulation of total phenolic compounds (Belwal et al., 2019). Furthermore, the substantial increase in total phenolic content observed on the last two harvesting dates (6^{th} and 20^{th} April) agrees with the results obtained in previous studies that identify the time of harvesting as a critical factor influencing the content of phenolic compounds in pepper fruits (Martí et al., 2011; Chávez-Mendoza et al., 2015). Furthermore, a substantial increase in TSS and TA was observed in the peppers (S12) harvested on 20^{th} April.

The ***publication 1*** also reported that the highest TA levels were found to be attributable to elevated concentrations of citric, malic, ascorbic, and succinic acids. It is noteworthy that a stronger positive correlation between TA and harvest date was evident in later harvests. However, postharvest storage at 8 °C for a 21-day period has been shown to induce a decrease in TA, which has been demonstrated to be inversely associated with an increase in TSS. This increase in TSS during the postharvest period is likely attributable to polysaccharide degradation and the subsequent accumulation of sugars. Furthermore, an increase in glucose and fructose levels was observed in the storage samples, whilst most organic acids (citric, malic, ascorbic, and succinic) decreased, except for fumaric acid, which demonstrated an increase. In this sense, TSS and TA

levels increased significantly at the S12 phenological stage. The harvest date demonstrated a significant correlation with TA, with elevated levels being observed in subsequent dates.

Following the harvesting of peppers, a decline in both AA and DHA content was evident when stored at 7 ºC for a 21-day storage period (***publication 1***). Conversely, an enhancement in TPC and both H-TAA and L-TAA was observed, with increases of 1.12-, 1.21-, and 2.03-fold, respectively. These results are consistent with those reported by Raffo et al. (2008), Barbagallo et al. (2012) and Barzegar et al. (2018), who observed an initial increase in antioxidant activity of green peppers during storage, attributed to the accumulation of hydroxycinnamic acid derivatives. Furthermore, fruits harvested at the most advanced phenological stage (S12) and at later harvest dates (20th April) exhibited the highest concentrations of antioxidant compounds and organic acids, including citric, malic, ascorbic, and succinic acids. However, a significant decline in these organic acids was observed during postharvest storage. These findings corroborate previous research suggesting that metabolic changes during storage affect the chemical composition and functional properties of peppers (Medlicott & Thompson, 1985).

In conclusion, the findings of the present PhD thesis, specifically those reported in the ***publication 1***, demonstrate that later phenological stages (S12) and later harvest dates (20th April) are associated with higher levels of TA, AA, DHA, TPC and H-TAA. It is hypothesised that if the fruit is permitted to continue developing on the plant, its nutritional and functional properties may be enhanced. It is recommended that fruits be harvested at the most advanced phenological stage and at later harvest dates. This knowledge forms the basis for investigating opportunities to improve the crop yield of green pepper fruit at harvest throughout the elicitation strategies applied in preharvest, in which SA and MeJA are encompassed (***publications 2 and 4***, respectively).

In this sense, the ***publication 2*** evaluated the effect of foliar preharvest treatment of SA at 0.5, 1, 5 mM on crop yield throughout the development and growth cycle of Lamuyo-type green pepper fruit, cv. 'Herminio'. The results obtained demonstrated that SA application at low concentrations (0.5 and 1 mM) significantly increased the accumulated crop yield (kg per plant) in comparison with the control. This increase was evident across ten harvest dates from April to July in the 2020 season. Conversely, the study also revealed a negative impact of higher SA concentrations (5 mM) on accumulated crop yield, with a significant reduction observed at the end of the crop cycle. It is worthy of note that the lowest concentration of 0.5 mM-SA tested was the most effective, showing a 2.0-fold increase at the first harvest date and a 0.87 kg per plant increase on the final accumulated yield compared to control pepper fruits. The enhanced crop yield was attributed to a significantly higher number of harvested fruits per plant and an increased average fruit weight. The administration of the treatments preceded the flowering stage. This finding suggests that the increase in fruit number observed in response to SA treatments could be related to several possibilities, including an elevated rate of flowering, an increased rate of fruit set, or a reduced incidence of fruit abscission, which occurs naturally during fruit development.

As demonstrated in earlier studies, the application of 0.001 mM SA resulted in a 1.70-fold increase in flower production, 40 % fructification, and enhanced net photoassimilate production in habanero pepper plants (Tucuch-Hass et al., 2017). This, in turn, led to an increase in fruit weight.

Furthermore, SA modulates various physiological and biochemical processes, including stomatal regulation, ion transport, membrane permeability, and photosynthetic efficiency (Barkosky et al., 1993; Khan et al., 2003; Gunes et al., 2005). In sweet pepper, the application of SA has been reported to stimulate vegetative growth, enhance the accumulation of photosynthetic pigments and mineral nutrients, and regulate endogenous hormone levels (El-Yazied, 2011). Furthermore, SA has been linked to elevated Rubisco activity and augmented yield in both maize and mustard crops (Fariduddin et al., 2003). Furthermore, its potential role in mitigating fruit abscission in pepper plants has been demonstrated, with similar effects previously reported in pomegranate fruit (García-Pastor et al., 2020a). Conversely, the higher concentration of SA (5 mM) exhibited an inhibitory effect, resulting in a reduction in both the number of fruits and their respective average weight. Consequently, preharvest treatments in table grapes with high concentrations of salicylates (5 and 10 mM) have been demonstrated to result in delayed berry ripening and reduced crop yield. Conversely, ripening was accelerated and crop yield increased at lower concentrations (García-Pastor et al., 2020d).

