MIGUEL HERNÁNDEZ UNIVERSITY OF ELCHE

DOCTORAL PROGRAMME IN AGRICULTURAL, AGRI-ENVIRONMENTAL AND FOOD RESOURCES AND TECHNOLOGIES

PhD THESIS

Characterization of Commercial Cultivars of Artichoke, Cardoon and their Wild Relatives for their Productive and Commercial Optimization

Marina Giménez Berenguer

DIRECTOR OF THE THESIS: Dr. Pedro Javier Zapata Coll

CO-DIRECTOR OF THE THESIS: Dra. María José Giménez Torres

– 2025 –

TESIS DOCTORAL

CHARACTERIZATION OF COMMERCIAL CULTIVARS OF ARTICHOKE, CARDOON AND THEIR WILD RELATIVES FOR THEIR PRODUCTIVE AND COMMERCIAL OPTIMIZATION

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UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE

Escuela Politécnica Superior de Orihuela

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

– 2025 –

PUBLICATIONS INCLUDED IN THIS DOCTORAL THESIS

This Doctoral Thesis, entitled "Characterization of Commercial Cultivars of Artichoke, Cardoon and their Wild Relatives for their Productive and Commercial Optimization", is presented under the modality of **thesis by compendium of publications** of the following research articles previously published:

Giménez, M. J., **Giménez-Berenguer, M.**, Guillén, F., Serna-Escolano, V., Gutiérrez-Pozo, M., & Zapata, P. J. (2023). Effect of Cysteine with Essential Oils on Quality Attributes and Functional Properties of 'Blanca de Tudela' Fresh-Cut Artichoke. *Foods*, *12*(24), 4414. https://doi.org/10.3390/foods12244414

Giménez-Berenguer, M., Giménez, M. J., Serna-Escolano, V., & Zapata, P. J. (2025). Impact of Flower Head Order and Phenolic Content on the Quality of Three Different Artichoke Cultivars for Fresh-Cut Products. *Agronomy,* 15(2), 322. https://doi.org/10.3390/agronomy15020322

Giménez-Berenguer, M., García-Pastor, M. E., García-Martínez, S., Giménez, M. J., & Zapata, P. J. (2022). Evaluation of 'Lorca' Cultivar Aptitude for Minimally Processed Artichoke. *Agronomy*, *12*(2), 515. https://doi.org/10.3390/agronomy12020515

Giménez, M.J., **Giménez-Berenguer, M.**, García-Pastor, M.E., Castillo, S., Valverde, J.M., Serrano, M., Valero, D., & Zapata, P.J. (2022). Influence of Flower Head Order on Phenolic Content and Quality of Globe Artichoke at Harvest and during Twenty-One Days of Cold Storage. *Sci. Hortic*., 295, 110846. https://doi.org/10.1016/j.scienta.2021.110846

Giménez-Berenguer, M., Gutiérrez-Pozo, M., Serna-Escolano, V., Giménez, M. J., & Zapata, P. J. (2023). Influence of Artichoke Antioxidant Activity in Their Susceptibility to Suffer Frost Injury. *Antioxidants*, *12*(11), 1960. https://doi.org/10.3390/antiox12111960

ADDITIONAL PUBLICATIONS
INCLUDED IN THIS DOCTORAL THESIS

This Doctoral Thesis, entitled "Characterization of Commercial Cultivars of Artichoke, Cardoon and their Wild Relatives for their Productive and Commercial Optimization", also includes the following article, which has already been submitted to a scientific journal and is currently under peer review.

Giménez-Berenguer, M., Salicola, S.A., Formenti, C., Giménez, M.J., Mauromicale, G. Zapata, P.J., Lombardo, S. & Pandino, G. (2025). Seeds Mineral Profile and Ash Content of Thirteen Different Genotypes of Cultivated and Wild Cardoon Over Three Growing Seasons. *Submitted to Agriculture.*

El Dr. D. *Pedro Javier Zapata Coll*, director, y la Dra. Dña. *María José Giménez Torres*, codirectora de la tesis doctoral titulada **"CHARACTERIZATION OF COMMERCIAL CULTIVARS OF ARTICHOKE, CARDOON AND THEIR WILD RELATIVES FOR THEIR PRODUCTIVE AND COMMERCIAL OPTIMIZATION"**

INFORMAN:

Que Dña. *Marina Giménez Berenguer* ha realizado bajo nuestra supervisión el trabajo titulado **"CHARACTERIZATION OF COMMERCIAL CULTIVARS OF ARTICHOKE, CARDOON AND THEIR WILD RELATIVES FOR THEIR PRODUCTIVE AND COMMERCIAL OPTIMIZATION"** 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 firmamos para los efectos oportunos, en Orihuela a 20 de mayo de 2025.

Director de la tesis	Codirectora de la tesis
Dr. D. *Pedro Javier Zapata Coll*	Dra. Dna. *María José Giménez Torres*

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

CERTIFICA:

Que la Tesis Doctoral titulada "**CHARACTERIZATION OF COMMERCIAL CULTIVARS OF ARTICHOKE, CARDOON AND THEIR WILD RELATIVES FOR THEIR PRODUCTIVE AND COMMERCIAL OPTIMIZATION"** de la que es autora la graduada en Ciencia y Tecnología de los Alimentos **Dña. Marina Giménez Berenguer**, ha sido realizada bajo la dirección del **Dr. Pedro Javier Zapata Coll** y la codirección de la **Dra. María José Giménez Torres**, actuando como tutor de la misma el Dr. **Salvador Castillo García**. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA, siendo por tanto apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a 20 de mayo de 2025.

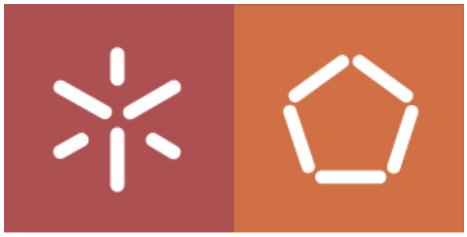
Dra. Dña. Juana Fernández López

Coordinadora del Programa Doctorado ReTos-AAA

This PhD thesis was conducted at Miguel Hernández University in the Postharvest Research Group of Fruit and Vegetables.

Marina Giménez Berenguer was granted a PhD-Scholarship (Grant number: ACIF/2021/178) for four years, as well as an International Mobility Scholarship (Grant number: ACIF/2021/178) to support her five-month research stay at the University of Catania (Catania, Italy), both funded by the Generalitat Valenciana.

Additionally, she was granted a Santander 2023 Scholarships for Internships Abroad (Resolution 03633/2023), which provided financial support for her two-month research stay at the University of Minho (Braga, Portugal).



Universidade do Minho
Escola de Engenharia

"Research is to see what everybody else has seen,

and to think what nobody else has thought."

"Investigar es ver lo que todo el mundo ha visto y

pensar lo que nadie más ha pensado."

– Albert Szent-Györgyi –

(1893-1986)

AGRADECIMIENTOS

AGRADECIMIENTOS

Escribo estas líneas con una mezcla de emoción y gratitud. Quién me iba a decir, cuando entré en esta universidad con 18 años, que este camino me llevaría a completar una carrera, un máster y, finalmente, un doctorado. Han sido casi diez años de aprendizaje, retos y crecimiento personal y profesional, una auténtica montaña rusa de momentos inolvidables, tanto buenos como difíciles, que han culminado en esta tesis, el eje central de mi vida durante los últimos cuatro años.

Este logro no habría sido posible sin todas las personas que, de una manera u otra, han dejado su huella en mí y han contribuido a que hoy llegue hasta aquí. Es imposible mencionarlas a todas, pero quiero intentar al menos reflejar mi agradecimiento a quienes han sido fundamentales en este viaje.

En primer lugar, quiero expresar mi más sincero agradecimiento a mis directores de tesis, el **Dr. Pedro Javier Zapata Coll** y la **Dra. María José Giménez Torres**. Su guía, dedicación y compromiso han sido fundamentales en cada etapa de esta investigación.

A ti, **Pedro**, gracias por darme la oportunidad de realizar esta tesis doctoral, por confiar en mí desde el TFG y el máster, y por haberme acompañado en este camino desde el inicio. Siempre estaré agradecida por tu apoyo y por haberme enseñado tanto a nivel profesional como personal. Además de ser un referente en investigación, tu capacidad de ver siempre un paso más allá y buscar mejoras constantes hacen que trabajar contigo sea un reto y un aprendizaje continuo del que he disfrutado mucho estos años. Asimismo, no puedo dejar de mencionar tu maestría en el pelado de alcachofas, un talento que ha sido clave en más de un ensayo. Muchas gracias por todo.

A ti, **MaríaJo**, por tu apoyo durante esta tesis, tanto en lo personal como en lo profesional. Muchas gracias por tus innumerables correcciones y por estar siempre ahí para resolver cualquier duda, por mínima que fuera. Tu ojo crítico y tu precisión a la hora de revisar han sido invaluables para mejorar cada parte de este trabajo y para mi crecimiento como investigadora. No son pocas las veces que te he dicho que aspiro a alcanzar ese nivel de detalle y rigor que tanto admiro en ti. Junto con Pedro, has sido un pilar fundamental en este proceso, y por ello te estaré siempre agradecida.

También quiero agradecer al **Dr. Salvador Castillo**, Salva para los amigos. Mi tutor en esta tesis y el fotógrafo oficial de la EPSO. Con tu característico saludo a lo "Mari", siempre dispuesto a ayudar en lo que hiciera falta y, no vamos a negarlo, también a echar alguna que otra bronca por el colorímetro, especialmente en los

primeros días de todo aquel o aquella que se incorporaba al laboratorio. A pesar de ese primer susto, siempre estabas dispuesto a resolver cualquier problema y, cómo no, a inmortalizar con tu cámara cada paso de esta tesis, rematando con tu icónica frase: *"A mandar… y no mucho."*

A todo el grupo de Post-Recolección de Frutas y Hortalizas de la UMH, gracias por haberme acogido y por el ambiente de trabajo tan enriquecedor. A los **doctores Daniel Valero**, **María Serrano**, **Fabián Guillén**, **Domingo Martínez** y **Juan Miguel Valverde**, mi gratitud por vuestras enseñanzas. Además de compañeros de grupo, os he tenido a todos como profesores y sin duda, habéis contribuido enormemente a mi formación como investigadora, guiándome con vuestro conocimiento y experiencia. Muchas gracias a todos.

A mis compañeros de laboratorio, que han sido parte esencial de este viaje: A **Alicia**, con quien he coincidido en innumerables muestreos de alcachofa. A lo largo de estos años, nos hemos ayudado mutuamente en nuestras tesis, yo con sus pimientos y ella con mis alcachofas, demostrando que, al final, la investigación también es trabajo en equipo. A **Jennifer** y **Fernando**, con quienes inicié este viaje y que pronto también alcanzarán esta meta. A la **Dra. Mª Emma García**, por su alegría y el buen ambiente que siempre ha transmitido en el laboratorio. A la **Dra. María Gutiérrez**, por su constante predisposición a ayudar y enseñar, siempre con una sonrisa y un buen rollo que hacían más llevaderas las horas. Al **Dr. Vicente Serna**, por su experiencia, conocimientos y metodología de trabajo, que han sido un ejemplo de disciplina y eficiencia. Y, por supuesto, a todos los estudiantes de prácticas, TFG y TFM (**Anabel**, **Fernando**, **Andrea**, ente muchos otros) que han pasado por el laboratorio y han aportado su esfuerzo y energía en los ensayos de alcachofa. Muchas gracias a todos.

A **Adrián Grau**, por su colaboración en nuestras numerosas recolecciones de alcachofa a lo largo de estos años. Un primer paso fundamental antes de cualquier ensayo. Muchas gracias.

I would also like to express my gratitude to the University of Minho, where I had the opportunity to conduct a two-month research stay in Braga (Portugal). My sincere thanks to **Dr. António Vicente** for his supervision and guidance, and to **Dr. Ana Cristina Pinheiro** for her constant support. A special mention to **João Araújo** for sharing his knowledge and teaching me the analytical techniques used in the laboratory. Muito obrigada!

En el contexto de Braga, quiero hacer una mención especial a **Sara Venina Ribeiro**, quien empezó siendo mi casera y acabó convirtiéndose en una gran amiga, haciéndome sentir como en casa durante mi estancia. Beijinhos e saudade!

I would also like to extend my thanks for my five-month research stay at the Università di Catania (Italy), an experience that was enriching both professionally and personally. To **Dr. Gaetano Pandino**, **Dr. Sara Lombardo** and **Dr. Giovanni Mauromicale**, for their guidance, time, and for giving me the opportunity to learn in their laboratory. To my lab mates, **Mirko La Iacona**, **Salvo Salicola**, and **Claudia Formenti**, for welcoming me from day one, making me feel like part of the team. Grazie mille!

A mis padres, **Agustín** y **Cristina**, por su amor incondicional y su apoyo constante en todo este proceso. No hay palabras suficientes para agradeceros por estar siempre ahí, por creer en mí incluso en los momentos en los que yo dudaba, por animarme a seguir adelante y recordarme, con cada gesto, que siempre tendría un refugio en vosotros. Gracias por vuestra paciencia, por vuestras palabras de aliento y por todo lo que habéis hecho para que pudiera llegar hasta aquí.

A mi hermano **Carlos** y a **Mila**, por acompañarme en este camino, por vuestro apoyo y por estar siempre presentes.

A toda mi familia y amigos, por formar parte de este proceso, por interesaros, por preguntar cómo iba la tesis y por hacerme sentir siempre arropada, sin importar la distancia o el tiempo.

Y, por último, pero no por ello menos importante, a mi compañero de vida y mejor amigo, **Adrián**. Desde el momento cero en que la vida nos volvió a unir, has sido un pilar fundamental de este proceso, apoyándome en cada momento y, en especial, en los más difíciles. Junto con mis directores de tesis, has sido la persona que más ha tenido que escuchar mis ensayos para las presentaciones de congresos y ponencias, con una paciencia infinita y aunque estuvieran lejos de tu campo. Por todo eso, y porque siempre sumas y nunca restas, te quiero y te estaré eternamente agradecida.

Para finalizar, esta tesis no habría sido posible sin todos vosotros y sin muchos otros que, aunque no estén mencionados aquí, han formado parte de este camino de una u otra manera.

A todos, de corazón, muchas gracias.

– Marina –

PUBLICATION QUALITY INDEX

PUBLICATION 1

Effect of Cysteine with Essential Oils on Quality Attributes and Functional Properties of 'Blanca de Tudela' Fresh-Cut Artichoke.

Authors

María J. Giménez, Marina Giménez-Berenguer, Fabián Guillén, Vicente Serna-Escolano, María Gutiérrez-Pozo and Pedro J. Zapata

Journal

FOODS

Date of publication

8 December 2023

DOI

10.3390/foods12244414

Publisher name

MDPI

JCR ® Category

FOOD SCIENCE & TECHNOLOGY

Quartile	**Rank**
Q1	38/173

Impact factor (2023)	**Impact factor (5 years)**
4.7	5.1

PUBLICATION 2

Impact of Flower Head Order and Phenolic Content on the Quality of Three Different Artichoke Cultivars for Fresh-Cut Products.

Authors

Marina Giménez-Berenguer, María J. Giménez, Vicente Serna-Escolano, and Pedro J. Zapata

Journal

AGRONOMY

Date of publication

27 January 2025

DOI

10.3390/agronomy15020322

Publisher name

MDPI

JCR ® Category

AGRONOMY

Quartile

Q1

Rank

20/126

Impact factor (2023)

3.3

Impact factor (5 years)

3.7

PUBLICATION 3

Evaluation of 'Lorca' Cultivar Aptitude for Minimally Processed Artichoke.

Authors

Marina Giménez-Berenguer, María E. García-Pastor, Santiago García-Martínez, María J. Giménez, and Pedro J. Zapata

Journal

AGRONOMY

Date of publication

18 February 2022

DOI

10.3390/agronomy12020515

Publisher name

MDPI

JCR ® Category

AGRONOMY

Quartile

Q1

Rank

20/126

Impact factor (2023)

3.3

Impact factor (5 years)

3.7

PUBLICATION 4

Influence of flower head order on phenolic content and quality of globe artichoke at harvest and during twenty-one days of cold storage.

Authors

María J. Giménez, Marina Giménez-Berenguer, María E. García-Pastor, Salvador Castillo, Juan M. Valverde, María Serrano, Daniel Valero and Pedro J. Zapata

Journal

SCIENTIA HORTICULTURAE

Date of publication

24 December 2021

DOI

10.1016/j.scienta.2021.110846

Publisher name

ELSEVIER

JCR ® Category

HORTICULTURE

Quartile

Q1

Rank

5/38

Impact factor (2023)

3.9

Impact factor (5 years)

4.4

PUBLICATION 5

Influence of Artichoke Antioxidant Activity in Their Susceptibility to Suffer Frost Injury.

Authors

Marina Giménez-Berenguer, María Gutiérrez-Pozo, Vicente Serna-Escolano, María J. Giménez, and Pedro J. Zapata

Journal

ANTIOXIDANTS

Date of publication

2 November 2023

DOI

10.3390/antiox12111960

Publisher name

MDPI

JCR ® Category

FOOD SCIENCE & TECHNOLOGY

Quartile

Q1

Rank

20/173

Impact factor (2023)

6

Impact factor (5 years)

6.7

PUBLICATION 6

Seeds Mineral Profile and Ash Content of Thirteen Different Genotypes of Cultivated and Wild Cardoon Over Three Growing Seasons.