In a similar manner, the foliar preharvest application of MeJA, as reported in ***publication 4***, also demonstrated a favourable effect on accumulated crop yield in two growing seasons (2020 and 2021) in this PhD thesis. The accumulated crop yield was found to be significantly higher in MeJA-treated plants at 0.1 and 1 mM in the 2020 season, showing a 0.78- and 0.47-fold increase in total production compared to untreated plants, respectively. The preharvest treatment at lower concentration exhibited the higher accumulated total yield, being the most effective in enhancing total production in the 2021 season. In addition, the 0.1 mM-MeJA concentration was identified as the most effective treatment, resulting in a yield per plant of 5.01 ± 0.07 kg, which is significantly higher than the control plants, yielding 4.09 ± 0.054 kg per plant. This is the first study to detail the effect of preharvest foliar MeJA application on total Lamuyo-type pepper plant yield. Furthermore, ***publication 4*** emphasises the potential of MeJA as a yield-enhancing tool at specific concentrations. This finding is consistent with the results of previous studies on other crops, which have suggested that MeJA enhances productivity by modulating physiological and metabolic processes, including flowering, fruit set, and reducing the impact of plant abiotic stress and photosynthetic activity (Martínez-Esplá et al., 2014; Lotfi et al., 2018; García-Pastor et al., 2019; García-Pastor et al., 2020b; Saadati et al., 2021; Baek et al., 2021). Nevertheless, in a manner analogous to SA, other studies have been documented which report that elevated doses of MeJA have a detrimental effect on crop yield, resulting in a substantial decrease in fruit weight and size, whilst concomitantly delaying the ripening process (Rudell et al., 2005; García-Pastor et al., 2019).

As demonstrated in ***publications 2 and 4***, in combination with the available literature, both SA and MeJA have the capacity to positively modulate pepper crop yield when applied preharvest at concentrations that are most effective at low levels, *i.e.* 0.5 and 0.1 mM, respectively. The timing of elicitor application is of paramount importance, as studies have demonstrated that pre-flowering treatments can influence crop yield. Furthermore, the harvest date, which is pivotal in determining the duration of fruit development post-elicitor application, plays a significant role in the crop yield. The concentration-dependent effects that have been observed thus far necessitate further research to establish precise application protocols that are adapted to specific phenological stages and harvest dates to maximise crop yield benefits.

5.2. Influence of elicitors on physiological and physiochemical quality at harvest and during postharvest storage

Maintaining the physiochemical quality properties of Lamuyo-type green pepper fruits is imperative in order to prolong shelf-life, enhance marketability and ensure consumer acceptance. The present PhD thesis is based on studies across several growing seasons and two methods of elicitor application (foliar spraying or irrigation). The findings of these studies have revealed the significant effects of preharvest treatments with SA and MeJA on important physiological and physiochemical traits of green pepper fruits at harvest and during postharvest storage at both optimal and suboptimal temperatures, which have been compiled with the ***publications 2, 3, 4 and 5***. The preharvest applications of SA and MeJA were found to have a significant effect on the physiological and physicochemical quality parameters of green bell peppers at harvest. Specifically, the preharvest application of SA at 0.5 mM, both through foliar spraying and irrigation methods, has shown the capacity to enhance fruit firmness, TSS, and TA at harvest (***publications 2, 3 and 5***). A similar effect has been observed with the preharvest application of MeJA at 0.1 mM that has shown to lead to augmented firmness, higher hue^{o} values (indicative of greenness), increased TSS, and enhanced TA in comparison to control fruits at harvest (***publications 4 and 5***).

In relation to postharvest quality during storage at 7 ºC, the impact of SA and MeJA on physiological and physiochemical parameters is pertinent to the extension of shelf-life. As demonstrated in the results, the application of SA (0.5, 1, and 5 mM) and MeJA (0.1 and 1 mM) prior to harvest has shown to effectively delay the onset of quality losses, including weight loss, respiration rate, and maintained firmness, as can be observed in ***publications 2, 3 and 4***. These quality losses are determinants of pepper shelf-life during storage at an optimum temperature of 7 ºC (Asghari & Hasanlooe, 2016; Ezzat et al., 2017; Wang et al., 2022a). These treatments were especially effective at the 0.5 mM-SA and 0.1 mM-MeJA concentrations.

In ***publication 2***, SA demonstrated to possess the capacity to reduce respiration rate at harvest and during storage, likely attributable to its anti-senescent properties and inhibitory effects on ethylene biosynthesis. This mechanism has been hypothesised to retard the activity of enzymes associated with ripening and susceptibility to pathogens, thereby preserving the integrity of cell walls. Furthermore, the application of SA postharvest treatments at 1-2 mM has been associated with reduced fruit shrinkage (Rao et al., 2011), as evidenced in green pepper fruits, where untreated fruits demonstrated evident shrinkage by the end of postharvest storage. In addition, the foliar application of SA to green pepper plants in the ***publication 2*** showed a significant reduction in weight loss and an increase in fruit firmness at harvest, particularly at 0.5- and 1-mM SA concentrations. This finding indicates that SA is the most effective agent, causing a 1.20-fold increase in comparison to untreated fruits. The results suggest that SA may enhance water retention in fruit tissues, possibly by modulating cell wall integrity and transpiration processes, as previously reported in citrus and pomegranate fruit (García-Pastor et al., 2020a; Serna-Escolano et al., 2021). Notwithstanding a decline in firmness during storage, SA-treated fruits demonstrated higher levels of firmness in comparison to control fruits, irrespective of the application method employed (***publications 2 and 3***). The delayed loss of fruit weight and firmness in green pepper fruits could be attributed to SA's role in modulating the activity of cell wall-modifying enzymes, such as

polygalacturonase (PG) and pectin methyl esterase (PME). As demonstrated in the research conducted by Rao et al. (2011), the application of SA and $CaCl_2$ treatments has been shown to inhibit the activity of these enzymes, thereby delaying fruit softening and maintaining fruit firmness.

Furthermore, the impact of SA application on the colouration of green peppers was observed at harvest and during postharvest storage, with a colour preservation effect being noted, thereby maintaining higher hue^{o} values, indicating delayed chlorophyll degradation (Almela et al., 1996). However, all SA treatments resulted in a delay in colour loss during storage, with the most significant effect observed in fruits treated with 0.5 mM SA, which exhibited reduced yellowing (***publication 2***). This phenomenon can be attributed to the SA-mediated retardation of fruit senescence. Conversely, no substantial disparities were detected in TSS content; nevertheless, all SA-treated fruits demonstrated elevated TA levels at harvest. Following a 21-day storage period, a significantly higher level of TA was observed in fruits from plants treated with 0.5- and 1-mM SA (***publication 2***). In accordance with the findings of other studies (El-Yazied, 2011; Hanieh et al., 2013; Koner et al., 2015; Jamiołkowska et al., 2016), the effects of SA on these quality parameters appear to be cultivar-dependent and influenced by growth conditions.