Authors

Marina Giménez-Berenguer, Salvatore A. Salicola, Claudia Formenti, María J. Giménez, Giovanni Mauromicale, Pedro J. Zapata, Sara Lombardo, and Gaetano Pandino

Journal

AGRICULTURE

Date of publication

Under review

DOI

/

Publisher name

MDPI

JCR ® Category

AGRONOMY

Quartile

Q1

Rank

20/126

Impact factor (2023)

3.3

Impact factor (5 years)

GENERAL INDEX

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LIST OF ABBREVIATIONS

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1,3-diCQA	1,3-di-O-caffeoylquinic acid (cynarin)
3,4-diCQA	3,4-di-O-caffeoylquinic acid
3,5-diCQA	3,5-di-O-caffeoylquinic acid
3-CQA	3-O-caffeoylquinic acid
4,5-diCQA	4,5-di-O-caffeoylquinic acid
5-CQA	5-O-caffeoylquinic acid (chlorogenic acid)
ABTS	2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ANOVA	Analysis of Variance
BI	Browning Index (%)
Ca	Calcium
CA	Controlled Atmosphere
CC	Cultivated Cardoon
Cu	Copper
DM	Dry Matter
EOs	Essential Oils
Fe	Iron
FW	Fresh Weight
GA_3	Gibberellic Acid
GAE	Gallic Acid Equivalents
GC-TCD	Gas chromatograph with thermal conductivity detector
HPLC-DAD-ESI/MS^n	High-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry
H-TAA	Hydrophilic Total Antioxidant Activity
K	Potassium
L-TAA	Lipophilic Total Antioxidant Activity
Lut 7-glc	Luteolin 7-O-glucoside
Lut 7-gluc	Luteolin 7-O-glucuronide
Lut 7-gluc 3-glc	Luteolin 7-O-glucuronide 3-O-glucoside
MAP	Modified Atmosphere Packaging
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
NaF	Sodium Fluoride
NaF	Sodium Fluoride
PAL	Phenylalanine Ammonia-Lyase
PDO	Protected Designation of Origin
ppm	Parts Per Million
PPO	Polyphenol Oxidase

PVP	Polyvinylpyrrolidone
RH	Relative Humidity
ROS	Reactive Oxygen Species
RP-HPLC-DAD	Reverse-Phase High-Performance Liquid Chromatography With Diode Array Detection
RR	Respiration Rate
SE	Standard Error
TAA	Total Antioxidant Activity
TPC	Total Phenolic Compounds
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
U	One Unit: amount of enzyme that caused an increase in absorbance of 0.001 per minute under the assay conditions
UniCT	University of Catania
WL	Weight Loss
Zn	Zinc

STRUCTURE OF THE DOCTORAL THESIS

STRUCTURE OF THE DOCTORAL THESIS

This Doctoral Thesis has been written in accordance with the current regulations of Miguel Hernández University of Elche under the modality of a thesis by compendium of publications. Therefore, this dissertation is structured as follows:

- **Abstract**: A brief description of the most relevant results and conclusions obtained in this doctoral thesis.
- **Introduction**: A concise overview of the scientific background of the doctoral thesis, establishing the relationship between the current state of knowledge and the challenges related to artichokes and cardoons. Additionally, the importance of the studied crops is analysed to justify their selection for this research.
- **Objectives**: General aim and specific objectives of the PhD Thesis have been established in this section, each of which is accompanied by a specific working hypothesis.
- **Summary of the Methodology**: A brief description of the plant material used, the experimental design, and the analytical methods applied to conduct the experiments included in this doctoral thesis.
- **Scientific Publications**: Literal transcriptions of the five scientific publications that constitute this thesis and one under review are presented in the following order:

    1. *Effect of Cysteine with Essential Oils on Quality Attributes and Functional Properties of 'Blanca de Tudela' Fresh-Cut Artichoke.*
    2. *Influence of the Artichoke Cultivar on the Aptitude for the Development of Fresh-Cut Artichoke.*
    3. *Evaluation of 'Lorca' Cultivar Aptitude for Minimally Processed Artichoke.*
    4. *Influence of Flower Head Order on Phenolic Content and Quality of Globe Artichoke at Harvest and During Twenty-One Days of Cold Storage.*

5. *Influence of Artichoke Antioxidant Activity in Their Susceptibility to Suffer Frost Injury.*
6. *Seeds Mineral Profile and Ash Content of Thirteen Different Genotypes of Cultivated and Wild Cardoon Over Three Growing Seasons.*

- **Summary of Results and Discussion**: This section presents and discusses the main findings of the doctoral thesis.
- **Conclusions**: A synthesis of the key conclusions drawn from this doctoral thesis.
- **Future Research Lines**: A brief description of potential future research directions that may emerge from the conclusions of this study.
- **References**: Bibliography used to support and justify this doctoral thesis.

ABSTRACT

RESUMEN

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ABSTRACT

This doctoral thesis, titled *"Characterization of Commercial Cultivars of Artichoke, Cardoon, and their Wild Relatives for Productive and Commercial Optimization"*, provides a comprehensive investigation into the genetic, agronomic, and environmental factors influencing the quality and commercial potential of artichokes (*Cynara cardunculus* var. *scolymus*) and cardoons (*Cynara cardunculus* var. *altilis*). These species, which belong to the *Asteraceae* family, are economically significant crops in Mediterranean agriculture, valued not only for their culinary applications but also for their high nutritional and bioactive compound content. Despite their agronomic importance and increasing interest in their health-promoting properties, artichokes and cardoons remain relatively underexplored compared to other vegetable commodities. The characterization of their biochemical, physiological, and post-harvest properties is crucial for enhancing their competitiveness, optimizing their industrial applications, and ensuring their sustainability within modern agricultural systems.

Artichokes and cardoons play a crucial role in Mediterranean agriculture. Spain, Italy, and Egypt are the leading producers of artichokes, with Spain ranking third worldwide. The artichoke industry is particularly relevant in the Valencian Community and Murcia, where it contributes significantly to local economies. Artichoke cultivation follows both annual and perennial production systems, with vegetative propagation being the dominant method in traditional farming. However, seed-propagated cultivars have gained interest due to their potential advantages in terms of uniformity, yield, and disease resistance.

Cardoons, on the other hand, are less commonly consumed as vegetables but hold significant agronomic value due to their adaptability to marginal lands and their tolerance to drought and extreme temperatures. They are cultivated mainly in Mediterranean countries, where they are used in traditional cuisine, functional food applications, and as a source of bioactive compounds. Moreover, cardoon cultivation has been associated with soil health benefits, such as enhanced organic matter and improved microbial activity.

Both artichokes and cardoons are highly regarded for their bioactive composition. Artichokes are particularly rich in polyphenols, including chlorogenic acid, cynarin, and flavonoids, which contribute to their strong antioxidant capacity. These compounds not only enhance the nutritional value of artichokes but also play a role in determining their post-harvest stability and processing suitability. Similarly, cardoon seeds have been identified as a valuable source of minerals such as calcium, magnesium, and potassium, making them a promising ingredient for functional food and feed applications.

Despite their well-documented nutritional and agronomic benefits, both artichokes and cardoons face several research gaps that limit their commercial and productive optimization. In the case of artichokes, post-harvest browning due to enzymatic oxidation remains a significant challenge for fresh-cut processing. While polyphenols are essential for their antioxidant properties, they also act as substrates for polyphenol oxidase leading to rapid discoloration and reduced consumer acceptability. This balance between nutritional quality and post-harvest stability is crucial in determining the suitability of different cultivars for fresh or processed markets. For cardoons, research has primarily focused on their agronomic traits and medicinal properties, while fewer studies have explored the stability of their mineral composition across different genotypes and growing conditions. Understanding these variations is essential for optimizing cardoon seed utilization in food, feed, and industrial applications.

This doctoral thesis aims to address these knowledge gaps by providing a comprehensive characterization of artichoke and cardoon cultivars, focusing on their genetic, biochemical, and post-harvest properties. Through six interconnected studies, this research aims to deepen the understanding of the productive and commercial aspects of these crops, addressing key challenges related to fresh-cut processing, post-harvest quality, environmental stress tolerance, and cardoon seed nutritional composition. By integrating physiological, biochemical, and agronomic perspectives, this thesis offers valuable insights that contribute optimizing production strategies, improving post-harvest management, and enhancing the competitiveness of these crops in agricultural and industrial sectors.

The first set of studies focuses on fresh-cut artichokes, investigating enzymatic browning, phenolic content, and storage stability. A novel approach combining *L*-cysteine and essential oils (eugenol, thymol and carvacrol) at an equivalent concentration of 75, 150 and 300 µL, was evaluated for its efficacy in minimizing enzymatic browning while preserving the bioactive properties and sensory attributes of 'Blanca de Tudela' fresh-cut artichokes. Results of the application of cysteine and 150 µL of EOs, confirmed a significant reduction in oxidative browning, extending shelf-life and improving visual quality without compromising phenolic retention.

Further research examined the suitability of three artichoke cultivars ('Lorca,' 'Tupac,' and 'Green Queen') for fresh-cut processing, with particular emphasis on the influence of flower head order (main, secondary, tertiary). Results demonstrated that tertiary heads, particularly from the 'Green Queen' cultivar, exhibited the highest phenolic content but also showed greater susceptibility to browning. In contrast, main heads, characterized by lower polyphenol levels, especially in the 'Lorca' and 'Tupac' cultivars, displayed reduced browning indices and higher consumer acceptability. A strong negative correlation coefficient was observed between these parameters, indicating that increased browning directly reduces sensory appeal. This study underscores the significance of cultivar selection and flower head order in optimizing the quality and commercial capability of minimally processed artichoke products.

Following this line of investigation, Lorca cultivar was selected for its viability as minimally processed product. Artichokes were classified according to the flower head orders (main, secondary, and tertiary) and three internal development stages (initial, intermediate, and advanced). For the first time, internal development stage was analysed in relation to total phenolic content. The results indicated that main heads and those in the advanced development stage exhibited the lowest phenolic concentration and PPO activity, resulting in reduced browning susceptibility, and therefore, potentially enhancing its suitability for fresh-cut processing.

Another core investigation conducted a phytochemical characterization of artichoke heads based on their position on the plant (main, secondary, and tertiary heads) and harvest date throughout an entire growing season in 'Blanca de Tudela'

artichoke cultivar. The results indicated that tertiary heads exhibited the highest total identified polyphenol concentration, primarily due to their elevated levels of hydroxycinnamic acid and luteolin derivatives compared to secondary and main heads. Furthermore, two postharvest storage experiments were conducted using main, secondary, and tertiary artichoke heads harvested in winter and spring. Tertiary heads demonstrated the lowest weight loss, minimal firmness reduction, and the lowest respiration rate during cold storage, which may be attributed to their higher antioxidant compound content. In conclusion, tertiary heads exhibit greater suitability for low-temperature storage from harvest to consumption, as they maintain quality attributes for a longer period and contain higher levels of bioactive compounds.

Expanding on this research, the following study explored the role of phenolic compounds and antioxidant activity in mitigating frost damage in 'Blanca de Tudela' artichokes. In northern hemisphere countries, artichoke harvesting takes place during the winter months, exposing the crop to low temperatures. This exposure can lead to frost injury, manifested as cuticle blistering and detachment of the outer bracts, which may subsequently develop brown or black discoloration. Such injuries can result in significant economic and production losses. However, to the best of our knowledge, no previous studies have specifically addressed this issue in artichokes. Several parameters were analysed, including flower head order, weight and size of the artichokes, total phenolic content, phenolic profile, and total antioxidant activity. The results indicated that tertiary heads, which are the smallest in size, contained the highest levels of total phenolic compounds and antioxidant activity. Consequently, these characteristics provided enhanced protection against frost damage. Conversely, larger artichokes, particularly primary heads, were more susceptible to frost injury. These findings suggest that a well-developed antioxidant system, characterized by elevated phenolic content, plays a key role in reducing artichoke susceptibility to frost injury.

Beyond artichokes, this thesis also evaluates the variations in mineral composition and ash content of thirteen distinct genotypes of cultivated and wild cardoon seeds (four commercial, four wild, and five self-developed genotypes from the University of Catania) over three consecutive growing seasons. The findings

revealed that ash content, as well as macro- and micro-element concentrations, were significantly influenced by environmental conditions, genetic factors, and their interactions. Notably, ash content exhibited substantial fluctuations across the three seasons, with the lowest value observed in the second season, likely associated with higher rainfall levels compared to seasons one and three. The self-developed genotypes demonstrated the highest mean concentrations of all micro-mineral elements analysed. Overall, cardoon seeds were confirmed to be a valuable source of essential macro- and micro-elements, with the self-developed genotypes from the University of Catania showing particularly high nutritional potential. This study provides, for the first time, a comprehensive assessment of the long-term consistency and variability in mineral content and ash composition in cardoon seeds, offering new insights into their nutritional value and potential applications.

Collectively, the findings of this thesis contribute to a more comprehensive understanding of artichoke and cardoon quality determinants. Through a multidisciplinary perspective, this research provides practical insights for breeders, growers, and processors seeking to optimize production strategies.

Furthermore, the thesis reveals a fundamental misalignment in artichoke commercialization, where larger main heads, which exhibit lower browning susceptibility, should be prioritized for industrial applications, while tertiary heads, richer in bioactive compounds, should be directed toward fresh consumption due to their potential health benefits. These insights lay the foundation for a paradigm shift in the artichoke industry, promoting a more rational and scientifically guided approach to production, consumption, and processing strategies.

RESUMEN

Esta tesis doctoral, titulada *"Caracterización de Variedades Comerciales de Alcachofa, Cardo y sus Parientes Silvestres para su Optimización Productiva y Comercial"*, presenta una investigación exhaustiva sobre los factores genéticos, agronómicos y ambientales que influyen en la calidad y el potencial comercial de las alcachofas (*Cynara cardunculus* var. *scolymus*) y los cardos (*Cynara cardun*culus var. *altilis*). Estas especies, que pertenecen a la familia *Asteraceae*, son cultivos de gran importancia económica en la agricultura mediterránea, valorados no solo por sus aplicaciones culinarias, sino también por su alto contenido en nutrientes y compuestos bioactivos. Sin embargo, a pesar de su relevancia agronómica y el creciente interés en sus propiedades beneficiosas para la salud, las alcachofas y los cardos siguen siendo relativamente poco investigados en comparación con otras hortalizas. La caracterización de sus propiedades bioquímicas, fisiológicas y post-cosecha es crucial para mejorar su competitividad, optimizar sus aplicaciones industriales y garantizar su sostenibilidad dentro de los sistemas agrícolas modernos.

Las alcachofas y los cardos desempeñan un papel fundamental en la agricultura mediterránea. España, Italia y Egipto son los principales productores de alcachofas, siendo España el tercer mayor productor mundial. La industria de la alcachofa es particularmente relevante en la Comunidad Valenciana y Murcia, donde contribuye significativamente a las economías locales. El cultivo de alcachofas sigue sistemas de producción anuales y perennes, siendo la propagación vegetativa el método dominante en la agricultura tradicional. Sin embargo, las variedades propagadas por semillas han ganado interés debido a sus ventajas potenciales en términos de uniformidad, rendimiento y resistencia a enfermedades.

Por otro lado, los cardos no son tan comúnmente consumidos como hortalizas, pero tienen un gran valor agronómico debido a su adaptabilidad a tierras marginales y su tolerancia a la sequía y temperaturas extremas. Se cultivan principalmente en países mediterráneos, donde se utilizan en la cocina tradicional, en aplicaciones de alimentos funcionales y como fuente de compuestos bioactivos. Además, el cultivo de cardos se ha asociado con beneficios para la salud del suelo, como el aumento de la materia orgánica y la mejora de la actividad microbiana.

Tanto las alcachofas como los cardos son altamente valorados por su composición bioactiva. Las alcachofas son particularmente ricas en polifenoles, como el ácido clorogénico, la cinarina y los flavonoides, que contribuyen a su fuerte capacidad antioxidante. Estos compuestos no solo mejoran el valor nutricional de las alcachofas, sino que también juegan un papel importante en la determinación de su estabilidad post-cosecha y aptitud para el procesado. De manera similar, las semillas de cardo se han identificado como una fuente valiosa de minerales como calcio, magnesio y potasio, lo que las convierte en un ingrediente prometedor para aplicaciones de alimentos funcionales y piensos.

A pesar de sus bien documentados beneficios nutricionales y agronómicos, tanto las alcachofas como los cardos presentan varias incógnitas en su investigación que limitan su optimización productiva y comercial. En el caso de las alcachofas, el pardeamiento post-cosecha sigue siendo un desafío importante para el procesado de productos frescos debido a la oxidación enzimática. Aunque los polifenoles son esenciales para sus propiedades antioxidantes, también actúan como sustratos para la enzima polifenoloxidasa, dando lugar a una rápida decoloración y reducción de la aceptación por parte del consumidor. Este equilibrio entre la calidad nutricional y la estabilidad post-cosecha es crucial para determinar la aptitud de las diferentes variedades para el mercado de producto fresco o procesado. En el caso de los cardos, la investigación se ha centrado principalmente en sus características agronómicas y propiedades medicinales, mientras que se han realizado menos estudios sobre la estabilidad de su composición mineral en diferentes genotipos y condiciones de cultivo. Comprender estas variaciones es esencial para optimizar la utilización de las semillas de cardo en aplicaciones alimentarias, piensos e industriales.

Esta tesis doctoral tiene como objetivo abordar estas lagunas de conocimiento mediante una caracterización integral de variedades de alcachofa y cardo, con énfasis en sus propiedades genéticas, bioquímicas y post-cosecha. A través de seis estudios interconectados, esta investigación tiene como objetivo profundizar en la comprensión de los aspectos productivos y comerciales de estos cultivos, abordando los principales desafíos relacionados con el procesado de productos frescos, la calidad post-cosecha, la tolerancia al estrés ambiental y la composición nutricional de las semillas de cardo. Al integrar perspectivas fisiológicas, bioquímicas y

agronómicas, esta tesis ofrece valiosos conocimientos que contribuyen a optimizar las estrategias de producción, mejorar la gestión post-cosecha y aumentar la competitividad de estos cultivos en los sectores agrícola e industrial.

El primer conjunto de estudios se centra en alcachofas en IV gama o mínimamente procesadas y listas para consumir o cocinar, investigando el pardeamiento enzimático, el contenido fenólico y la estabilidad durante el almacenamiento. Se evaluó un enfoque novedoso que combina L-cisteína y aceites esenciales (eugenol, timol y carvacrol) a una concentración equivalente de 75, 150 y 300 µL, para determinar su eficacia en minimizar el pardeamiento enzimático mientras se preservan las propiedades bioactivas y atributos sensoriales de las alcachofas 'Blanca de Tudela'. Los resultados de la aplicación de cisteína en combinación con 150 µL de aceites esenciales confirmaron una reducción significativa del pardeamiento oxidativo, extendiendo la vida útil y mejorando la calidad visual sin comprometer la concentración de compuestos fenólicos.

Investigaciones adicionales examinaron la aptitud de tres variedades de alcachofa ('Lorca', 'Tupac' y 'Green Queen') para el procesado en fresco, con especial énfasis en la influencia del orden floral (principal, secundario, terciario). Los resultados demostraron que las alcachofas terciarias, especialmente de la variedad 'Green Queen', presentaron el mayor contenido fenólico, pero también una mayor susceptibilidad al pardeamiento. En contraste, las alcachofas principales, caracterizadas por niveles más bajos de polifenoles, especialmente en las variedades 'Lorca' y 'Tupac', mostraron menores índices de pardeamiento y una mayor aceptabilidad por parte de los consumidores. También se observó un fuerte coeficiente de correlación negativa entre estos parámetros, lo que indica que el aumento del pardeamiento reduce directamente la apariencia visual. Este estudio pone de manifiesto la importancia de la selección de variedades y del orden floral para optimizar la calidad y la capacidad comercial de los productos de alcachofa mínimamente procesados.

Siguiendo esta línea de investigación, se seleccionó la variedad 'Lorca' por su viabilidad como producto mínimamente procesado. Las alcachofas se clasificaron según el orden floral (principal, secundario, terciario) y en tres estados de desarrollo interno (inicial, intermedio, avanzado). Por primera vez, se evaluó el estado de

desarrollo interno en relación con el contenido fenólico total. Los resultados indicaron que las alcachofas principales y aquellas en el estado de desarrollo avanzada mostraron la menor concentración de compuestos fenólicos y actividad de la PPO, lo que resultó en una menor susceptibilidad al pardeamiento y, por lo tanto, podría mejorar su aptitud para el procesado mínimo.

La siguiente investigación se centró en realizar una caracterización fitoquímica de las alcachofas según su posición en la planta (alcachofas principales, secundarias y terciarias) y la fecha de recolección a lo largo de toda una temporada de cultivo en la variedad de alcachofa 'Blanca de Tudela'. Los resultados indicaron que las alcachofas terciarias exhibieron la mayor concentración total de polifenoles identificados, principalmente debido a sus elevados niveles de ácido hidroxicinámico y derivados de luteolina en comparación con las alcachofas secundarias y principales. Además, se realizaron dos experimentos de almacenamiento post-cosecha utilizando alcachofas principales, secundarias y terciarias recolectadas en invierno y primavera. Las alcachofas terciarias mostraron la menor pérdida de peso, reducción mínima de firmeza y la tasa de respiración más baja durante el almacenamiento en frío, lo que puede atribuirse a su mayor contenido en compuestos antioxidantes. En conclusión, las alcachofas terciarias tienen una mayor aptitud para el almacenamiento a bajas temperaturas desde la cosecha hasta el consumo, ya que mantienen las propiedades de calidad durante un periodo más largo y contienen mayores niveles de compuestos bioactivos.

Ampliando esta investigación, el siguiente estudio exploró el papel de los compuestos fenólicos y la actividad antioxidante en la mitigación del daño por heladas en las alcachofas 'Blanca de Tudela'. En los países del hemisferio norte, la recolección de alcachofas se realiza durante los meses de invierno, exponiendo el cultivo a bajas temperaturas. Esta exposición puede provocar daños por heladas, manifestados como ampollas en la cutícula y apertura de las brácteas exteriores, que pueden desarrollar decoloración marrón o negra. Dichos daños pueden resultar en importantes pérdidas económicas y de producción. Sin embargo, hasta donde sabemos, no existen estudios previos que aborden específicamente este problema en alcachofas. Es por ello por lo que en nuestra investigación se analizaron varios parámetros, incluidos el orden floral, el peso y tamaño de las alcachofas, el contenido

fenólico total, el perfil fenólico y la actividad antioxidante total. Los resultados indicaron que las alcachofas terciarias, las más pequeñas en tamaño, contenían los niveles más altos de compuestos fenólicos totales y actividad antioxidante. En consecuencia, estas características proporcionaron una mayor protección contra el daño por heladas. Por el contrario, las alcachofas más grandes, especialmente las principales, fueron más susceptibles al daño por heladas. Estos hallazgos sugieren que un sistema antioxidante bien desarrollado, caracterizado por un contenido elevado de fenoles, juega un papel clave en la reducción de la susceptibilidad de las alcachofas al daño por heladas.

Más allá de las alcachofas, esta tesis también evalúa las variaciones en la composición mineral y el contenido de cenizas de trece genotipos distintos de semillas de cardo cultivado y silvestre (cuatro comerciales, cuatro silvestres y cinco auto-desarrollados por la Universidad de Catania) a lo largo de tres temporadas consecutivas de cultivo. Los resultados revelaron que el contenido de cenizas, así como las concentraciones de macro- y micro-elementos, fueron significativamente influenciados por las condiciones ambientales, los factores genéticos y sus interacciones. Específicamente, el contenido de cenizas mostró fluctuaciones sustanciales entre las tres temporadas, observando el valor más bajo en la segunda temporada, lo cual probablemente estuvo relacionado con los niveles más altos de precipitaciones en comparación con la primera y tercera temporada. Los genotipos auto-desarrollados mostraron las concentraciones medias más altas de todos los micro-minerales analizados. En general, las semillas de cardo se confirmaron como una fuente valiosa de macro- y micro-elementos esenciales, con los genotipos desarrollados de la Universidad de Catania mostrando un potencial nutricional particularmente alto. Este estudio ofrece, por primera vez, una evaluación integral de la consistencia y variabilidad a largo plazo en el contenido mineral y la composición de cenizas en las semillas de cardo, proporcionando nuevos conocimientos sobre su valor nutricional y aplicaciones potenciales.

En conjunto, los hallazgos de esta tesis contribuyen a una comprensión más completa de los determinantes de calidad de la alcachofa y el cardo. A través de una perspectiva multidisciplinaria, esta investigación ofrece valiosas perspectivas para mejoradores, productores y procesadores que buscan optimizar las estrategias de

producción. Además, la tesis revela una desalineación fundamental en la comercialización de la alcachofa, donde las alcachofas principales que son las más grandes, presentan menor susceptibilidad al pardeamiento, por lo que deberían priorizarse para aplicaciones industriales. Por el contrario, las alcachofas terciaras, más ricas en compuestos bioactivos, deberían orientarse al consumo fresco debido a sus posibles beneficios para la salud. Estos hallazgos establecen las bases para un cambio de paradigma en la industria de la alcachofa, promoviendo un enfoque más racional y científicamente guiado para las estrategias de producción, consumo y procesado.

INTRODUCTION

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INTRODUCTION

The Globe Artichoke and Cardoon

The globe artichoke (*Cynara cardunculus* L. var. *scolymus*) and cardoon (*Cynara cardunculus* L. var. *altilis*), both members of the *Asteraceae* family, are herbaceous perennials plants that originated in the Mediterranean region. These botanical varieties are the descendants of their primary ancestor (*Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori), so-called wild cardoon or wild artichoke (Pandino et al., 2011b; Pesce & Mauromicale, 2019) They are highly valued not only for their culinary uses but also for their rich nutritional and medicinal profiles, offering a rich source of bioactive compounds, fiber, and essential minerals (Borah et al., 2009; Brat et al., 2006; Cabezas-Serrano et al., 2009; de Falco et al., 2015).

This section delves into both the economic and cultural significance of these plants, as well as their botanical differences, cultivation practices, nutritional benefits, post-harvest management and growing global demand.

1. Globe Artichoke (*Cynara cardunculus* L. var. *scolymus*)

1.1. Economic and Cultural Significance of Artichokes

The artichoke's distinctive structure includes edible inflorescences, also known as "heads" or "capitula", composed of fleshy bracts and a tender receptacle. The inflorescence of artichoke plants consists of a hierarchically organized structure, comprising a primary head or capitulum and several secondary and tertiary heads.

The primary capitulum, which is generally the largest, develops at the apex of the main stem, while the secondary and tertiary heads are smaller and emerge along the lateral branches (Lombardo et al., 2018). In annual cultivation systems, each plant typically produces a single primary capitulum and may yield up to twenty additional secondary and tertiary heads (de Falco et al., 2015; Feiden et al., 2023).

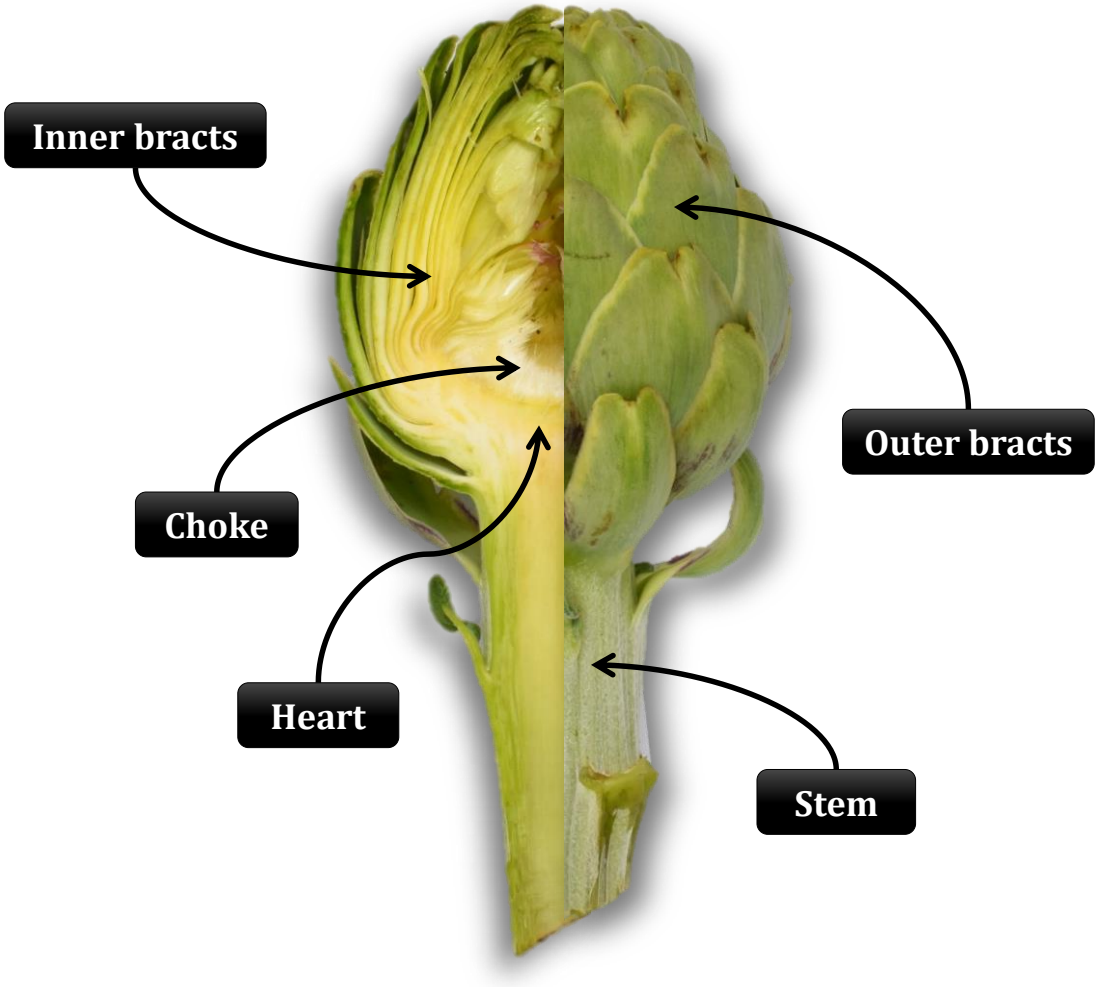

Figure 1. Artichoke head parts and cross section.

Globally, the artichoke holds a prominent place in the agricultural economies of Mediterranean countries, where Italy, Egypt, and Spain are the main producers. In Spain, approximately 13,862 hectares are dedicated to artichoke cultivation, yielding nearly 196,965 tons annually, with most of this production concentrated in the regions of the Comunidad Valenciana and Murcia (MAPA, 2020).

The territory of the Vega Baja del Segura, located in the northernmost part of the Valencian Community, accounts for approximately 80% of the total artichoke production in the province of Alicante. Additionally, in Benicarló (Castellón), artichoke cultivation is protected under the Protected Designation of Origin (PDO) Alcachofa de Benicarló, underscoring its economic and cultural significance in the area. These place Spain as the third-largest artichoke producer worldwide, while Italy and Egypt lead, reflecting the long-standing cultural and economic importance

of artichoke cultivation in the Mediterranean basin (Giménez et al., 2022; Pandino et al., 2011a). The region's warm, dry summers and mild, moist winters provide ideal conditions for artichoke growth, supporting both annual and perennial planting systems (Lombardo et al., 2015a).

Globe artichoke production predominantly relies on vegetative propagation through basal and lateral offshoots (Grabowska et al., 2018), as exemplified by the widely cultivated Spanish cultivar 'Blanca de Tudela'. This cultivar exhibits a reflowering capacity that allows for two distinct production cycles: the primary one during the autumn-winter season (early production), originating from the initial plant emerging from the rhizome, and a second one in spring season (late production), derived from a secondary plant that regenerates from the senescing first shoot. Although 'Blanca de Tudela' is classified as an early-maturing cultivar, vegetative propagation presents several limitations, including increased vulnerability to pathogen transmission, physiological variability among propagated material, low propagation efficiency, restricted transplanting flexibility, elevated planting costs, and a considerable risk of establishment failure (Giménez et al., 2021; Mauromicale et al., 2018). In response to these disadvantages, alternative propagation strategies have been explored to enhance crop yield, improve harvest timing, and optimize head quality in accordance with market demands. Over the past three decades, these efforts have led to the development of seed-propagated cultivars, initially through open-pollinated lines and subsequently via hybrid breeding programs (Giménez et al., 2021; Mauromicale & Ierna, 2000).

Seed-propagated globe artichoke cultivars offer several agronomic and economic benefits, including enhanced uniformity, greater yield potential, improved resistance to diseases, and increased profitability (Baixauli et al., 2012; Giménez et al., 2021; Lombardo et al., 2012). Nonetheless, a major limitation associated with seed-propagated genotypes is their delayed onset of production, primarily due to an extended juvenile phase (Macua et al., 2012). Despite this constraint, cultivation from seedlings has demonstrated the potential to achieve substantial yields even in areas characterized by shorter vegetation periods (Giménez et al., 2021; Sałata et al., 2012).

Figure 2: Seed-propagated artichoke cultivar ('Green Queen') vs. Vegetative propagated artichoke cultivar ('Blanca de Tudela').

However, seed propagation introduces challenges in terms of delayed flowering and lower phenolic content compared to vegetatively propagated varieties. In this sense, gibberellic acid (GA_3) treatments have been investigated to offset these limitations by promoting early flowering and increasing yield. GA_3 has complex effects on phenolic composition, often reducing hydroxycinnamic acid levels while enhancing luteolin derivatives, which vary according to flower head order and cultivar (Giménez et al., 2021).

Approximately 60% of the global artichoke production is destined for the fresh market. In general terms, standard commercial head weighs between 200 and 500 grams, although they may reach up to 700 grams. Smaller-sized heads can also be found, ranging from 150 to 200 grams (Grabowska et al., 2018). Heads are sold with a peduncle length of 4 to 6 cm. The cultural significance of artichokes goes beyond their economic value; they are integral to Mediterranean cuisine and culture.

Artichokes are valued for their distinct taste and versatility in dishes, from traditional salads and soups to more elaborate preparations. This cultural affinity has supported a steady demand in Europe, where artichokes are marketed fresh, canned, or frozen (Feiden et al., 2023; Lattanzio et al., 2009; Macua, 2007; Macua et al., 2012; Salekzamani et al., 2019). In addition, the Mediterranean diet's emphasis on plant-based foods has increased consumer awareness of artichokes as a functional food with notable health benefits (Ceccarelli et al., 2010; de Falco et al., 2015).

1.2. Nutritional Composition and Health Benefits

Artichokes are an essential crop in the Mediterranean, renowned for their culinary applications and substantial nutritional and medicinal benefits (Pandino et al., 2011a). These vegetables offer an abundant source of bioactive compounds, including polyphenols and flavonoids (Brat et al., 2006; Giménez et al., 2021), as well as inulin (Lattanzio et al., 2009), essential vitamins, minerals, and dietary fibre (Borah et al., 2009; Cabezas-Serrano et al., 2009; Gagliardi et al., 2020; Lombardo et al., 2017; Rocchetti et al., 2020; Rouphael et al., 2017).

The polyphenolic and flavonoid content, particularly the presence of caffeoylquinic acids and luteolin derivatives, has been associated with potent antioxidant activity, which provides critical protection against oxidative stress and inflammation-related diseases (Ceccarelli et al., 2010; Lattanzio et al., 2009; Silva et al., 2022). These properties are essential for cardiovascular health, digestive support, and liver function (de Falco et al., 2015; Salem et al., 2015; Santos et al., 2018), making artichokes a valuable dietary addition with preventative health benefits (Muratore et al., 2015).

Polyphenols play a pivotal role in mediating health effects, with several studies suggesting that these compounds reduce cellular oxidative stress by neutralizing free radicals and stabilizing reactive oxygen species (ROS) (Silva et al., 2022). Research has also highlighted the role of inulin and dietary fibre in promoting gut health, supporting cardiovascular health by reducing cholesterol levels, and aiding in glycaemic control, which is of particular benefit in diabetic diets (Brat et al., 2006;

Cabezas-Serrano et al., 2009; Gagliardi et al., 2020). These beneficial compounds have led to an increased focus on developing fresh-cut and ready-to-eat products, that maintain the bioactive properties of artichokes, which align with consumer preferences for convenient, functional and health-promoting foods (Muratore et al., 2015).

1.3. Quality Preservation of entire artichoke

1.3.1. Challenges in Post-Harvest Handling of fresh entire artichoke

Despite their nutritional profile, artichokes are highly perishable and prone to rapid quality degradation following harvest. After harvest, globe artichoke heads exhibit a high degree of perishability, primarily due to their elevated respiration and transpiration rates. This accelerated metabolic activity predisposes the artichokes to several physiological changes that significantly affect their post-harvest quality. Key issues include weight loss, firmness reduction, and the hardening and opening of bracts. Besides, the degradation process in artichokes is largely driven by the breakdown of chlorophyll, which leads to noticeable colour changes in the bracts, particularly the apical parts. This chlorophyll degradation results in a browning effect, forming brown pigments that detract from the product's freshness and visual quality (Ruíz-Jiménez et al., 2014).

Furthermore, artichokes are notably sensitive to cold-induced damage, which can occur even under controlled storage conditions designed to extend shelf-life. When exposed to temperatures below their optimal storage range, typically between 0 °C and 1 °C, artichokes suffer from cold injury. Symptoms of this damage include increased tissue softening, water-soaking, and further discoloration. Such stress not only accelerates senescence but also predisposes the produce to microbial invasion. Among the most common postharvest fungal pathogens are *Sclerotinia sclerotiorum* (white mold) and *Botrytis cinerea* (grey mold). *Sclerotinia sclerotiorum* is responsible for white mold development, characterized by cottony mycelial growth and water-soaked lesions that can rapidly lead to tissue collapse (Marcucci et al., 2010). *Botrytis cinerea*, on the other hand, causes grey mold, which manifests as

greyish fungal spores on the surface of the bracts and stems, often following minor wounds or chilling injury (Larran et al., 2004).

These alterations not only compromise the visual appeal but also the structural integrity of the artichoke, diminishing its marketability and consumer acceptability (Ruíz-Jiménez et al., 2014).