The ***publication 3*** corroborates the effects of preharvest 0.5 mM SA application, administered through foliar spraying or irrigation, on the ripening and senescence processes of green pepper fruit stored at 7 ºC for 28 days. The findings indicate that both SA treatments significantly reduce weight loss and delay the decline in firmness and colour changes compared to untreated fruits. Furthermore, an enhancement in the levels of TSS and TA was observed, resulting in a lower RI. It has been demonstrated that SA treatments have the capacity to delay postharvest senescence, as evidenced by the preservation of essential physicochemical properties. In conclusion, the comparison of application methods, foliar spraying or irrigation, as outlined in the ***publication 3***, provides a valuable practical perspective. It is suggested that irrigation of SA is a potentially more advantageous method due to its ease and cost-effectiveness.

Conversely, green pepper fruits treated with MeJA exhibited a reduced weight loss and respiration rate after 28 days of postharvest storage at 7 ºC. Furthermore, it was observed that these pepper fruits maintained higher levels of firmness (26 % for 0.1 mM and 14 % for 1 mM) and delayed the softening process during storage in the 2020 season. The delay of softening was also observed in green pepper fruits treated with MeJA at 0.1 mM during the 2021 season (***publication 4***). The reduced softening of MeJA-treated green pepper fruit may be ascribed to MeJA-induced suppression of cell wall degrading enzymes (Venkatachalam & Meenune, 2015). Furthermore, the application of MeJA resulted in the preservation of green colouration in peppers over an extended period, indicating a retardation in chlorophyll degradation. These results are consistent with earlier research demonstrating the role of MeJA in the preservation of colour and firmness in different fruits, including plums, apples, pomegranates and mangoes (Rudell et al., 2005; Martínez-Esplá et al., 2014; Ozturk et al., 2015; Muengkaew et al., 2016; García-Pastor et al., 2020b). As demonstrated in the ***publication 4***, MeJA treatment has been shown to enhance the content of TSS and TA, which are considered to be essential indicators of green pepper fruit quality. The potential correlation of these enhancements with MeJA's role in regulating sugar metabolism and organic

acid preservation, as evidenced by studies on citric fruits (Serna-Escolano et al., 2021; Dhamia et al., 2022), merits further investigation.

Generally, SA and MeJA preharvest treatments diminished the respiration rate at harvest in comparison to untreated peppers (***publications 2 and 4***). This effect on decreasing the respiration rate at harvest would indicate an effect of both elicitors on reducing the cell metabolism rate in green pepper fruit. This, in turn, could be attributed to a lower metabolic activity induced by both preharvest treatments. On the other hand, the finding appreciated on the delay of firmness losses is consistent with the established role of SA and MeJA in preserving cell integrity (***publications 2, 3 and 4***). The observed effects could be also attributed to the modulation of cell wall-modifying enzymes, such as PG and PME. In the context of green pepper fruits, colour preservation emerges as a pivotal consideration. The application of preharvest treatments with SA and MeJA at 0.5 and 0.1 mM, respectively, has demonstrated to be efficacious in preserving the green pigmentation of Lamuyo-type green pepper fruits, thereby reducing their colour losses at 7 ºC (***publications 2, 3 and 4***). This discolouration delay suggests a potential role for these elicitors in decelerating senescence-related pigmentation changes, which could be beneficial in reducing chlorophyll degradation (***publication 3***). Furthermore, the application of both elicitation strategies tested resulted in a significant increase in the levels of both TSS and TA in SA- and MeJA-treated green pepper fruits, in comparison to untreated peppers at harvest (***publications 2, 3 and 4***). In addition, it was noted that the levels of TSS and TA in the treated peppers remained higher than those in the non-treated peppers during postharvest storage (***publications 2, 3 and 4***). This finding suggests that both elicitors had a significant effect on reducing the RI in green pepper fruits during storage at 7 ºC (***publications 2, 3 and 4***).

Considering the substantiated benefits observed in terms of enhanced quality upon harvest and extended shelf-life during postharvest storage with the foliar application of SA and MeJA at concentrations of 0.5 and 0.1 mM, respectively, across two consecutive seasons (2020 and 2021; ***publications 2, 3 and 4***), a novel experimental design was instituted in the 2022 season (***publication 5***). The ***publication 5*** compared both treatments applied by two methods of application (foliar and irrigation) on the effects of the incidence of CI in green peppers stored at 2 °C. As stated in the ***publication 5*** of the present PhD thesis, all results concerning physiological and physiochemical quality were corroborated. The application of 0.5 mM-SA and 0.1 mM-MeJA via foliar spray or irrigation during the storage period generally resulted in a reduction in weight losses, while maintaining firmness and colour, as compared to the control pepper fruits. However, the efficacy of the application method exhibited variation according to the specific parameter.

Regarding the study's objective of weight loss, it was found that both application methods were equally efficacious for both phytohormones (***publication 5***). Conversely, the foliar pulverization of MeJA resulted in a greater reduction in firmness losses compared to irrigation, while no significant differences were observed between the two methods for SA (***publication 5***). The method of application also influenced the resultant green colour (***publication 5***). While both foliar SA and MeJA treatments demonstrated an improvement in colour preservation, the irrigation method exhibited superior hue^{o} values. This finding indicates that a more systemic uptake of the phytohormones via irrigation may exert a more sustained effect on pigment stability. The effects

on TSS were found to be similar between treatments and application methods (***publication 5***). However, for TA, foliar application of both MeJA and SA resulted in the highest levels after 28 days of storage, suggesting that foliar spray might be more effective in maintaining TA levels, potentially by directly interacting with the leaf physiology and influencing source-sink relationships during fruit development (***publication 5***).

The overall conclusion of this objective of the present PhD thesis is that preharvest treatments with SA and MeJA significantly enhance the physiological and physicochemical quality of Lamuyo-type green pepper fruits both at harvest and during postharvest storage. Specifically, SA at 0.5 mM and MeJA at 0.1 mM, applied through either foliar spraying or irrigation, demonstrated the capacity to improve fruit firmness, TSS and TA at harvest. Furthermore, these treatments effectively extended the shelf-life of green peppers stored at 7 ºC by delaying weight loss, reducing respiration rate, maintaining firmness, and preserving green colour. The present PhD thesis suggests that both application methods showed efficacy, with irrigation of SA appearing to be more practical, and the optimal method for MeJA application varying depending on the specific quality parameter. This research provides substantial evidence for the efficacy of SA and MeJA preharvest treatments in preserving the quality and extending the shelf-life of green pepper fruits. Consequently, the aforementioned benefits could enhance the marketability and consumer acceptance of 'Lamuyo'-type green pepper fruit, as well as reduce the food waste.