1.3.2. Pre- and Post-harvest Management Strategies

To mitigate these issues, postharvest management strategies must focus on minimizing respiration and transpiration rates through optimal storage conditions, including temperature control and humidity regulation. To preserve the quality of globe artichoke heads and extend their shelf-life to approximately 3 to 4 weeks, it is imperative to cool the artichokes immediately after harvest. Optimal storage conditions involve maintaining a temperature of 0 to 1 °C with a relative humidity of 90–95% (Gil-Izquierdo et al., 2002). These conditions help mitigate the rapid physiological and metabolic changes that can degrade product quality.

However, during cold storage, significant changes in secondary metabolism occur, impacting the overall quality of the artichoke heads. One critical biochemical response to cold storage is the induction of phenylalanine ammonia-lyase (PAL) activity. This enzyme plays a pivotal role in the biosynthesis of phenolic compounds, particularly chlorogenic acid, which contributes to the antioxidative capacity of the plant tissues (Lattanzio, 2003). Enhanced PAL activity has been linked to an increase in the concentration of phenolics, particularly chlorogenic acid, which has been observed in artichokes stored at 0, 2, 5, and 7 °C (Gil-Izquierdo et al., 2001).

Additionally, further studies have demonstrated that preharvest treatments with compounds such as oxalic acid or methyl jasmonate can significantly influence postharvest quality. These treatments have been shown to enhance the phenolic content at the time of harvest, which correlates with delayed senescence and extended shelf-life. Specifically, artichokes subjected to these treatments maintained better quality traits during storage, including reduced browning and improved firmness and texture (Martínez-Esplá et al., 2017a, 2017b).

Thus, incorporating both optimal storage practices and strategic preharvest treatments forms a comprehensive approach to managing the postharvest period of globe artichokes. This integrated strategy not only reduces the susceptibility to physiological and microbial degradation but also preserves the bioactive properties of the artichokes, thereby ensuring that the nutritional and sensory qualities meet consumer expectations. Continued research and innovation in postharvest technology will be essential to refine these methods and develop new solutions that address the challenges posed by the perishable nature of globe artichokes. This will ultimately enhance their marketability and extend their reach in both local and global markets.

1.4. Quality Preservation of fresh-cut artichokes

If whole artichokes already present significant post-harvest preservation challenges due to their high respiration rate, susceptibility to dehydration, and rapid quality deterioration, these issues are further exacerbated when artichokes are processed into fresh-cut products, as the cutting and trimming processes further disrupt their cellular integrity, accelerating deterioration, complicating their storage and reducing their shelf-life. Besides, artichokes pose considerable challenges in processing due to their morphology. The preparation of fresh-cut artichokes primarily involves obtaining the "heart" of the artichoke, which is the most desirable part of the plant. Obtaining this edible portion generates substantial waste, as approximately 80-85% of the harvested biomass is discarded (Cannas et al., 2024; Corrias et al., 2023; Feiden et al., 2023). These plant waste, which include inedible components such as outer bracts, stems, leaves, and the fuzzy choke (Feiden et al., 2023; Llorach et al., 2002; López-Molina et al., 2005; Zayed and Farag, 2020), present waste management challenges. This issue is further complicated by the fact that, while larger, globe-type artichokes produce less waste due to their dense, meatier hearts, smaller or fibrous varieties tend to have higher discard rates.

Consequently, the development of processed artichokes as fresh-cut products offers a dual benefit. Firstly, it enhances consumer convenience by minimizing the waste typically generated during home preparation, which could, in turn, boost overall consumption. This is particularly relevant given the high proportion of plant

material discarded due to the complexity of trimming and preparation. Secondly, the residual by-products from processing present valuable opportunities for utilization within the food industry, contributing to a more sustainable and efficient use of resources.

1.4.1. Challenges in Post-Harvest Handling of Fresh-Cut Processed Artichokes

Despite their nutritional appeal, fresh-cut artichokes present significant post-harvest management challenges as these vegetables are highly perishable and susceptible to quality degradation (Cabezas-Serrano et al., 2009; Cefola et al., 2012).

Enzymatic browning is a major quality concern that is primarily catalysed by polyphenol oxidase (PPO) enzyme, resulting from the exposure of polyphenols to oxygen when plant tissues are damaged during processing and storage. This oxidation of phenolic compounds yields to the formation of brown pigments, called quinones, which affect the visual and sensory quality of the product (Amodio et al., 2020a; Cefola et al., 2012; Constabel and Barbehenn, 2008).

The resulting discoloration and browning diminish market appeal and reduces the perceived freshness and nutritional value, factors that are particularly critical for consumers preferences seeking minimally processed foods (Del Nobile et al., 2009; Pandino et al., 2017a).

In addition to browning, artichokes' high moisture content makes them susceptible to microbial spoilage, which is exacerbated by the physical disruption of tissues during processing and intensify the decline in quality (Restuccia et al., 2014). The combination of enzymatic browning and microbial spoilage compounds the difficulty in maintaining visual and microbiological quality during storage (Licciardello et al., 2017). Furthermore, cutting operations further compromise the structural integrity of artichoke tissues, leading to moisture loss, texture degradation, and accelerated decline in sensory attributes like taste and colour (Cabezas-Serrano et al., 2009; Cefola et al., 2012; Tortosa-Díaz et al., 2025). These factors collectively limit the shelf-life of fresh-cut artichokes, challenging their quality preservation over extended storage periods.

1.4.2. Post-Harvest Innovations for Fresh-Cut Artichokes

To address these issues and cope with the growing demand for convenient, ready-to-eat products, substantial research has focused on developing innovative post-harvest strategies and techniques aimed at extending the shelf-life of fresh-cut artichokes while preserving their nutritional attributes, bioactive content and sensory - organoleptic properties.

Techniques such as controlled atmosphere (CA) and modified atmosphere packaging (MAP) with low storage temperatures have shown efficacy in reducing oxidative processes and maintaining moisture levels, effectively delaying quality deterioration (Amodio et al., 2020a; Capotorto et al., 2020; García-Martínez et al., 2017; la Zazzera et al., 2012). In conjunction with MAP, the use of anti-browning agents, edible coatings, and innovative packaging systems has also been explored to mitigate enzymatic browning and extend shelf-life (El-Mogy et al., 2020; Ghidelli et al., 2013; Gómez di Marco et al., 2012; la Zazzera et al., 2012; Muratore et al., 2015). Several studies have demonstrated the potential of L-cysteine, ascorbic acid, and other chemical treatments to inhibit PPO activity (Amodio et al., 2011; Cabezas-Serrano et al., 2009; Calabrese et al., 2012; Del Nobile et al., 2009; Ghidelli et al., 2013; Ruíz-Jiménez et al., 2014).

Edible coatings infused with essential oils (EOs) and natural antioxidants represent another promising approach, offering antimicrobial and moisture-retentive properties that can enhance shelf-life (Alvarez et al., 2022; Bakkali et al., 2008; Burt, 2004; Cháfer et al., 2012; Fisher and Phillips, 2008; Pérez-Alfonso et al., 2012; Yan et al., 2020). EOs components such as eugenol, thymol, and carvacrol have shown synergistic effects in delaying microbial spoilage and extending the storage life of fresh-cut products (Amodio et al., 2020a; Nikkhah et al., 2017; Regnier et al., 2014; Rizzo and Muratore, 2020). However, the use of such coatings in artichokes remains mainly underexplored, with most studies focusing on the impact on sensorial attributes without fully examining the effect on other key quality parameters like weight loss and respiration rate.

1.5. Influence of Genetic and Environmental Factors on Post-Harvest Quality

The effectiveness of post-harvest treatments is intricately tied to genetic, agronomic and environmental factors, among others, that influence artichoke quality at the time of harvest. (Ceccarelli et al., 2010; Giménez et al., 2021; Lattanzio et al., 2009; Palma et al., 2023; Pandino et al., 2012; Spanu et al., 2018). All these factors may influence the phenolic content, a key factor in browning and aptness for fresh-cut processing artichokes.

On the one hand, various studies have shown that genotypic diversity among artichoke cultivars affects phenolic content, enzyme activity, and susceptibility to browning, with certain cultivars displaying inherent traits that make them more suitable for minimal processing (Cabezas-Serrano et al., 2009; Cefola et al., 2012; Licciardello et al., 2017; Lombardo et al., 2015b; Pandino et al., 2017a; Todaro et al., 2010).

Additionally, flower head order, a unique aspect of artichoke morphology, categorized as primary, secondary, and tertiary heads, play a significant role in determining the biochemical composition, particularly the phenolic content critical to post-harvest quality (Giménez et al., 2021). In this sense, the research of Giménez et al. (2021) was the first one to evaluate this parameter serving as a foundational study and a preliminary exploration for this thesis, providing essential insights into the complex interactions between genotype, head order, and pre-harvest treatments that influence the quality and bioactive profile of artichokes. In that study, eight distinct cultivars of globe artichokes with different propagated methods (2 vegetatively propagated cultivars, 2 seed-propagated open-pollinated cultivars and 4 seed-propagated hybrids) were analysed to assess how flower head order and GA_3 treatment influenced the levels of two key groups of bioactive compounds: hydroxycinnamic acids and luteolin derivatives. These phenolic compounds are recognized for their potent antioxidant properties, which are associated with numerous health benefits. The research highlighted significant variations in phenolic content depending on the position of the flower head on the plant. Tertiary heads consistently contained the highest levels of phenolic compounds, including

chlorogenic acid, followed by secondary and main heads. This hierarchical distribution underscored the impact of flower head order on the biochemical composition and nutritional value of artichokes, suggesting that selectively using specific head orders could enhance both their functional quality and shelf-life in fresh and processed forms. Additionally, the study demonstrated that GA_3 treatment played a distinct role in modulating phenolic composition; while it reduced hydroxycinnamic acid levels, it increased luteolin derivatives. This effect was found to be cultivar-dependent, emphasizing the importance of careful genotype selection and post-harvest treatment strategies to optimize the health-promoting properties of artichokes (Giménez et al., 2021). The findings directly informed the broader and more in-depth analyses conducted in the present thesis, setting a groundwork for developing targeted approaches to enhance artichoke quality for fresh consumption and value-added product applications.

On the other hand, environmental conditions, such as temperature and sunlight exposure, also significantly influence the biochemical composition and the phenolic profile. Seasonal fluctuations and agronomic practices, including harvest timing and fertilization, further impact the biosynthesis of antioxidant compounds, which are essential for mitigating oxidative stress (Ceccarelli et al., 2010; Giménez et al., 2021; Lattanzio et al., 2009; Palma et al., 2023; Pandino et al., 2012; Petropoulos et al., 2018b; Spanu et al., 2018).

1.6. Impact of frost injury in artichokes

In this respect, frost stress emerges as a crucial factor affecting artichoke crops, particularly given the seasonal production cycles in regions like Spain, where cold temperatures and frost events can occur during the early winter harvest. Within these regions, the typical harvesting period for artichokes extends from October to May, with the dual harvest cycle of cultivars like 'Blanca de Tudela' allowing the harvest in the coldest months, January and February. This early harvest period, however, poses a heightened risk of frost injury, also named frostbite, which can significantly affect both crop yield and quality.

Frost injury in plants results from an accumulation of ROS within cells, causing oxidative stress and cellular damage (Janda et al., 2003). The degree of frost injury varies depending on cultivar sensitivity, developmental stage, and specific frost conditions, including intensity, duration, and frequency (Dalezios, 2017). In artichokes, initial frost damage can begin at temperatures as low as −1.2 °C, which may cause symptoms like cuticle blistering, bronzing, and detachment of outer bracts (Figure 3). As temperatures drop further, below −4 °C, the damage becomes more severe, leading to water-soaked bracts, necrosis, and the formation of brown or black spots (Amodio et al., 2020b).

Figure 3. Artichoke frost injury damage.

The economic impact of frost injury is well-documented across various crops, where quality and yield are both negatively affected (Alharbi et al., 2021; Fuller et al., 2007; Mougiou et al., 2020; Neugart et al., 2012; Oh et al., 2009; Weil et al., 2019). However, frost injury in artichokes has not yet been thoroughly studied, despite its relevance for regions with winter production cycles.

In artichokes, secondary metabolites such as phenolic compounds, which play a key role in the regulation of development and tolerance mechanisms against biotic and abiotic stresses (Kumar et al., 2023), such as the plant's defence against environmental stressors, may be influenced by frost exposure. Frost stress below 0

°C stimulates the biosynthesis of antioxidant compounds that work to mitigate ROS damage, thus preserving cell integrity and limiting oxidative damage within tissues (Toscano et al., 2019). The antioxidant defence mechanisms in plants include both enzymatic components (e.g., catalase, ascorbate peroxidase, superoxide dismutase) and non-enzymatic molecules (e.g., phenolic compounds, ascorbic acid, and flavonoids) (Das and Roychoudhury, 2014). In this context, phenolics and flavonoids are essential due to their ability to act as electron donors, thereby countering ROS accumulation (Bendary et al., 2013; Noreen et al., 2017; Shrestha and Dhillion, 2006; Turkiewicz et al., 2019). Moreover, certain phenolic compounds, including hydroxycinnamic acids and luteolin derivatives, can stimulate the production of endogenous antioxidants within cells, providing a robust defence against frost-induced oxidative stress (Côté et al., 2010).

Numerous studies emphasize the role of phenolic compounds in neutralizing free radicals, breaking down peroxides, deactivating metal ions, scavenging oxygen in biological systems, and thus preventing oxidative damage (Aryal et al., 2019; Babbar et al., 2015). These bioactive compounds may play a fundamental role in protecting plants against environmental stressors, underscoring the importance of understanding and leveraging their natural antioxidative properties in the context of artichoke cultivation and post-harvest management.

2. Cardoon (*Cynara cardunculus* L. var. *altilis*)

2.1. Cultural and Historical Significance of Cardoon Cultivation in the Mediterranean Basin

While less commonly consumed as a vegetable, cardoon (*C. cardunculus* var. *altilis*) holds significant economic and ecological value. Cardoon is closely related to the globe artichoke but differs primarily in its growth form and utilization. They are typically larger than artichokes and are cultivated for their robust stalks, which, when blanched, are edible.

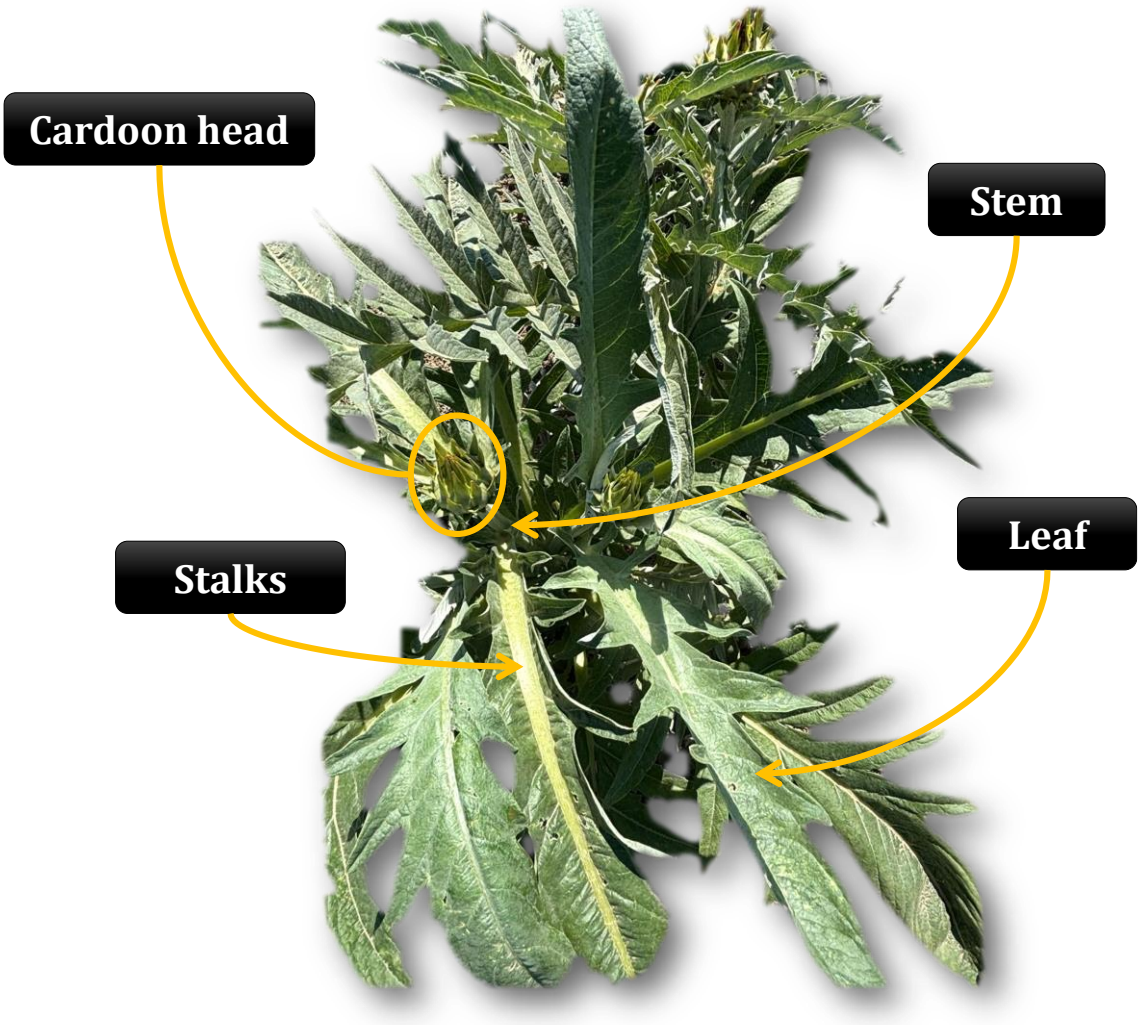

Figure 4. Cardoon plant parts.

The cultivation of both cultivated and wild cardoon is primarily concentrated in Southern European countries, notably Italy, Spain, Portugal, Greece, and France (Ierna and Mauromicale, 2010; Mandim et al., 2023). These regions benefit from a Mediterranean climate, characterized by hot, dry summers and mild, humid winters, that is highly conducive to the growth of cardoon, a crop particularly well-adapted to such challenging environmental conditions. Notably, cardoon's robust tolerance to various abiotic stresses makes it an especially valuable crop in these climates, where resilience to extreme temperatures and drought is vital. Beyond its adaptability, the cultivation of cardoon has shown positive effects on soil health. Studies reveal that it contributes to the soil fertility-enhancing properties, including weed suppression, promoting the growth of beneficial soil bacteria and nutrient enhancement, such as organic matter, exchangeable potassium, total nitrogen, organic carbon, and assimilable phosphorus (La Iacona et al., 2024; Mandim et al., 2023; Mauromicale et al., 2014; Scavo et al., 2019). Additionally, cardoon cultivation offers potential benefits for soil conservation, as it reduces erosion and can serve as a cover crop in areas susceptible to land degradation (Rossi et al., 2022). This dual role as both a resilient crop and a soil-enhancer underpins cardoon's growing importance within sustainable agriculture frameworks in Mediterranean countries.

In addition, cardoon has a rich historical lineage, with evidence of its cultural and medicinal significance dating back to ancient civilizations, such as the Egyptians, Greeks, and Romans (Sałata et al., 2024, 2023). Throughout the Mediterranean region, cardoon has been deeply integrated into culinary traditions, often featured in traditional recipes, where its immature inflorescences are consumed fresh or preserved by canning or freezing (Pandino and Mauromicale, 2020). Beyond its culinary use, cardoon has long been employed in traditional medicine, where it is prized for its purported benefits in treating liver diseases, diabetes and cardiovascular diseases, and for acting as a choleretic and antihemorrhoidal agent (Ben Salem et al., 2017; Gostin and Waisundara, 2019; Mandim et al., 2022b). In recent years, scientific investigations have substantiated many of these traditional claims by revealing that cardoon is a rich source of bioactive compounds, including dietary fibers, minerals, inulin, and antioxidant phenolic compounds (Ben Amira et al., 2018; Brás et al., 2015; Mandim et al., 2022a; Petropoulos et al., 2018c). These

findings position cardoon not only as a functional food but also as a potential source of health-promoting phytochemicals.

2.2. Economic and Industrial Applications of Cardoon

Cardoon has garnered substantial economic interest due to its diverse applications across multiple industries. In Mediterranean cuisine, it serves as a staple ingredient in various soups and salads, capitalizing on its nutrient-rich profile and functional properties (Conceição et al., 2018; Elsebai et al., 2016; Gominho et al., 2018; Ierna et al., 2020; Khaldi et al., 2021). An especially notable use of cardoon flowers is as a natural rennet in goat and sheep cheese production. This imparts unique flavours and textures to PDO cheeses, including those from Portugal, Spain, and Italy (Almeida and Simões, 2018; Conceição et al., 2018; Gominho et al., 2018). The agricultural by-products from cardoon cultivation, such as green fodder and residues from oil extraction, also present significant nutritional value as animal feed, contributing to a sustainable agriculture that does not interfere with plant growth cycles (Barbosa et al., 2020; Barracosa et al., 2019; Petropoulos et al., 2018c; Zumbo et al., 2022). These by-products are especially promising for livestock feed due to their high protein and energy content, as well as the potential health benefits derived from phenolic compounds within the plant (Cabiddu et al., 2019).

Beyond food and feed applications, cardoon is emerging as a renewable energy source due to its high biomass yield and relatively low cultivation costs. Studies on bioethanol, biomethane, and biodiesel production from cardoon demonstrate its potential to contribute to sustainable bioenergy production, thereby supporting circular economy objectives and reducing greenhouse gas emissions (De Menna et al., 2016; Gominho et al., 2018; Ierna et al., 2020; Mauromicale et al., 2019; Mehmood et al., 2017; Pesce et al., 2017). Moreover, cardoon's high cellulose content renders it suitable for the pulp and paper industry, offering an alternative to traditional woody plants for paper manufacturing. Various delignification techniques have been explored to optimize cellulose extraction, positioning cardoon as a potentially sustainable resource for the pulp and paper sector (Abrantes et al., 2007; Gominho et al., 2018; Lourenço et al., 2017; Vergara et al., 2018). The

cosmetics industry has also taken interest in cardoon for its antioxidant, anti-inflammatory, and UV-protective properties, employing its extracts in body oils and creams (Barbosa et al., 2020; Conceição et al., 2018; Yahya et al., 2018). Furthermore, the ornamental appeal of cardoon, coupled with a growing consumer demand for novel plant varieties, has led to its cultivation as both a garden plant and a source of cut flowers (Lanteri et al., 2012). These diverse applications underscore cardoon's status as a multifunctional crop, meeting consumer demands across culinary, cosmetic, ornamental, and industrial markets.

2.2.1. Specialized Industrial Uses of Cardoon Seeds

Cardoon seeds hold specific industrial applications, most notably in oil production, where they serve as a source of oil for human consumption and as feedstock for biodiesel production (Barbanera et al., 2021; Bartocci et al., 2016; De Domenico et al., 2016; Fernández et al., 2006; Khaldi et al., 2021). The solid by-products from oil extraction, also known as cardoon oil cakes, are particularly valuable as animal feed due to their high protein, fiber, and energy content, along with their digestibility (Barbosa et al., 2020; Cabiddu et al., 2019; Gominho et al., 2018; Zumbo et al., 2022). Emerging applications for these oil cakes include their use in the production of biodegradable and edible plastics, contributing to eco-friendly technological developments that align with the circular economy objectives (Barbosa et al., 2020; Gominho et al., 2018; Mirpoor et al., 2021). Additionally, recent studies have highlighted the pharmacological and nutraceutical properties of cardoon seeds, which offer further economic value to this versatile crop (Silva et al., 2022; Zayed et al., 2020). All these possible uses contribute to the economic value of cardoon crops, making them a multifunctional plant with diverse industrial applications.

2.3. Nutritional Importance of Mineral Content in Cardoon Seeds

Cardoon seeds are of significant nutritional interest, particularly due to their mineral content, which is essential for both human and livestock health (Cajarville

et al., 2000; Petropoulos et al., 2019; Silva et al., 2022). In humans, mineral elements are fundamental for various biochemical functions that support overall health and well-being. They play critical roles in the regulation of blood pressure, contribute to cardiovascular health, aid in structural support for bones and teeth, and play regulatory roles in metabolism, immune defence, and more (Gonçalves et al., 2022). Ensuring sufficient intake of these essential minerals, either through diet or supplementation, is vital for preventing deficiency-related diseases and maintaining optimal physiological function (Silva et al., 2022).

From a farming perspective, the role of minerals is equally decisive in animal and livestock health, especially given the increasing global demand for animal-source foods driven by population growth and rising consumption in developing countries (Delgado et al., 2001). Over the past few decades, livestock production systems have transitioned from traditional, small-scale operations to intensive, large-scale, commercially focused systems. These specialized units rely on highly controlled nutrition regimens, which are often concentrated feeds sourced from international suppliers (López-Alonso, 2012). Consequently, adequate mineral supplementation in concentrated feed has become essential, not only in intensive systems where it is often the sole feed source but also as a complement in traditional farming practices.

Minerals are necessary for nearly all biochemical processes in animal organisms, serving as structural and regulatory components within numerous enzymatic systems. Optimal nutrition with adequate intake of these elements is integral to sustaining vital physiological, catalytic, and regulatory functions essential to maintaining animal health and productivity (Suttle, 2010).

2.4. Factors Affecting Nutrient Concentration and Bioavailability in Cardoon

The nutritional composition of cardoon, particularly its mineral content, is influenced directly or indirectly by various environmental and genetic factors (Lombardo et al., 2017). Research has shown that soil composition, climate conditions, genotype, and developmental stages play a crucial role in determining

the mineral profile of cardoon seeds. Understanding such factors is fundamental for optimizing cardoon's value as a nutritionally food source, as significant variations are observed across different genotypes and growing environments (Borah et al., 2009; Kopsell et al., 2004; Mandim et al., 2022a; Pandino et al., 2011a; Petropoulos et al., 2019; Raigón et al., 2008).

2.5. Current Research on Cardoon Seed Composition and Unexplored Areas

Previous investigations into cardoon seeds have primarily focused on their chemical composition, bioactive properties, and potential applications. Mandim et al. (2022a) conducted detailed analyses of a commercial cultivated cardoon (CC) genotype, examining chemical profiles and biological activities across four maturity stages, while in an earlier study by the same group (Mandim et al., 2020) another commercial CC genotype was used to assess its chemical composition and in vitro bioactivities. Petropoulos et al. (2018a, 2018c) made significant contributions to the field through multiple studies, which expanded on chemical analysis and bioactivity evaluations. Specifically, they analysed the chemical composition of a commercial CC genotype and assessed the effects of various extraction methods. Further research included both wild and commercial genotypes, examining differences in bioactivities, chemical profiles, and nutritional values, highlighting the versatility of cardoon seeds in various contexts (Petropoulos et al., 2019). In a parallel study, Nemska et al. (2019) also explored a commercial CC genotype, focusing on antioxidant capacity and chemical composition, while Piluzza et al. (2020) examined phenolic compounds and antioxidant levels of seeds derived from different flower head orders. Additionally, several reviews have documented the broader uses and applications of cardoon and cardoon seeds as a valuable herbaceous species with extensive uses, reinforcing cardoon's wide-ranging industrial potential (Barbosa et al., 2020; De Domenico et al., 2016; Fernández et al., 2006; Gominho et al., 2018; Mauromicale et al., 2019, 2014; Mirpoor et al., 2021; Pandino and Mauromicale, 2020; Pesce and Mauromicale, 2019; Silva et al., 2022; Zayed et al., 2020; Zumbo et al., 2022).

Despite these advances, a notable research gap exists regarding the mineral composition of cardoon seeds, an area with scarce studies. Although a few investigations have examined mineral profile (Nemska et al., 2019; Petropoulos et al., 2019, 2018c), these studies are limited in scope, examining only isolated genotypes and single harvest periods, which does not account for the potential impact of environmental factors and genotype-specific characteristics over time.

To address these gaps, there is a clear need for research that examines the influence of genotype and environmental conditions over multiple growing seasons on the mineral composition and ash content of cardoon seeds. Understanding these variations would provide crucial insights into the stability and variability of nutrient content, informing the nutritional potential of different cardoon genotypes. This could maximize cardoon's potential in agricultural and commercial applications, enhancing their economic value and supporting more targeted breeding and cultivation practices tailored to specific nutritional goals.

OBJECTIVES

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OBJECTIVES OF THE THESIS

This thesis, titled "Characterization of Commercial Varieties of Artichoke, Cardoon, and Their Wild Relatives for Productive and Commercial Optimization", aims to address the research gap in artichoke and cardoon studies, two crops with high economic and agricultural relevance in Mediterranean countries like Spain, Italy, and Egypt. Despite their significant role as valuable sources of bioactive compounds and functional foods, artichokes and cardoons remain under-researched relative to other crops. A search on the Web of Science (WOS, 2025) shows considerably fewer studies on artichokes (3.625) and cardoons (1.372) compared to more widely researched vegetables, such as tomatoes (153.633). This discrepancy highlights a gap in understanding these crops' genetic, nutritional, and functional properties, even though both are increasingly recognized for their health-promoting benefits, high polyphenol content, and suitability as antioxidant-rich, functional foods.

Driven by the growing consumer demand for functional foods that offer both convenience and health benefits, this thesis hypothesizes that artichoke and cardoon varieties with high phenolic content are likely to exhibit superior qualities for fresh-market commercialization. Conversely, varieties with lower phenolic content could be better suited for fresh-cut products, as reduced browning susceptibility and enhanced shelf-life are key attributes for minimally processed formats.

1. General Objective

The overarching objective of this research plan is to enhance the competitiveness of artichokes across all phases of the production chain, from the commercialization of seeds or planting materials, agricultural production, industrial processing, and ultimately, to the market stage. This research seeks to provide valuable insights for genetic breeders, growers, and processors alike, spotlighting a vegetable that, though minor in cultivation volume, holds significant importance for the agricultural economies of the Valencian Community and Murcia. By comprehensively characterizing different artichoke and cardoon cultivars, this study aims to support industry and agricultural sectors in maximizing the productive and commercial potential of these crops.

2. Specific Objectives

I. To evaluate the efficacy of L-cysteine combined with essential oils (eugenol, thymol, and carvacrol) in reducing enzymatic browning, while preserving the bioactive properties and quality attributes of fresh-cut 'Blanca de Tudela' artichokes stored under refrigeration for nine days.

It is hypothesized that this combination could significantly reduce browning and contribute to better preservation of quality and functional properties compared to untreated controls.

II. To compare the aptitude of multiple artichoke cultivars ('Lorca,' 'Tupac,' and 'Green Queen') for fresh-cut processing, focusing on the influence of flower head order on phenolic content, browning evolution, and quality maintenance during storage.

The working hypothesis is that 'Lorca' and 'Tupac', particularly in primary heads, could perform better than 'Green Queen', as they might exhibit lower phenolic content and reduced enzymatic browning.

III. To assess the phenolic profile and browning susceptibility in different head orders and developmental stages of the 'Lorca' cultivar, with the aim of defining its suitability for fresh-cut market formats.

It is expected that artichokes at more advanced developmental stages and from primary heads may be more suitable for minimally processed presentations due to their lower phenolic content and, therefore, a potentially lower susceptibility to browning.

IV. To characterize the influence of flower head order on the phenolic content, antioxidant activity, and overall quality of 'Blanca de Tudela' artichokes at harvest and during cold storage, determining the best-suited head orders for fresh market sale versus industrial processing.

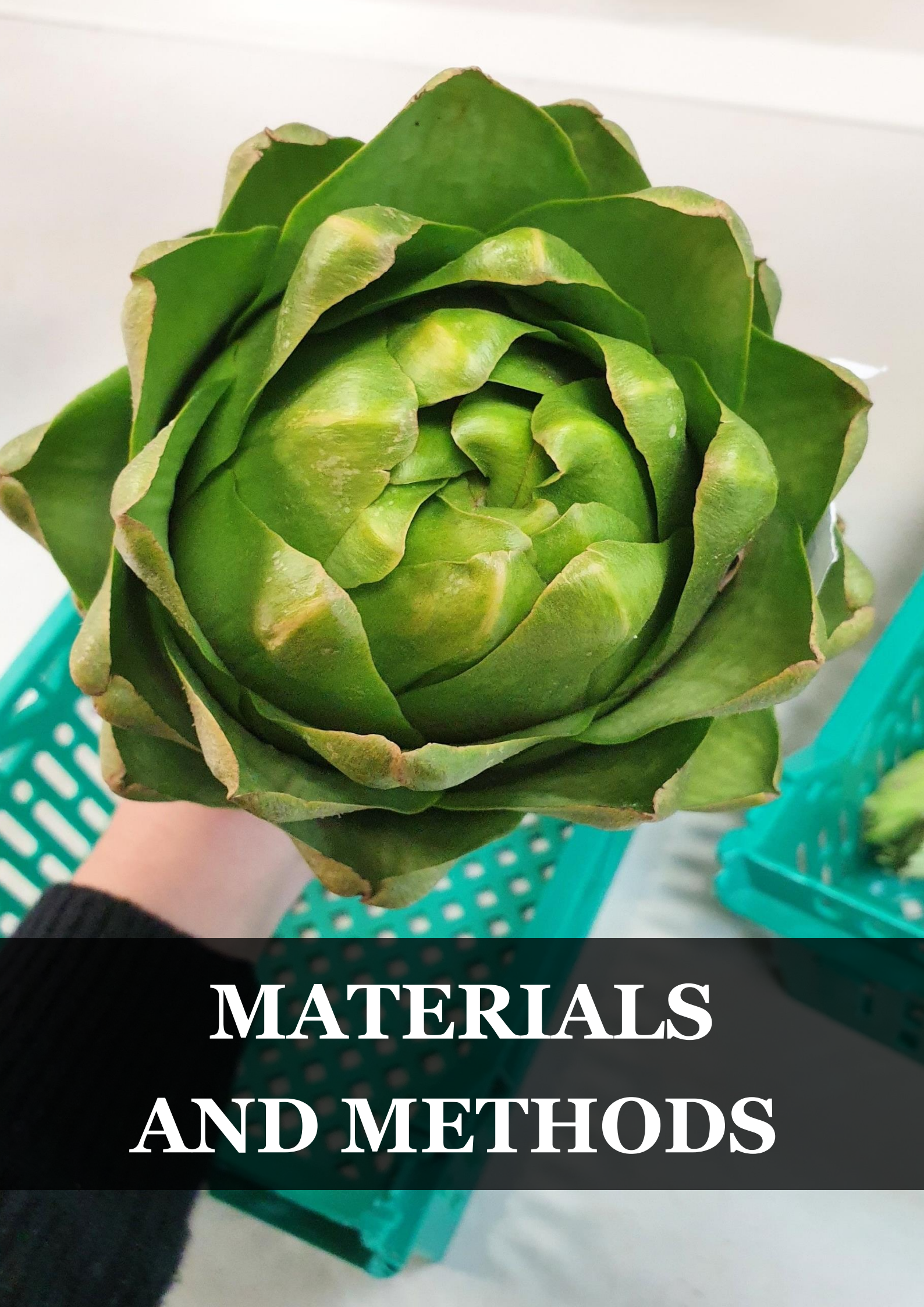
It is hypothesized that tertiary heads could present higher phenolic content and antioxidant activity, thus potentially offering better postharvest quality and being more appropriate for fresh consumption, while primary heads might be more suitable for industrial use.

V. To investigate the relationship between antioxidant activity and frost injury resistance in 'Blanca de Tudela' artichokes, analysing how total phenolic content may enhance tolerance to low temperatures and contribute to post-harvest resilience.

It is expected that heads with higher phenolic content and antioxidant capacity could display greater resistance to frost damage, suggesting a protective role of these compounds.

VI. To analyse the mineral profile and ash content in thirteen different genotypes of cultivated and wild cardoon seeds over three consecutive growing seasons.

The hypothesis is that identifying genotypic and seasonal variations may contribute to improved nutritional consistency and enhance their suitability for use in food, feed, and bio-industrial applications.



MATERIALS AND METHODS

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MATERIALS AND METHODS

This doctoral thesis includes a total of six in-depth comprehensive studies, with a primary focus on artichokes (*Cynara scolymus*) and cardoon (*Cynara cardunculus*), plants of significant agronomic and nutritional interest within Mediterranean agriculture.

Five studies concentrate on various aspects of artichoke production, quality maintenance and postharvest characteristics. These include two investigations focused on fresh-cut processing and preservation techniques, examining approaches to optimize shelf-life and maintain quality attributes under minimally processed conditions. The remaining three artichoke-focused studies target whole artichokes, addressing physiological, biochemical, and agronomic factors that influence their suitability for both fresh market and industrial applications.

The sixth study, cantered on cardoon, represents the initial research in a compendium of three future publications emerging from a predoctoral research stay of 5 months at the University of Catania, Italy, made possible through collaborative efforts between institutions. This cardoon-focused study pioneers an in-depth assessment of cardoon seed properties, exploring their biochemical composition, in particular the mineral profile, and potential applications in food and industrial sectors.

Each experiment in this thesis adheres to a systematic experimental design, tailored to address specific research questions and ensure comprehensive data collection. For this reason, initially, the study site and growing conditions, common to all articles, will first be described for both artichoke and cardoon, followed by a description of the plant materials used in each study. Subsequently, a brief explanation of the specific experimental designs will be presented to provide context on the conditions of cultivation, selection and post-harvest management. This section will outline the distinct setups employed across the six studies, reflecting their unique research objectives and methodological needs. Finally, a consolidated description of the common analytical techniques and assessment methods applied across the studies will be provided.