5.3. Effect of elicitors on the modulation of the antioxidant system of green pepper fruit at harvest and during postharvest storage

The effects of preharvest applications of SA and MeJA on functional quality and accumulation of bioactive compounds in green pepper fruits have been studied in the present PhD thesis (***publications 2, 3, 4 and 5***). The results obtained in those studies provide compelling evidence that SA and MeJA elicitors can significantly modulate the metabolic pathways involved in the quality and antioxidant defence mechanisms of green pepper fruits, both at harvest and during postharvest storage. All preharvest treatments with SA and MeJA enhanced the concentration of total phenolics and TAA due to an increase on the H-TAA and L-TAA of Lamuyo-type green pepper fruits (***publications 2, 3, 4 and 5***). It is important to note that significant differences were observed in the lowest doses of the treatments tested, which proved to be the most effective in increasing the TPC and the TAA of those green peppers treated with SA and MeJA (***publications 2 and 4***). Consequently, these concentrations (0.5 mM-SA and 0.1 mM-MeJA) were subjected to further testing in the subsequent growing seasons (2021 and 2022) through a range of application methods, including foliar and irrigation treatments (***publications 3 and 5***). The findings of the ***publications 3 and 5*** corroborate the initial results from ***publications 2 and 4***, thereby validating the research and establishing a foundation for conclusions and recommendations regarding the optimal method of application.

The preharvest application of SA resulted in an increase in the content of bioactive compounds, including total phenolics, AA, DHA and chlorophylls (***publications 2, 3 and 5***). Furthermore, TAA was enhanced in both the hydrophilic and lipophilic phases, suggesting that SA-treated fruits possess a more robust antioxidant defence system (***publications 2, 3 and 5***). The foliar

application of SA was found to be the most effective at harvest. However, following a 28-day storage period, irrigation SA demonstrated a notable enhancement in the maintenance of H-TAA levels, suggesting a potential interaction between the application method and the antioxidant preservation capacity during storage (***publications 3 and 5***). The significant correlation between TPC and H-TAA further supports the hypothesis that SA-induced phenolic biosynthesis plays a crucial role in extending fruit shelf-life (Wei et al., 2011). The results included in the present PhD thesis demonstrate that the content of chlorophyll a and b was found to be significantly higher in fruits treated with SA at harvest and after 28 days of storage (***publications 3 and 5***). This finding suggests that SA exerts a protective effect against chlorophyll degradation. These findings are consistent with those of previous studies that reported SA mitigating pigment degradation and delaying colour changes associated with ripening (Pérez-Gálvez et al., 2020).

The enhancement of the antioxidant system was further evidenced by increased enzymatic antioxidant defences, particularly APX, CAT and POD, at harvest and during storage, suggesting a reduction in oxidative stress (***publication 3***). At harvest, SA-treated fruits exhibited a pronounced increase in APX and POD activity, an effect that is closely associated with the upregulation of *Ca*APX and *Ca*POD gene expression (***publication 3***). However, while irrigation SA enhanced CAT activity at harvest, POD activity remained consistently enhanced after 28 days of storage in foliar SA-treated fruits, which suggests a prolonged antioxidant response induced by this application method (***publication 3***). The present PhD thesis hypothesises that the activation of enzymes contributes to the regulation of reactive oxygen species (ROS) homeostasis in Lamuyo-type green pepper fruit. The maintenance of this homeostasis is crucial for mitigating oxidative damage and delaying fruit senescence (Gomes et al., 2021; Serna-Escolano et al., 2021).

At the metabolomic modulation level, SA treatment resulted in the upregulation of the expression of key antioxidant-related genes, including *Ca*APX, *Ca*POD, *Ca*PAL, and *Ca*DHAR2, particularly at harvest (***publication 3***). While both foliar spraying and irrigation methods exhibited comparable efficacy in enhancing fruit quality and delaying senescence, irrigation was identified as the more advantageous approach due to its ease of application and cost-effectiveness. These findings emphasise the potential of SA treatment as a preharvest strategy to extend the shelf-life of green pepper fruit, cv. 'Herminio', by modulating antioxidant metabolism and gene expression, thus providing valuable insights into improving postharvest preservation strategies. Furthermore, the application of SA influenced the biosynthesis of phenolic compounds through the phenylpropanoid pathway, as evidenced by the upregulation of the *Ca*PAL gene at harvest in foliar SA-treated fruits (***publication 3***), such as oranges, table grapes and watermelons (Blanch et al., 2020; Amiri et al., 2021; Wu et al., 2021). The stimulation of this metabolic pathway resulted in a significant increase in TPC, thereby reinforcing the role of SA in enhancing fruit antioxidant capacity. Furthermore, the implementation of SA through both methodologies resulted in the upregulation of *Ca*DHAR2 gene expression at harvest, thereby contributing to an augmented content of AA and DHA (***publication 3***), which are imperative for scavenging reactive oxygen species (ROS) and enhancing oxidative stress tolerance (Amiri et al., 2021).

In relation to the preharvest application of MeJA (***publication 4***), it was found that this resulted in a significant enhancement of TPC at harvest, which was maintained at higher levels

during postharvest storage at 7 ºC, highlighting MeJA at 0.1 mM concentration. The stimulation of phenolic accumulation by MeJA treatment has been observed in studies on different fruits, including apricots, apples, papaya, grape berries and blueberries (Ezzat et al., 2017; Wang et al., 2019; Lv et al., 2023; Bron et al., 2023; Zhang et al., 2024). The aforementioned authors hypothesise that this phenomenon is attributable to the potential activation of enzymatic activities and gene expression within the phenylpropanoid pathway. The significant increase in both H-TAA and L-TAA in MeJA-treated peppers is a direct consequence of the increased phenolic content, as phenolics are a major contributor to non-enzymatic antioxidant capacity (***publication 4***). In addition, the impact of MeJA was found to result in a substantial enhancement in the activity of key antioxidant enzymes in green pepper fruits, both at harvest and during storage (***publication 4***). The results of ***publication 4*** demonstrated a 40 % increase in APX activity, a 35 % increase in CAT activity, and a 28 % increase in POD activity in the treated green pepper fruits in comparison with the control fruits. This enhancement of antioxidant defence mechanisms is of significance. The 0.1 mM concentration was determined to be the most effective in stimulating these enzymatic activities (***publication 4***). It is imperative to emphasise the pivotal function of these enzymes in scavenging ROS that are generated during the postharvest ripening and senescence processes. This process serves to mitigate the potential oxidative damage that may arise from these processes. This observation is consistent with the findings reported in eggplant, plums, table grapes, pomegranates and lemons, where MeJA treatment has also shown to promote antioxidant enzyme activity, potentially contributing to an extended shelf-life (Cheong & Choi, 2003; Wang et al., 2015; García-Pastor et al., 2019).

As stated in the last experimental research of this PhD thesis (***publication 5***), which pertains to the preharvest application of SA and MeJA to Lamuyo-type green pepper fruits to study the chilling tolerance effect, the results of the antioxidant systems were corroborated through the implementation of two application methods: foliar spraying and irrigation. These methods were employed over a period of 28 days in cold storage. With regard to bioactive compounds, SA and MeJA, irrespective of the method, exhibited a marked increase in TPC at harvest and following storage in comparison to untreated peppers (***publication 5***). It is noteworthy that the application of SA by both foliar or irrigation and MeJA by irrigation were the most effective in increasing TPC (***publication 5***). For H-TAA, all treatments resulted in improved levels at harvest, although the highest levels were obtained after storage with SA applied by foliar spraying (***publication 5***). In a similar manner, both phytohormones enhanced L-TAA, irrespective of the application method at harvest and after storage, with no significant differences observed between the two methods (***publication 5***). Regarding the TCC, no discrepancies were detected at harvest between the treated and control fruits (***publication 5***). However, following storage, treatments involving SA and MeJA increased TCC, with foliar SA proving to be the most effective (***publication 5***). The findings suggest that the method of application can differentially affect the accumulation and retention of different bioactive compounds, with the method via irrigation being effective in this enhancement for both phytohormones.

5.4. Impact of elicitors on decay and chilling injury incidence of green pepper fruit during postharvest storage

The ***publication 2*** evaluated the effect of preharvest foliar application of SA at concentrations of 0.5, 1, and 5 mM on the postharvest decay incidence of Lamuyo-type green pepper fruits, cv. 'Herminio', stored at 7 ºC for 21 days. The findings of ***publication 2*** indicated that the preharvest administration of SA led to a substantial reduction in the incidence of decay in green pepper fruits during postharvest storage. Specifically, the lowest percentage of decay (*ca.* 2%) at the end of the 21-day storage period was recorded for pepper fruits treated with 0.5 mM SA, which was significantly lower than that of untreated control fruits (16.6 %) and fruits treated with 1 mM (3.7 %) and 5 mM (9.3 %) SA (***publication 2***). This finding indicates that preharvest SA treatment, particularly at lower concentrations, can induce fruit tolerance against fruit decay during storage. The induction of tolerance has been hypothesised to be associated with the stimulation of POD and polyphenoloxidase (PPO) enzymes activities, as well as the increased accumulation of H_2O_2 and the expression of pathogenesis-related proteins. The most effective concentration for reducing postharvest decay incidence in Lamuyo-type green pepper fruits was determined to be 0.5 mM of SA, applied as a preharvest foliar spray.

The present PhD thesis explores the effectiveness of SA and MeJA treatments in reducing the incidence of CI, a physiological disorder in green pepper fruit (***publication 5***). As demonstrated in ***publications 2, 3 and 4***, the application of SA and MeJA at concentrations of 0.5- and 0.1-mM, respectively, in a preharvest manner yielded optimal results in enhancing crop yield, as well as the physiochemical and functional quality of green pepper fruit during postharvest storage at 7 ºC. It can thus be concluded that the application of both elicitors in the ***publication 5*** was achieved at the same concentrations by means of foliar spraying and irrigation methods and under cold storage at 2 ºC. The results obtained demonstrate that both foliar and irrigation SA and MeJA treatments have a significant impact on maintaining membrane structure (***publication 5***). This is evidenced by the observation that lower CI symptoms values were recorded in treated green peppers compared to untreated peppers (***publication 5***). The foliar application of MeJA and SA treatments exhibited a decline in CI incidence of 1.24- and 1.32-fold, respectively, compared to the control group following a duration of 28 days in cold storage (***publication 5***). Nevertheless, the most substantial decrease was evident in the irrigation treatment, exhibiting an approximate 1.40-fold reduction (***publication 5***).

It is well established that membrane damage and subsequent changes in lipid composition are correlated with the occurrence of CI. These alterations in lipid composition are primarily characterised by a decline in the unsaturated fatty acids (UFA) / saturated fatty acids (SFA) ratio. This could be affecting the phase transition of membrane lipids from a liquid-crystalline to a solid-gel state, and in turn leading to membrane peroxidation and damage, accelerating the occurrence of CI (Wongsheree et al., 2009). In the absence of discrepancies between treatments or application methods, it was decided at the conclusion of the functional analyses to investigate lipid metabolism

in the most accessible commercial application method, irrigation (***publication 5***). As outlined in the ***publication 5***, the irrigation application of SA and MeJA has demonstrated to be a highly effective preharvest strategy for enhancing chilling tolerance in green bell peppers. The analysis of the samples revealed significant variations in the SFA content depending on the treatment and storage (***publication 5***). At harvest, the application of SA and MeJA resulted in a substantial reduction of C14:0 and C16:0 fatty acids compared to the controls, especially in the case of irrigation SA (***publication 5***). In this context, irrigation with SA led to a significant reduction in the concentrations of the major SFAs, palmitic (C16:0), stearic (C18:0) and lignoceric (C24:0), at harvest and after storage (***publication 5***). After storage, a 30 % reduction in palmitic acid levels and a 48 % reduction in stearic acid levels were observed in SA-irrigated fruit in comparison with the control (***publication 5***). A comparable decline in stearic and lignoceric acids of 20 % and 52 %, respectively, was observed with MeJA irrigation, indicating a phytohormonal capacity to impede SFA accumulation under cold conditions (***publication 5***). Conversely, UFAs, which are pivotal for sustaining membrane fluidity and function under chilling stress, exhibited enhanced preservation in SA- and MeJA-treated fruits (***publication 5***). Whilst cold storage demonstrated to result in a decline in significant UFAs, including linoleic (C18:2n6c), α-linolenic (C18:3n3), and oleic (C18:1c9) acids, the extent of this reduction was significantly mitigated by SA and MeJA preharvest treatments (***publication 5***).