This approach allows for a clearer understanding of shared methodologies, highlighting their consistent application and relevance to the research goals. These studies, by evaluating critical parameters such as phenolic content, antioxidant capacity, browning susceptibility and storage viability, among others, aim to characterize and enhance the commercial potential of these Mediterranean crops, both as fresh products and as processed goods, in response to consumer demand for functional and nutritious food products.

In addition, for a better perception of the experimental framework, a graphical overview of the experimental designs and methodologies applied across the studies included in this thesis is provided, illustrating the progression between these research efforts. The experiments are categorized into three main groups: fresh-cut artichokes (Figure 5), entire artichokes (Figure 6), and cardoon (Figure 7), reflecting the distinct research approaches within each category.

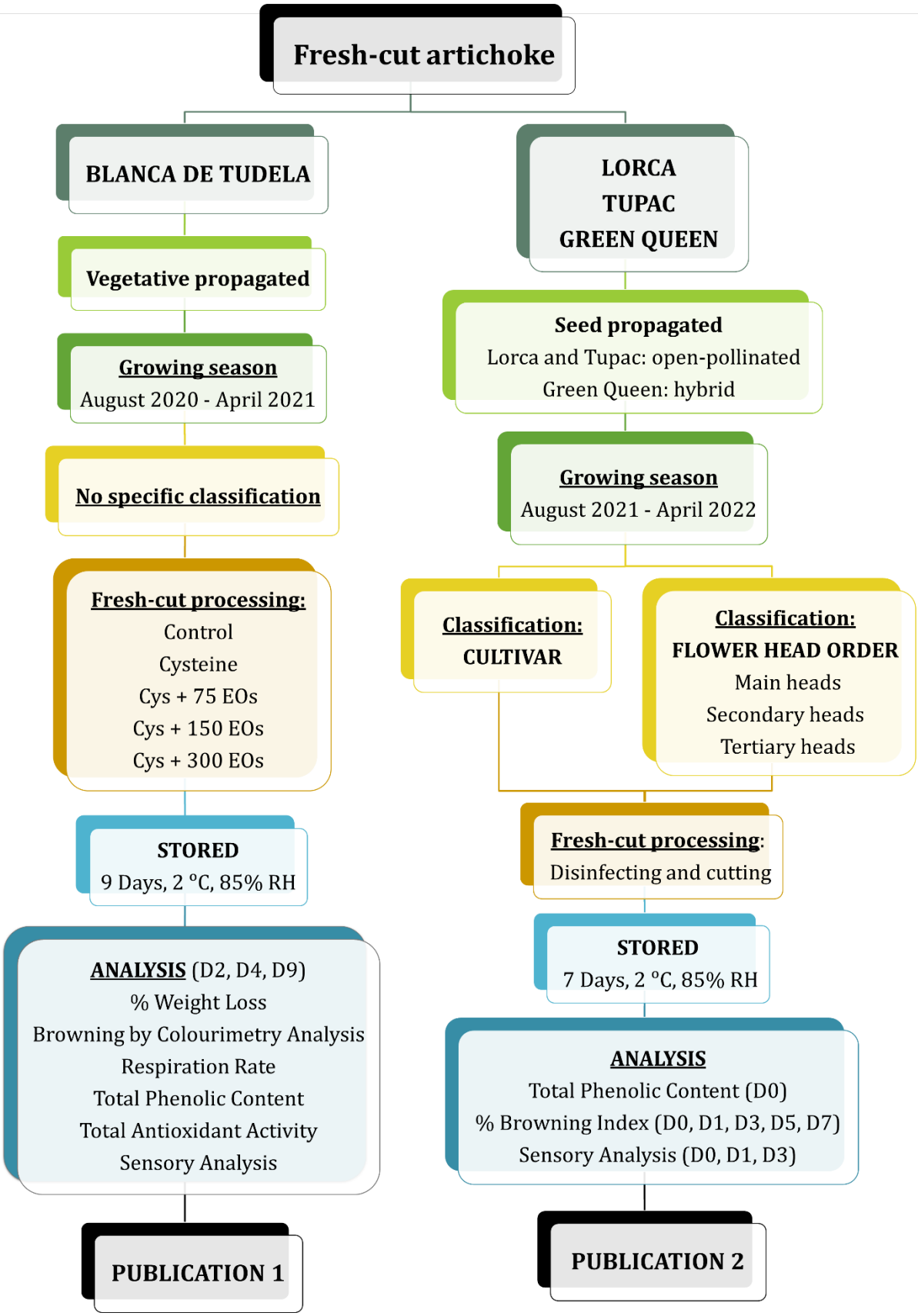

Figure 5: Experimental design scheme of the different research on fresh-cut artichoke included in this PhD Thesis.

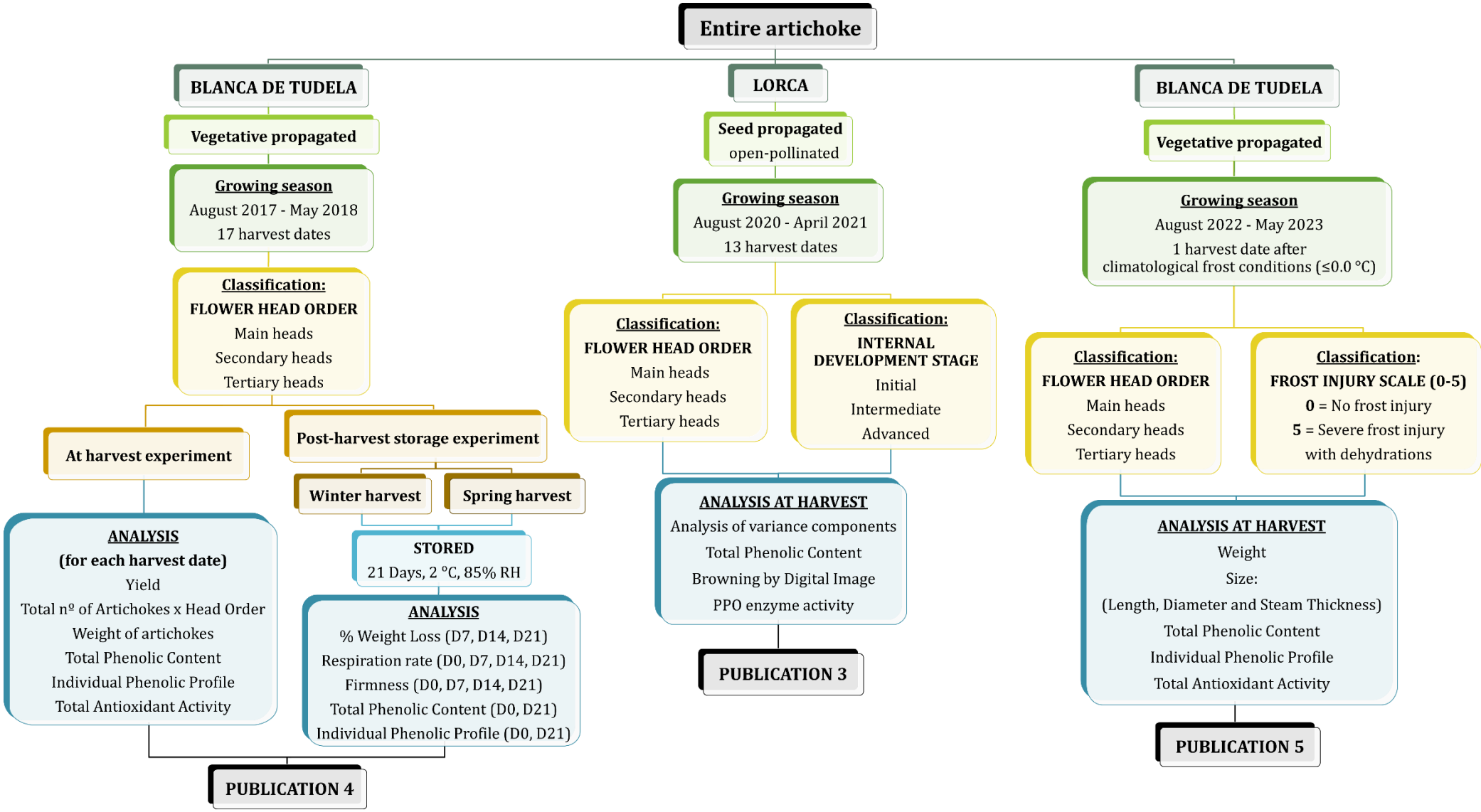

Figure 6: Experimental design scheme of the different research on entire artichoke included in this PhD Thesis.

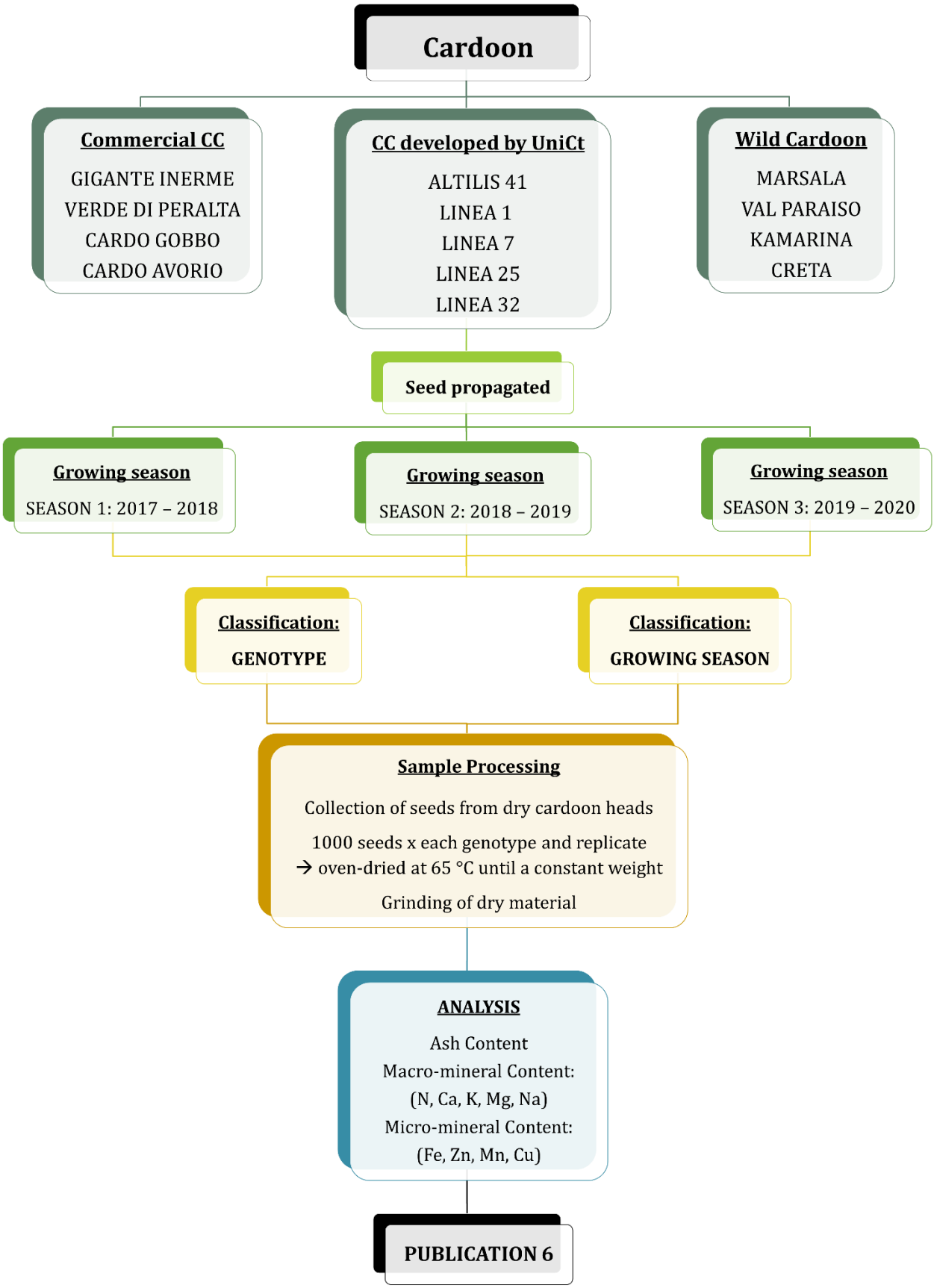

Figure 7. Experimental design scheme of the different research on cardoon included in this PhD Thesis.

1. Artichoke: Study Site and Growing Conditions

The experimental work with artichokes was conducted on an experimental plot at Miguel Hernández University in Orihuela, Southeast Spain (38°06′63.52″ N, −0°988′09.29″ W), with the exception of the experiment detailed in Publication 1, which took place on a commercial plot also located in Orihuela. The region is characterized by a Mediterranean climate, defined by hot, dry summers and mild, humid winters.

Figure 8. Artichoke experimental field.

Artichokes were planted during the month of August of each growing season, following a planting frame of 0.8 m apart within a row and 1.2 m apart between nearby rows. Standard agronomic practices commonly used by local growers in Southeast Spain were followed for crop management. Prior to transplanting, a base application of sheep manure was incorporated into the soil at a rate of 2.5 kg/m^2. Insecticide and fungicide applications were implemented throughout the crop cycle as required, while fertilization was provided via drip irrigation with a nutrient profile of 250 kg N, 120 kg P_2O_5, and 300 kg K_2O per hectare. GA_3 was omitted from the growth regimen.

All artichoke heads were harvested according to the commercial standards, when flower heads were firm and tightly closed and reached their average commercial size and morphology suitable for commercial distribution. Each artichoke was collected along with a 15–20 cm of the floral stem.

2. Plan Material: Artichoke Cultivars

The plant material used in this doctoral thesis consisted of four commercial artichoke (*Cynara cardunculus* var. *scolymus*) cultivars: 'Blanca de Tudela', 'Lorca', 'Tupac', and 'Green Queen'. These cultivars were chosen based on their agronomic performance and commercial relevance. The selection of these cultivars aimed to evaluate their physiological, biochemical, and technological properties, providing insights into their suitability for fresh consumption, fresh-cut processing, and other industrial applications.

2.1. Blanca de Tudela

This is the most widely cultivated artichoke cultivar in Spain, particularly in the Vega Baja del Segura region (Alicante). It is characterized by its compact, medium-sized, round-shaped flower heads with tightly closed bracts. A distinctive feature of 'Blanca de Tudela' is the central cavity (hollow centre) inside the flower head, which differentiates it from other cultivars (Figure 9). It is highly valued in the fresh market due to its distinctive sensory characteristics.

Figure 9. 'Blanca de Tudela' characteristic hollow centre.

Unlike modern cultivars, 'Blanca de Tudela' is traditionally propagated vegetatively, meaning it is reproduced using basal and lateral offshoots. While this method ensures genetic uniformity, it also entails several drawbacks, such as heightened risk of pathogen transmission, physiological variability among plant material, low multiplication efficiency, high planting costs, and a high percentage of planting failures. This cultivar is known for its reflowering ability, allowing for two production periods: the first coinciding with the autumn-winter season (early production) and a subsequent one in spring (late production). Despite being characterized as an early cultivar, the limitations of vegetative propagation have driven the development of seed-propagated cultivars in recent years.

Figure 10. 'Blanca de Tudela' artichoke cultivar.

2.2. Lorca and Tupac

Both of them are open-pollinated, seed-propagated cultivars, meaning they are grown from seeds obtained through natural pollination. This type of propagation has been developed to address the challenges associated with vegetative propagation, offering benefits such as increased uniformity, high productivity, disease resistance, and greater flexibility in planting schedules. However, a key drawback of seed-propagated cultivars is their delayed entry into production, as they require a prolonged juvenile phase before reaching maturity.

- **'Lorca'** is a green artichoke cultivar characterized by a medium-vigour, highly uniform plant. It produces medium round to elongated flower heads, compared to 'Blanca de Tudela', with tightly packed bracts. The outer bracts may exhibit a slight anthocyanin pigmentation, which becomes more pronounced under low temperatures. This cultivar is valued for its exceptional flavour and is suitable for both fresh consumption and processing. Additionally, Lorca's adaptability allows for transplanting at different times of the year, making it a versatile choice for growers.

Figure 11. 'Lorca' artichoke cultivar.

- **'Tupac'** is characterized by a medium-vigour plant, well-suited for both fresh markets and the canning industry. One of its key advantages is its low vernalization requirement, allowing it to produce artichokes even with minimal exposure to cold temperatures. The flower heads of 'Tupac' have a distinctly conical shape, with a broad base and a narrow apex. They are compact and densely covered with numerous fleshy bracts, contributing to a high specific weight and excellent yield for the processing industry. The adaptability of this cultivar enables production over an extended period and across diverse growing regions, provided proper crop planning is implemented.

Figure 12. 'Tupac' artichoke cultivar.

The ability to cultivate artichokes from seeds has allowed for high yields even in regions with short growing seasons, making these cultivars valuable alternatives to traditionally propagated varieties.

2.3. Green Queen

This cultivar is a hybrid seed-propagated variety and the largest among the four studied, producing significantly bigger flower heads than 'Blanca de Tudela', 'Lorca', and 'Tupac'. Hybrid artichokes are developed through controlled crossbreeding between two genetically distinct parent lines to obtain superior agronomic traits such as higher yield, uniformity, and improved adaptation to environmental conditions. Unlike open-pollinated cultivars, hybrid artichokes are typically sterile and do not produce viable seeds, meaning they cannot be reproduced from their own seeds. Instead, new crops must be established from commercially produced hybrid seeds each season.

'Green Queen' forms medium to large, globular flower heads with tightly packed, bright green bracts. It is characterized by excellent field-holding capacity, allowing for flexible harvesting at different sizes to meet the demands of both domestic and export markets. This extended field-holding ability, enhances its market adaptability and logistical efficiency. Additionally, 'Green Queen' exhibits

high uniformity in fruit size and shape, reducing the percentage of discarded heads and optimizing commercial yield. Its high yield potential and uniformity make it an excellent option for large-scale commercial production.

Figure 13. 'Green Queen' artichoke cultivar.

2.4. Classification of artichokes attending to the floral head order

The classification of the floral head order in artichokes in the corresponding experiments (Publications 2 – 5) was determined primarily by their growth position within the plant structure, emphasizing the hierarchical organization and morphological distinctions among the different head orders (Figure 14) (Giménez-Berenguer et al., 2023).

- **Primary heads**, also referred to as **main heads**, developed directly from the central stem of the plant. These artichokes represented the first order and were positioned closest to the plant base, achieving the lowest height.
- **Secondary heads** emerged from the lateral stalks originating from the main stem. These heads occupied an intermediate position and height within the plant structure, forming the second order of flower heads.
- Finally, **tertiary heads** grew from the stalks of the secondary stems, representing the most distal position from the central stem and the final level.