The UFA/SFA ratio is a crucial indicator of membrane fluidity and chilling tolerance. In ***publication 5***, both MeJA and SA irrigation enhanced the UFA/SFA ratio at harvest, with values of 3.36 ± 0.48 and 4.68 ± 0.41, respectively, compared to the control (2.31 ± 0.16). This ratio was found to be significantly lower in all treatments tested following a storage period of 28 days at 2 ºC, followed by 2 days at 20 ºC (***publication 5***). However, a substantially higher UFA/SFA ratio was exhibited by SA irrigation after storage in relation to both the control and MeJA-treated green pepper fruits (***publication 5***). The enhanced UFA/SFA ratio in 0.5 mM SA-irrigated peppers is likely to be a favourable contributor to their improved chilling tolerance by maintaining greater membrane fluidity and integrity at low temperatures due to a shift in lipid composition towards increased desaturation of fatty acids. This change is a probable underlying cause of the enhanced chilling tolerance observed in these fruits. It is evident from the results that SA applied via irrigation has proven to be a superior method for mitigating CI symptoms. This is strongly correlated with its ability to modulate the fatty acid profile and maintain a higher UFA/SFA ratio during cold storage.

5.5. Phytochemical characterisation and potential agrifood of pepper seeds

The ***publication 6*** has provided a comprehensive overview of the extant knowledge pertaining to the characterisation and potential applications of pepper seeds, a significant by-product of pepper (*Capsicum annuum* L.) processing, with a particular focus on their application in the agrifood sector. While the primary focus of the pepper seeds is on general aspects, including various pepper types and ripening stages, it is possible to extrapolate and critically discuss in the ***publication 6*** the findings relevant to green pepper seeds specifically.

As indicated in ***publication 6***, the analysis of pepper seeds reveals their substantial nutritional value, with the presence of notable nutrients including carbohydrates (43.60 to 80.89 g 100 g^{-1}), dietary fiber, proteins (6.30 to 28.30 g 100 g^{-1}), fats, vitamins, minerals and essential amino acids (Embaby et al., 2011; Zou et al., 2015; Chouaibi et al., 2019; Cvetković et al., 2020; Anaya-Esparza et al., 2021; Adewole et al., 2022; Chen et al., 2024). Dietary fiber constitutes a substantial portion of the carbohydrate fraction (26 to 61 %), with insoluble fiber being the predominant form (Azabou et al., 2017; Gu et al., 2017). This finding is of particular significance, given the potential of insoluble fiber to be utilised as a functional ingredient in food products, thereby enhancing their nutritional value (***publication 6***). The incorporation of pepper seed flour into products, such as jams, sauces and soups could represent a promising avenue for future product development (Cvetković et al., 2022).

In relation to the content of crude fat in pepper seeds, the range observed was from 11.00 to 23.65 g per 100 g (***publication 6***). The fatty acid profile of pepper seed oil is characterised by a high proportion of UFA, such as linoleic and oleic acids (El-Adaway et al., 2001; Jarret et al., 2013; Cvetković et al., 2020). Despite the absence of a precise fatty acid profile for green pepper seeds, extant research suggests that the fatty acid composition of pepper seed oil remains constant, irrespective of cultivar or growing season (Koncsek et al., 2018). It can be deduced that green pepper seeds are likely to have a similar profile, providing a valuable source of essential fatty acids (***publication 6***). Nevertheless, to confirm this extrapolation, further research directly analysing green pepper seed oil would be advantageous. Moreover, the amino acid profiling reveals the presence of both essential and non-essential amino acids (***publication 6***). The high leucine and glutamic acid content is of particular interest (Embaby et al., 2011; Zou et al., 2015; Chen et al., 2024). This diverse amino acid composition suggests that pepper seeds could be used as a protein source in various food applications, with the potential to enhance the nutritional quality of other food products (***publication 6***).

The potential of natural antioxidants, such as by-products of pepper processing, as well as polyphenol phytochemicals, present in these plant materials, has also been reviewed in the ***publication 6***. With regard to green pepper seeds specifically, Ahmad et al. (2024) reported a significantly higher total phenolic content [88.60 mg of gallic acid equivalents (GAE) 100 g^{-1} in dry weight (DW) basis] in green pepper fruit seeds compared to red pepper seeds (22.30 mg GAE 100 g^{-1} DW). This observation suggests that green pepper seeds may be a more abundant source of phenolic antioxidants than red pepper seeds (***publication 6***). This result is consistent with the prevailing trend observed in *Capsicum spp.*, where the presence of phenolics and flavonoids is frequently more pronounced in the green and immature stages (Hamed et al., 2019; Guilherme et al., 2020). The higher phenolic content found in green pepper seeds suggests the need for further research into their potential for nutraceutical and cosmetic applications, as well as their suitability as natural antioxidants in food preservation or plant biostimulants (***publication 6***).

The potential of pepper seeds for various agrifood industrial applications, such as food condiments, biostimulants, and biomass for energy, has also been investigated (Yilmaz et al., 2015; Valdez-Morales et al., 2021; Cvetković et al., 2022). The higher phenolic content found in pepper seeds enhances their potential as a source of organic plant biostimulants, given the established role

of phenolic compounds in inducing plant defence responses and promoting growth (Sodaeizadeh et al., 2009; Abdalla et al., 2013; Czerniewicz et al., 2016; Ertani et al., 2016; Laquale et al., 2020). This is a particularly salient area for future research, focusing on the extraction and application of bioactive compounds from green pepper seed waste to support sustainable agriculture (***publication 6***). In relation to volatile organic compounds (VOCs), Chen et al. (2024) reported the profile of chopped pepper seeds, identifying aldehydes, esters, and alcohols as the predominant groups. They also observed the presence of some common major VOCs among different cultivars (***publication 6***). In a related finding, Ahmad et al. (2024) discovered divergent phenolic profiles between green and red pepper seeds, which may be indicative of disparities in their VOCs and, consequently, their prospective utilisation as flavouring agents (***publication 6***). Further investigation into the specific VOCs profile of green pepper seeds is required to fully understand their aroma potential and suitability as natural flavouring agents in the food industry.