Artichokes produced beyond the tertiary flower head order were excluded from this study to ensure consistency in the analysis and to focus on the most commercially relevant floral heads.

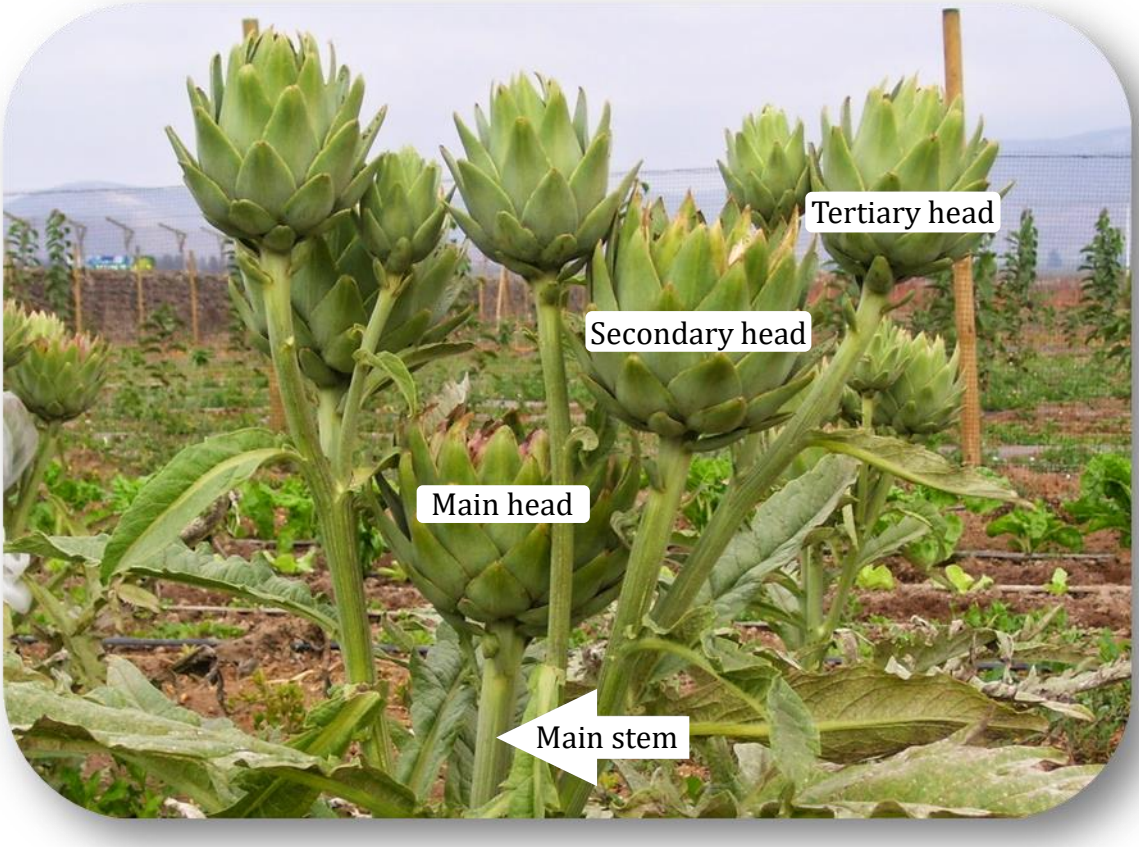

Figure 14. Flower head order disposal in artichoke plant. (Giménez-Berenguer et al., 2023).

This categorization was integral to understanding the distinct morphological and physiological characteristics associated with each head order, enabling the posterior exploration of their influence on phenolic content, antioxidant activity, and suitability for fresh-cut processing or commercial use. By prioritizing positional classification, it is highlighted the importance of structural organization in determining the functional and commercial attributes of artichokes.

3. Cardoon: Study site and Growing Conditions

The cultivation of cardoon plants was carried out over three consecutive growing seasons (2017–2018, 2018–2019, and 2019–2020; hereafter referred to as S1, S2, and S3, respectively) in an experimental field located in the Catania Plain, Sicily, southern Italy (37°30′ N, 15°4′ E, 10 m a.s.l.). The region is characterized by a Mediterranean climate, with mild winters and hot, dry summers.

Figure 15. Cardoon experimental field.

Seed sowing was performed in a greenhouse in early August 2017. Once the seedlings reached the three- to four-leaf stage, they were transplanted into the field in mid-September 2017. The experimental design followed a randomized complete block layout with four replicates, each consisting of 10 plants. The planting density was set at 1 plant m^{-2}, with a spacing of 1.25 m between rows and 0.8 m between plants within each row.

The crop was maintained in the field for three consecutive growing cycles. At the end of each cycle (early August), the aboveground biomass was cut, allowing natural regrowth to occur with the onset of autumn rains in September. Throughout the study, plants received approximately 50 mm of supplemental irrigation, 80 kg ha^{-1} of nitrogen fertilization, and manual weed control. The aerial biomass, including flower heads, was harvested at the end of July each season, when the plants had completely dried. The collected flower heads were subsequently processed to extract the achenes (seeds) for further analysis.

4. Plan Material: Cardoon Genotypes

The sixth study was conducted using seeds from a diverse selection of cardoon (*Cynara cardunculus* L.) genotypes, encompassing both cultivated and wild varieties (Figure 16). The plant material was categorized into three groups: commercial cultivated cardoon, cultivated cardoon developed by the University of Catania, and wild cardoon. These genotypes were chosen to represent a broad spectrum of genetic diversity and agronomic potential. The complete list of selected genotypes and their respective acronyms used in this experiment is provided in Table 1.

Figure 16. Cardoon seeds from diverse genotypes.

Table 1. List of genotype seeds selected, and acronyms used for this experiment attending to their plant material.

PLANT MATERIAL		GENOTYPE	ACRONYMS USED
***C. cardunculus* L. var. *altilis* DC**	**Commercial CC**	Gigante Inerme	GI
		Verde di Peralta	VdP
		Cardo Gobbo	CG
		Cardo Avorio	CA
	CC developed by Catania University (UniCt)	Altilis 41	A41
		Line 1	L1
		Line 7	L7
		Line 25	L25
		Line 32	L32
***C. cardunculus* L. var. *sylvestris* (Lamk) Fiori**	**Wild Cardoon**	Marsala	MA
		Val Paraiso	VP
		Kamarina	KA
		Creta	CR

4.1. Commercial Cultivated Cardoon

This category includes genotypes traditionally cultivated in Mediterranean countries for their enlarged blanched petioles, which are consumed as a vegetable, as well as for industrial applications. The four selected commercial cultivated cardoon (CC) genotypes were 'Cardo Avorio' (CA), 'Cardo Gobbo' (CG), 'Gigante Inerme' (GI), and 'Verde di Peralta' (VdP). These genotypes are widely recognized for their high biomass production, adaptability to Mediterranean growing conditions, and economic significance in traditional agricultural systems.

4.2. Cultivated Cardoon developed by Catania University

Five cultivated cardoon genotypes (Altilis 41 (A41), Linea 1 (L1), Linea 7 (L7), Linea 25 (L25), and Linea 32 (L32)) were specifically developed by the University of Catania (UniCT) through a controlled breeding program involving commercial cultivated varieties. These genotypes were derived from progenies obtained by selecting and crossing clonal lines of commercial cardoon varieties and selected based on their superior agronomic traits. The breeding process focused on

enhancing key characteristics, such as high biomass yield and enhanced phytochemical production, making them suitable for various industrial applications (Pandino et al., 2015).

4.3. Wild Cardoon

Wild cardoon (*Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori) represents the ancestral form of cultivated cardoon and is naturally distributed across different Mediterranean and South American regions. The four wild cardoon genotypes selected for this study were 'Creta' (CR), 'Kamarina' (KA), 'Marsala' (MA), and 'Valparaiso' (VP). These landraces genotypes were collected from diverse geographical locations, including the island of Crete ('Creta'), the coastal areas of Sicily ('Marsala' and 'Kamarina'), and central Chile ('Valparaiso'). Wild cardoon populations are well adapted to challenging environmental conditions and exhibit strong resilience to abiotic stresses.

5. Experimental Design

5.1. Research 1

Artichokes from the 'Blanca de Tudela' cultivar were harvested and immediately transported to the laboratory. Processing was conducted under hygienic conditions at 10 °C. After an initial inspection, defective heads were discarded, and the remaining ones were trimmed to remove the external tough, green bracts and upper portions. Each artichoke heart was then cut into eight equal segments (1/8). These segments were mixed and submerged in ice water containing 100 parts per million (ppm) of sodium hypochlorite for disinfection.

Following disinfection, all heart slices were drained, and 72 segments were randomly allocated to each treatment and replicate. The experimental treatments were the following (Table 2).

Table 2: Composition of treatments applied to fresh-cut artichoke in Publication 1.

Treatment	Composition
Control	Distilled water
Cys	L-cysteine at 0.028 M and pH 2.2
Cys + 75 EOs	L-cysteine + 75 µL/L EOs (25 µL eugenol, 25 µL thymol, 25 µL carvacrol)
Cys + 150 EOs	L-cysteine + 150 µL/L EOs (50 µL eugenol, 50 µL thymol, 50 µL carvacrol)
Cys + 300 EOs	L-cysteine + 300 µL/L EOs (100 µL eugenol, 100 µL thymol, 100 µL carvacrol)

Artichoke slices were dipped in their respective treatments for 1 minute, air-dried, and stored in open polyethylene trays at 2 °C and 85% of relative humidity (RH) for 9 days. Physicochemical (weight loss, colour, respiration rate), functional (total phenolic content, antioxidant activity), and sensory analyses were conducted on triplicate samples after 2, 4, and 9 days of storage.

5.2. Research 2

This study examined three seed-propagated artichoke cultivars: 'Green Queen' (hybrid) and 'Lorca' and 'Tupac' (open-pollinated). The experiment was conducted during the 2021–2022 growing season (August 2021–April 2022). Artichokes were harvested at commercial maturity and classified by flower head order (main, secondary, tertiary). Harvest dates varied based on availability: main and secondary heads were collected on February 22, 2022, tertiary heads of 'Lorca' and 'Tupac' on March 4, and tertiary heads of 'Green Queen' on March 14. The harvested artichokes were immediately transported to the laboratory for processing.

Ten artichokes per cultivar and head order were used for total phenolic content analysis. The remaining artichokes were trimmed into hearts, sliced, disinfected in a 100 ppm sodium hypochlorite solution for 5 minutes, rinsed, manually centrifuged, and air-dried. Slices were arranged in polyethylene trays (12 slices per tray, 10 trays per cultivar and head order), totalling 90 trays (1,080 slices). Trays were stored at 2 °C and 85% RH for 7 days.

Total phenolic content was analysed at harvest (day 0), while browning index was evaluated at days 0, 1, 3, 5, and 7. Sensory analysis was conducted until day 3, as the visual appearance no longer met the quality criteria.

5.3. Research 3

For this experiment, the developmental cycle of 'Lorca' artichoke cultivar was evaluated over the growing season of August 2020 to April 2021. A total of 40 seed-propagated, open-pollinated plants were grown in four replicates (10 plants per block). Harvesting followed commercial standards, starting on February 1 and continuing weekly until April 21, with 13 harvest dates and a total of 445 heads collected, classified by flower head order: 40 main, 278 secondary, and 127 tertiary heads.

The harvested artichokes were transported to the laboratory for immediate analysis. Each artichoke was first sliced longitudinally, and digital image analysis was performed to evaluate browning evolution during 180 seconds (3 minutes). Then, the internal developmental stage was assessed using a three-category scale based on the presence of internal hair and heart coloration. After classification, phenolic compounds were extracted and quantified. For PPO enzyme analysis, the remaining half of each artichoke heart was sliced, flash-frozen in liquid nitrogen, and stored at −80 °C until analysis.

5.4. Research 4

The experiment analysed the 'Blanca de Tudela' cultivar throughout its developmental cycle from autumn 2017 to spring 2018. A total of 150 first-year plants (50 per replicate) were planted in August, with blocks separated by borderlines. Seventeen harvests were conducted between November 29 and May 24, recording yield per plant (number and weight of heads) and categorizing them by flower head order (main, secondary, tertiary). For each harvest date, five heads per order and replicate were sampled to assess phenolic content and total antioxidant activity.

Additionally, artichokes harvested on February 26 (winter harvest) and April 23 (spring harvest) were used for postharvest storage studies. Following harvest, main, secondary, and tertiary heads without visible defects were selected, grouped into four lots of five artichokes per head order and replicate, and stored at 2 °C with 85% RH for 21 days. Quality parameters (weight loss, firmness, respiration rate) were evaluated at 0, 7, 14, and 21 days, while phenolic content, individual phenolic compounds, and total antioxidant activity were analysed at harvest and after storage.

5.5. Research 5

The experiment focused on the 'Blanca de Tudela' cultivar throughout its developmental cycle from autumn 2022 to spring 2023. In this sense, in early August, 300 offshoots from first-year artichoke plants were planted. Despite the growing area having a Mediterranean climate, the month preceding harvest, January, recorded an absolute maximum and minimum temperature of 23.4 °C and −1.3 °C, respectively. The average RH was 57%, and only 0.2 mm of precipitation was recorded (data from weather station 7244X, Orihuela-Desamparados, 38°04′04″ N, 00°58′53″ W). Therefore, conditions propitious to frost injury occurred on January 29 and 30, when temperatures dropped below 0.0 °C (−1.3 °C and −0.2 °C, respectively).

Harvesting was performed on February 1, 2023, when artichokes met commercial standards. A total of 330 artichokes were collected and classified by flower head order (main, secondary or tertiary) and then transported to the laboratory for analytical measurements: weight, size (artichokes length, diameter and steam thickness) and frost injury assessments with a 0 to 5 scale. Once evaluated, 10 frosted artichokes (affected by severe frost injury: grades 4–5) and 10 non-frosted artichokes (grade 0) were selected for phenolic compound identification and quantification, as well as total antioxidant activity analysis.

5.6. Research 6

At the end of each growing season, cardoon heads were harvested when the plants had completely dried. The harvested heads were transported to the laboratory, where the seeds (achenes) were manually extracted. For each genotype and replicate, approximately 1000 seeds were collected and subsequently oven-dried at 65 °C (Binder, Milan, Italy) until a constant weight was achieved. The dried seed material was then finely ground and used for the determination of ash content and the analysis of macro- and micro-mineral composition, including nitrogen (N), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu).

6. Physicochemical, Functional, and Quality determinations

6.1. Average Weight of Artichokes

The weight of each artichoke, including a floral stem length of 15–20 cm, was measured using a high-precision balance (Radwag WLC 2/A2, Radwag Wagi Elektroniczne, Radom, Poland) with an accuracy of 0.01 g. This ensured precise and consistent weight measurements across all samples. The average weight of the artichoke heads was calculated by averaging the weights of all measured samples for each flower head order. Results were reported as mean ± standard error (SE) and expressed in grams (g).

6.2. Size of Artichokes

The physical dimensions of each artichoke, including length, diameter, and stem thickness, were measured using a precision digital calliper (Digimatic Caliper CD-P15K, Mitutoyo Corporation, Kanagawa, Japan), accurate to 0.01 mm. Measurements were taken at the widest and longest parts of the heads and stems to ensure uniformity and reproducibility (Figure 17).

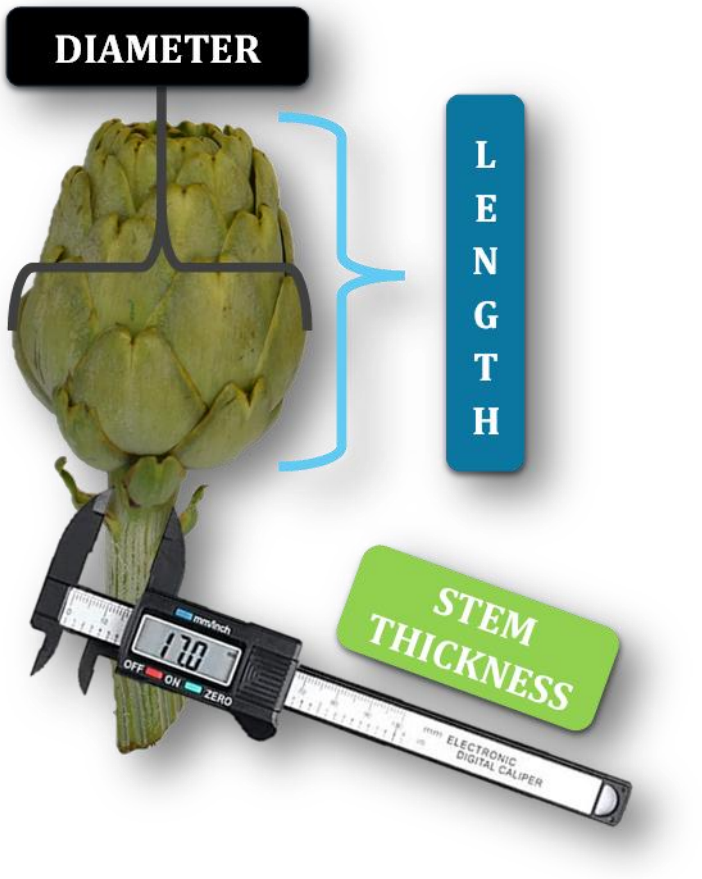

Figure 17. Size measurements of artichokes.

The average size of the artichoke heads, including length, diameter, and stem thickness, was calculated and expressed as the mean ± SE in millimetres (mm).

6.3. Total Yield

Yield was systematically quantified in Publication 4 as the total weight of artichoke heads per plant (kg · plant^{-1}) for each replicate, consisting of 50 plants (n = 3 replicates).

Besides, for every harvest date, the total number of harvested artichoke heads per plant was recorded. The harvested heads were then classified into three distinct floral head orders (main, secondary, and tertiary) based on their position within the plant structure, following the established hierarchical categorization.

All results were expressed as the mean ± SE, enabling statistical comparisons and robust interpretation of variability across replicates and head orders.

6.4. Weight Loss Determination

Weight loss (WL) was assessed by weighing fresh-cut artichoke samples (Publication 1) or individual artichoke heads (Publication 4) at day 0 (initial weight) and on subsequent storage days for each sampling date. WL was calculated as a percentage relative to the initial weight using the Equation 1:

Equation 1

$$WL\ (\%) = \frac{Initial\ Weight - Weight\ at\ Sampling\ Date}{Initial\ Weight} \times 100$$

Measurements were performed using a precision electronic balance (Radwag WLC 2/A2, Radwag Wagi Elektroniczne, Radom, Poland) with an accuracy of 0.01 g, to ensure consistency across all replicates. WL was expressed as the mean ± SE for each sample group.