In conclusion, the findings of the ***publication 6*** revealed the potential for applications of pepper seeds in several areas and the importance of valorising pepper by-products to promote sustainability and a circular bioeconomy approach. The higher phenolic content observed in green pepper seeds lends further support to their potential contribution to this goal by providing a valuable source of bioactive compounds for various applications. The utilisation of these fruits as a condiment or flavouring agent is substantiated by the presence of VOCs, which contribute to their distinctive aroma. Pepper seed oil has also been identified as a viable source for biodiesel production, offering a renewable alternative to fossil fuels. Furthermore, the utilisation of pepper seed biomass for energy generation offers a potential avenue for mitigating agricultural waste.

- 6 -

– Conclusions –

– Conclusiones –

6. CONCLUSIONS / CONCLUSIONES

The main aim of the present PhD thesis is to make a significant contribution to the advancement of knowledge on green pepper fruit. This is achieved by optimising its physiological developmental stage and harvest date, as well as the application of preharvest elicitors: SA and MeJA. In this regard, a set of guidelines has been formulated for utilisation by the production industry. In a similar manner, a review has been conducted for the purpose of synthesising information, with the objective of reinforcing the revaluation of pepper seeds within the agrifood industry in a near future. Therefore, the ***overall conclusions*** are:

I. Phenological stages and harvest dates have been shown to significantly influence the accumulation of bioactive compound content and antioxidant activity of Lamuyo-type green pepper fruit. Those that appear by S12 and 20th April could be more beneficial to health.

II. Preharvest applications at 0.5- and 1 mM-SA and 0.1- and 1 mM-MeJA were found to have a positive effect on crop yield in kg per plant. Furthermore, the quality of the green pepper fruit was improved, and the antioxidant systems at harvest were enhanced. Additionally, the onset of quality losses, fruit decay and chilling injury symptoms during postharvest storage was delayed under optimal or suboptimal temperature conditions. This resulted in an extension of the shelf-life of green pepper fruits.

III. Peppers seeds possess a valuable nutritional profile, providing significant amounts of carbohydrates, dietary fiber, proteins, fats, vitamins, minerals and essential amino acids. In addition, they have been demonstrated to be a substantial reservoir of natural antioxidants, indicating a probable application in a variety of sectors within the agrifood industry. Furthermore, they are well-suited to contributing to a circular bioeconomy approach.

Furthermore, the following ***specific conclusions*** have been obtained:

i. Foliar treatments of SA and MeJA at 0.5 mM and 0.1 mM, respectively, resulted in an enhancement of the crop yield of Lamuyo-type green pepper plants. This enhancement was observed in terms of kilograms per plant, number of fruits per plant, and average fruit weight.

ii. Foliar or irrigation SA and MeJA treatments at 0.5 mM and 0.1 mM, respectively, have shown to improve fruit quality at harvest and during postharvest storage through slowing down the respiration rate, increasing the values of firmness, colour, TSS and TA at harvest and delaying losses of fruit weight, firmness, colour and TA of Lamuyo-type green pepper fruit. Despite the absence of appreciable disparities between the two application methods, the irrigation method was deemed the most pragmatic.

iii. The preharvest application of 0.5 mM SA and 0.1 mM MeJA, predominantly via irrigation, has been shown to stimulate the accumulation of bioactive compounds and the

antioxidant capacity of Lamuyo-type green pepper fruit at harvest. This effect has been observed in both enzymatic and non-enzymatic systems, with the functional quality being maintained during postharvest storage. This phenomenon is evidenced by the upregulation of the relative response of certain targeted antioxidant enzymes-related genes. Consequently, it can be inferred that the impact on antioxidant metabolism has resulted in an augmentation of the shelf-life period during postharvest storage, both at 2 and 7 ºC.

iv. In relation to the subject of fruit decay and CI incidence, the following conclusions can be drawn: *1)* foliar treatments with SA, particularly at a concentration of 0.5 mM, resulted in a reduction in the percentage of decay at the end of storage at 7 ºC when compared to the control, and *2)* the application of SA and MeJA elicitors by foliar spraying or irrigation led to the induction of CI tolerance in Lamuyo-type green pepper fruit under cold storage conditions at 2 ºC. It was observed that the irrigation method and the SA treatment were the most efficacious since showed the highest UFA/SFA ratio.

6. CONCLUSIONS / CONCLUSIONES

El objetivo principal de la presente tesis doctoral es realizar una contribución significativa al avance del conocimiento sobre el pimiento verde. Esto se consigue optimizando su estado de desarrollo fisiológico y la fecha de recolección, así como mediante la aplicación de elicitores precosecha: AS y JaMe. En este sentido, se ha formulado un conjunto de directrices para su utilización por la industria productora. Del mismo modo, se ha realizado una revisión con el fin de sintetizar la información, con el objetivo de reforzar la revalorización de las semillas de pimiento dentro de la industria agroalimentaria en un futuro próximo. Por lo tanto, las ***conclusiones generales*** son:

I. Se ha demostrado que el estado fenológico y la fecha de recolección influyen significativamente en la acumulación del contenido de compuestos bioactivos y en la actividad antioxidante de los pimientos verdes tipo Lamuyo. Aquellos pertenecientes al estado S12 y recolectados el 20 de abril podrían ser los más beneficiosos para la salud.

II. Se observó que las aplicaciones precosecha de 0,5- y 1 mM-AS y 0,1- y 1 mM-JaMe tenían un efecto positivo sobre el rendimiento del cultivo en kg por planta. Además, se mejoró la calidad del pimiento verde y los sistemas antioxidantes en el momento de la recolección. Asimismo, se retrasó la aparición de pérdidas de calidad, pudrición del fruto y síntomas de daño por frío durante el almacenamiento postcosecha en condiciones de temperatura óptima o subóptima. El resultado fue una prolongación de la vida útil del pimiento verde.

III. Las semillas de pimiento poseen un valioso perfil nutricional, ya que aportan cantidades significativas de hidratos de carbono, fibra alimentaria, proteínas, grasas, vitaminas, minerales y aminoácidos esenciales. Además, se ha demostrado que son un importante reservorio de antioxidantes naturales, lo que indica una probable aplicación en diversos sectores de la industria agroalimentaria. En este sentido, son muy adecuadas para contribuir a un enfoque de bioeconomía circular.

Además, se han obtenido las siguientes ***conclusiones específicas***:

i. Los tratamientos foliares de AS y JaMe a las concentraciones de 0.5 mM y 0.1 mM, respectivamente, resultaron en un aumento del rendimiento de las plantas de pimiento verde tipo Lamuyo. Esta mejora se observó en términos de kilogramos por planta, número de pimientos por planta y peso medio de los pimientos.

ii. Los tratamientos foliares o de riego con AS y JaMe a las concentraciones de 0,5 mM y 0,1 mM, respectivamente, han demostrado mejorar la calidad de los pimientos en el momento de la recolección y durante el almacenamiento postcosecha mediante la ralentización de la tasa de respiración, el aumento de los valores de firmeza, color, SST y AT en la recolección y el retraso de las pérdidas de peso, firmeza, color y AT del pimiento verde tipo Lamuyo. A pesar de la ausencia de disparidades apreciables entre los dos métodos de aplicación, el método de riego se consideró el más pragmático.

iii. Se ha demostrado que la aplicación precosecha de 0,5 mM de AS y 0,1 mM de JaMe, predominantemente vía riego, estimula la acumulación de compuestos bioactivos y la capacidad antioxidante del pimiento verde tipo Lamuyo en el momento de la recolección. Este efecto se ha observado tanto en sistemas enzimáticos como no enzimáticos, manteniéndose la calidad funcional durante el almacenamiento postcosecha. Este fenómeno se evidencia por la regulación al alza de la respuesta relativa de ciertos genes específicos relacionados con los enzimas antioxidantes. En consecuencia, puede deducirse que el impacto sobre el metabolismo antioxidante ha dado lugar a un aumento de la vida útil durante el almacenamiento postcosecha, tanto a 2 como a 7 ºC.

iv. En relación con el tema de la incidencia de podredumbres y daños por frío, se pueden extraer las siguientes conclusiones: *1)* los tratamientos foliares con AS, particularmente a una concentración de 0,5 mM, produjeron una reducción del porcentaje de podredumbres al final del almacenamiento a 7 ºC en comparación con el control, y *2)* la aplicación de AS y elicitores JaMe mediante pulverización foliar o riego condujo a la inducción de tolerancia a los daños por frío en el pimiento verde tipo Lamuyo en condiciones de almacenamiento en frío a 2 ºC. Se observó que el método de riego y el tratamiento con AS fueron los más eficaces ya que mostraron la mayor relación entre ácidos grasos insaturados (AGI) y ácidos grasos saturados (AGS) (ratio AGI/AGS).

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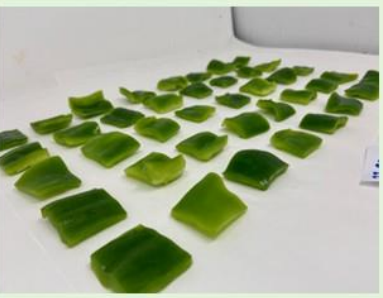
Future Research Lines




7. FUTURE RESEARCH LINES

In consideration of the results obtained during the present PhD thesis, new scenarios have been identified that merit further study. These scenarios are outlined below:

1. The objective of novel research lines could be to develop *in vitro* trials to evaluate the efficacy of preharvest treatments with SA and MeJA at their optimal concentrations. The main aim is to prevent and reduce the incidence of *Alternaria alternata* during postharvest storage at 7 ºC, since it is the main fungal pathogen that can cause fruit rot in pepper fruits.

2. The development of pre- and postharvest strategies for the handling of fresh-cut peppers is of paramount importance to extend their shelf-life. This is a popular addition to salads and various dishes due to the exponential growth of the processing industry.

3. As graphically shown in **Figure 7**, pepper seeds have the potential to be revalued as a by-product. It is recommended that these seeds be characterised in their fresh stage or after undergoing a roasting process, to enhance their use as a food ingredient (topping, additive, flavouring, supplement, etc.). Furthermore, it is endorsed as a source for the extraction of phenolic compounds, with the potential for the development of plant biostimulants for its application in pepper crops.

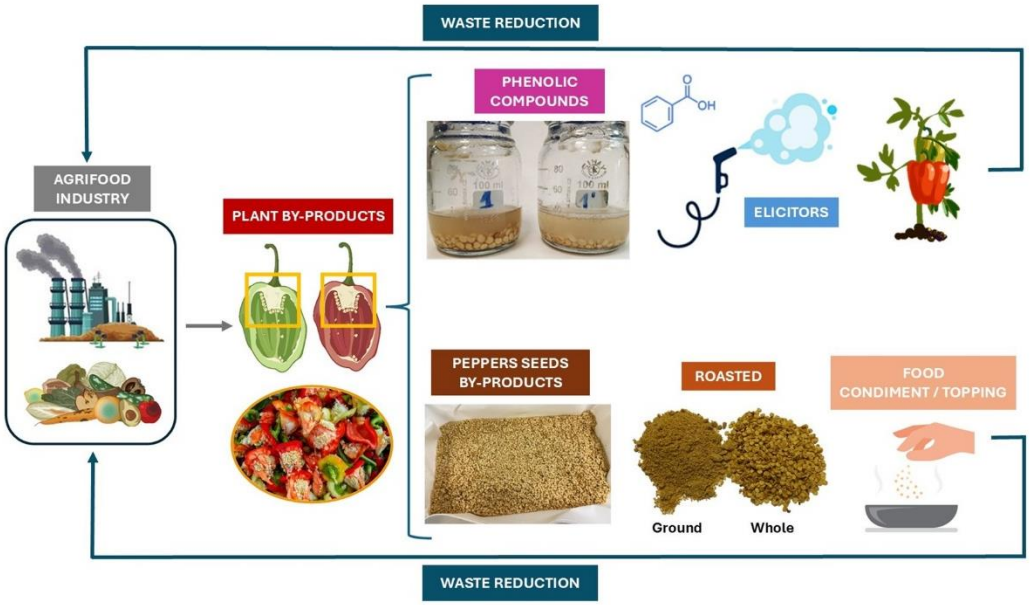


Figure 7. *Integral revalorization of pepper seeds as by-products for two different agrifood applications: 1) plant biostimulants in pepper crop, and 2) food condiment or topping. Source: The illustration was created by the authors with BioRender (Toronto, ON, Canada).*

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