6.5. Respiration Rate Measurement

Respiration rate (RR) was measured for artichoke heart slices (Publication 1) and whole artichoke heads (Publication 4).

For artichoke slices, eight 1/8 heart slices were placed in a 1 L glass jar, hermetically sealed for 30 minutes at room temperature. A 1 mL sample of the jar's headspace atmosphere was extracted and injected in a gas chromatograph (Shimadzu, Kyoto, Japan) with thermal conductivity detector (GC-TCD) to measure CO_2 concentration.

For whole artichokes, five heads from each flower order (main, secondary, and tertiary) were placed individually in 3 L glass jars, hermetically sealed for 60 minutes. A 1 mL sample of the jar atmosphere was withdrawn and analysed under the same conditions.

In both cases, RR was calculated and expressed as milligrams of CO_2 per kilogram per hour (mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$). Measurements were conducted in triplicate for each sample group, and results were reported as the mean ± SE.

6.6. Firmness

Firmness was assessed individually in five artichokes from each flower order (main, secondary, and tertiary) and replicate (Publication 4). The diameter of each artichoke head was measured prior to analysis. A force sufficient to achieve a 5% deformation of the head's initial diameter was then applied using a texture analyser (TX-XT2i Texture Analyzer, Stable Micro Systems, Godalming, UK).

The firmness was calculated as the ratio of force to deformation, expressed in newtons per millimetre ($N \cdot mm^{-1}$). Results were reported as the mean ± SE, ensuring accuracy and reproducibility in firmness evaluations across all samples.

6.7. Evaluation of the Internal Development Stage

The internal developmental stage of artichokes was assessed in Publication 3 after slicing the heads longitudinally using a sterilized stainless-steel knife. A self-designed, three-point scale was specifically developed for this research to evaluate the developmental progression of the artichoke's internal morphology, providing a standardized and replicable approach. The scale categorized artichokes into three distinct stages based on key physical characteristics observed in the heart and internal structure (Figure 18) (Giménez-Berenguer et al., 2022):

(a) = Initial Development Stage: Artichokes classified at this stage displayed no internal hair or a short minimal amount, undeveloped internal hair. The heart was characterized by a bright green colour and a tender texture, indicative of early morphological development.

(b) = Intermediate Development Stage: This stage was marked by a moderate presence of internal hair, representing a transitional phase. The heart remained green, reflecting a continued state of immaturity but with visible signs of further development compared to the initial stage.

(c) = Advanced Development Stage: Artichokes in this category exhibited abundant, fully developed internal hair. A notable change in coloration was observed, with the heart transitioning from green to purple, suggesting the progression toward physiological maturity.

Figure 18. Scale of internal development stage:
(a) initial developmental stage; **(b)** intermediate developmental stage;
(c) advanced developmental stage.
(Giménez-Berenguer et al., 2022).

6.8. Frost Injury Evaluation

Frost injury in individual artichoke heads was evaluated in Publication 5 using a detailed 0 to 5-point hedonic scale specially designed for this research to quantify the severity of frostbite symptoms based on visual inspection (Figure 19) (Giménez-Berenguer et al., 2023). This scale provided a standardized method for evaluating the extent of frost injury across all samples, ensuring consistency and reproducibility in the results. The criteria were as follows:

- **(0) = No appreciable frost injury**: Artichokes exhibited no visible symptoms of frost damage, maintaining their original appearance and quality.
- **(1) = Slight frost injury**: Minor and barely noticeable changes, such as subtle discoloration, were observed on the outer bracts.

(2) = Light frost injury: External bracts displayed slight browning concentrated at the ends, indicating the early stages of frost damage.

(3) = Moderate frost injury: Browning became more pronounced, affecting a larger portion of the outer bract ends, though still localized.

(4) = Severe frost injury: Significant brown spots were present across the external bracts, reflecting extensive frost damage.

(5) = Severe frost injury with dehydration: Widespread brown spots were accompanied by noticeable dehydration of the external bracts, indicating advanced frost damage and compromised structural integrity.

Artichokes were visually inspected under consistent lighting conditions to ensure accuracy in symptom detection. Digital images of representative samples from each category were captured to serve as references (Figure 19) (Giménez-Berenguer et al., 2023).

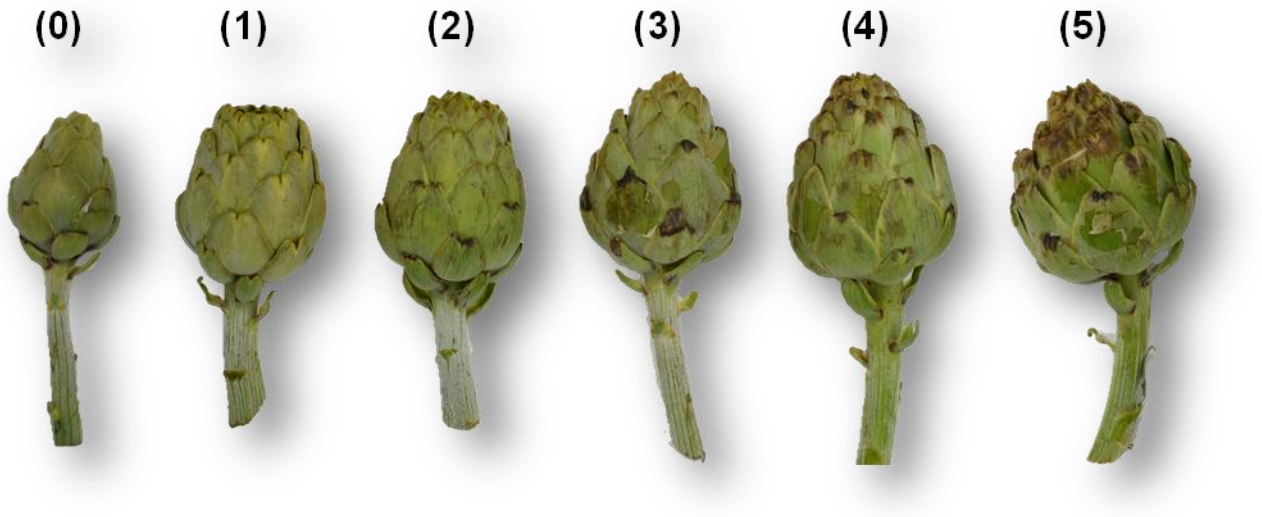

Figure 19. Frost injury scale:

(0) no appreciable frost injury; (**1**) slight frost injury; (**2**) light frost injury; (**3**) moderated frost injury; (**4**) severe frost injury; (**5**) severe frost injury with dehydrations in external bracts.

(Giménez-Berenguer et al., 2023).

6.9. Browning Evaluation of Artichokes

Browning of artichokes was evaluated using multiple methods, including colorimetric analysis (Publication 1), browning index calculation (Publication 2) and digital image analysis (Publication 3).

For fresh-cut artichokes, the colour of each slice was measured individually at room temperature on both sides of the external surface in triplicate using a Minolta colorimeter (CR-400; Konica Minolta, Osaka, Japan), after calibrating the instrument with a white plate. The colour parameters L*, a*, and b*, representing lightness, greenness/redness, and blueness/yellowness, respectively, were evaluated according to the CIE Lab System (Giménez-Berenguer et al., 2025).

For the first publication, the browning was studied using both, both L* (lightness) and b* (yellowness) colour parameters in artichoke slices.

For the second publication, browning evaluation was performed using the Browning index (% BI), which was calculated with the Equation 2 (Kasim and Kasim, 2015; Liu et al., 2022) presented below and results were expressed as mean ± SE.

Equation 2:

$$\%\ Browning\ index\ (\%\ \mathrm{BI}) = \frac{[100(x - 0.31)]}{0.17}$$

Where (in Equation 2) x:

$$x = \frac{(a^* + 1.75L^*)}{5.645L^* + a^* - 3.012b^*}$$

In publication 3, a real-time monitoring of browning after cutting entire artichokes was performed. Each artichoke head was longitudinally cut in half, and images were captured every 15 seconds for 3 minutes from the internal surface of the artichoke using a digital camera (Nikon D3400, Minato, Tokio, Japan) in a light box with a white background. These images were saved as JPEG files and analysed using ImageJ v1.52a software (NIH Image, National Institutes of Health, Bethesda, USA) (Giménez-Berenguer et al., 2022).

The hue angle (h°) used as the browning parameter, was calculated from the colour parameters a* and b* using the following equation (Equation 3):

Equation 3:

$$Hue\ angle\ (\mathrm{h}^{\circ}) = \arctan\left(\frac{b*}{a*}\right)$$

The camera setup conditions for this, and all the images of the samples recorded in this thesis included light provided by two LEDs with a colour temperature of 5600 K, a shutter speed of 1/5 s, ISO 100, focal aperture (f) 20 and length of 35 mm. All colour measurements and image analyses were conducted in triplicate, and results were expressed as the mean ± SE to ensure the accuracy and reproducibility of the browning evaluations.

6.10. Sensorial analysis

The sensory analysis of fresh-cut artichokes was conducted in two specific ways across Publication 1 and Publication 2. In both studies, the sensory analysis was performed by a trained panel of 10 judges (5 males and 5 females) from the Department of Agri-Food Technology at Miguel Hernández University (Orihuela, Alicante, Spain) (Giménez-Berenguer et al., 2025).

These two methods allowed for a comprehensive sensory assessment, evaluating both the specific organoleptic characteristics and overall acceptability of the artichokes at different storage intervals.

6.10.1. Descriptive Sensory Analysis

In the first publication, a descriptive sensory analysis was performed on 'Blanca de Tudela' fresh-cut artichoke. Prior to the fist evaluation, two preliminary sessions were held to determine the most relevant attributes for characterizing fresh-cut artichokes and to establish a common understanding of these sensory attributes that would provide a more precise description of the product.

Sensory attributes evaluated included visual appearance, odour, taste, and texture (Table 3) (Giménez et al., 2023), which were assessed based on the standardized methodology outlined by García-Martínez et al. (2017). The panellists

used a 0 to 5 hedonic scale for evaluation, as defined by Giménez et al. (2003), where scores of 1 to 2 indicated very poor quality, 3 to 4 represented fair quality, and 5 indicated excellent quality. Artichokes scoring 3 or lower for any sensory characteristic were deemed to have reached the end of their shelf-life. Results were reported as the mean ± SE.

Table 3. Organoleptic characteristics evaluated for fresh-cut artichokes. (Giménez et al., 2023).

Sensory Attribute	Description
Visual appearance	
Colour	Colours ranging from White to yellow.
Browning in cut areas	Presence of black areas in cut zones.
Dehydrated	High moisture loss.
Odour	
General Odour	Smell due to volatile compounds identified with artichoke odour
EOs odour	Smell due to residual volatile compounds from the EOs applied
Taste	
Sweet **Bitter** **Sour / Acid**	Primary tastes produced by substances such as sucrose (sweet), caffeine (bitter), and citric acid (sour or acid)
Texture	
Firmness	Force necessary to break the product and sound generated
General acceptability	**General impression of the sample**

6.10.2. Sensory evaluation of general acceptability

In the second publication, a general acceptability evaluation was conducted to assess the visual quality of different artichoke head orders (main, secondary, and tertiary) from the 'Lorca', 'Tupac', and 'Green Queen' cultivars during refrigerated storage. This evaluation involved a 0 to 5 self-made hedonic scale with 0.5-point increments, based on visual standards established from previously captured photos for this trial (Figure 20) (Giménez-Berenguer et al., 2025).

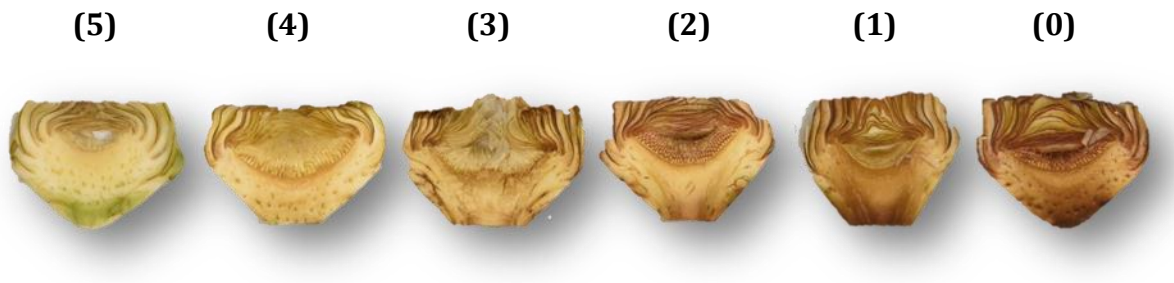

Figure 20. General acceptability scale:

(5) Optimum quality; **(4)** Moderate quality; **(3)** insufficient quality; **(2)** poor quality; **(1)** very poor quality; **(0)** extremely poor quality.
(Giménez-Berenguer et al., 2025).

The definitions of each sensory score are provided in Table 4 (Giménez-Berenguer et al., 2025). These descriptions helped to standardize the sensory assessment, allowing for objective evaluation of fresh-cut artichokes' organoleptic characteristics and aiding in determining their suitability for consumption over time.

A score of 3 or less was used to determine the end of the product's shelf-life, reflecting that the sample no longer met quality standards based on visual appearance. Results were reported as the mean ± SE.

Table 4. Description of the characteristics of each sensory score used to evaluate fresh-cut artichokes. (Giménez-Berenguer et al., 2025).

Sensory score	Description
(5) = Optimum quality	**No Browning - Excellent Freshness**: Artichoke heart slice appears in perfect condition, entirely free of browning, retaining its original pale green colour. There are no visible signs of dehydration, and the surface looks plump and moist. This indicates excellent freshness and quality.
(4) = Moderate quality	**Slight Browning - Good Freshness**: Artichoke heart slice shows a very slight brown tinge, barely noticeable. Browning is minimal, with only a few spots showing any darkening. There are no signs of severe dehydration or deterioration, and the surface still looks relatively moist.
(3) = Insufficient quality	**Light Brown - Not Fresh**: The artichoke heart slice displays a light brown colour, but it is spread throughout all the sample. The colour suggests initial quality deterioration and not freshness. A score of 3 or less indicate the end of the shelf-life.

(2) = Poor quality	**Moderate Brown - Slight Deterioration**: The artichoke heart slice exhibits a moderate brown colour, indicating mild browning. Browning is present and extensive, with some areas showing more intense coloration than others. The surface shows slight signs of quality deterioration.
(1) = Very poor quality	**Dark Brown - Moderate Deterioration**: The artichoke heart slice shows a deep brown colour, indicating moderate browning. Browning is noticeable across the surface, suggesting high level of oxidation. The surface appears moderately deteriorated.
(0) = Extremely poor quality	**Very Dark Brown - Severe Deterioration**: The artichoke heart slice appears very dark brown, almost black, with extensive browning throughout the surface. This indicates severe oxidation and deterioration. The surface looks highly deteriorated. This suggests poor freshness and a very unappealing visual appearance.

6.11. Extraction and Quantification of Total Phenolic Compounds

The extraction and quantification of total phenolic compounds (TPC) were performed following the methodology established by Giménez et al. (2021). Briefly, 5 grams of the edible portion of each sample (composed of the heart and inner bracts) were weighed and homogenized with 15 mL of an 80% (v/v) methanol solution. This extraction solvent was enhanced with 2 mM sodium fluoride (NaF) to inhibit PPO activity and minimize the enzymatic degradation of phenolic compounds during processing.

The homogenization process was conducted for 2 minutes at high-speed using an Ultra-Turrax® homogenizer (model TP 18, IKA, Staufen, Germany), ensuring thorough cell disruption and efficient extraction of phenolic compounds. Subsequently, the homogenates were subjected to centrifugation at 10,000 × g for 15 minutes at 4 °C to separate the liquid supernatant from solid residues. The supernatant, containing the extracted phenolic compounds, was carefully collected and stored on ice to prevent degradation before analysis.

Quantification of total phenolic content was carried out using the Folin-Ciocalteu colorimetric assay, a widely recognized and extensively validated method for determining phenolic compounds in plant matrices.

Calibration was performed using gallic acid as the standard, enabling the expression of results as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (FW) (g GAE · 100 g^{-1} FW) in Publication 1 and grams of GAE per kilogram of FW (g GAE · kg FW) in Publications from 2 to 5. All measurements were performed in duplicate, and the data were reported as mean values ± standard SE.

6.12. Identification and Quantification of the Individual Phenolic Profile

For the identification and quantification of phenolic profiles, the samples collected after the centrifugation in the previously extraction process were subjected to filtration prior to analysis through 0.45 µm CHROMAFIL® filters (Macherey-Nagel GmbH & Co., Düren, Germany), following the protocol described by Giménez et al. (2021). Once filtrated, the identification and quantification of phenolic profiles were performed using both, high-performance liquid chromatography systems coupled with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS^n, Bruker Daltonics Ultra HCT-ESI Ion Trap, Bremen, Germany) and reverse-phase high-performance liquid chromatography with diode array detection (RP-HPLC-DAD, Agilent HPLC 1200 Infinity series, Agilent Technologies, Waldbronn, Germany). A volume of 20 µL of each phenolic extract was injected into the chromatographic systems. Chromatographic separation was achieved using a Luna C18 column (250 × 4.0 mm, 5 µm particle size, Tenokroma, Barcelona, Spain).

The mobile phase consisted of 1% formic acid in ultra-pure water (solvent A) and acetonitrile (solvent B), with a flow rate of 1 mL · min^{-1}. The gradient program initiated with 1% solvent B, increased to 15% at 15 minutes, 30% at 30 minutes, 40% at 40 minutes, and 95% at 45 minutes, which was maintained until 50 minutes before returning to 1% at 60 minutes for column re-equilibration. Chromatograms were recorded at 320 nm for hydroxycinnamic acids and 360 nm for luteolin derivatives.

Compounds were identified based on their mass spectra and retention times by comparison with previous bibliographic data. Calibration curves were prepared

using 5-O-caffeoylquinic acid and 3-luteolin-O-rutinoside (Sigma-Aldrich, Darmstadt, Germany) as standards for quantifying hydroxycinnamic acids and luteolin derivatives, respectively. An example of an RP-HPLC-DAD chromatogram for hydroxycinnamic acids and luteolin derivatives identified is provided in Figure 21 (Giménez et al., 2021).

The hydroxycinnamic acids identified included:

- 3-O-caffeoylquinic acid (**3-CQA**; tr ≈ 7.3 min)
- 5-O-caffeoylquinic acid (**chlorogenic acid, 5-CQA**; tr ≈ 11.5 min)
- 1,3-di-O-caffeoylquinic acid (**cynarin, 1,3-diCQA**; tr ≈ 14.7 min)
- 3,4-di-O-caffeoylquinic acid (**3,4-diCQA**; tr ≈ 22.2 min)
- 3,5-di-O-caffeoylquinic acid (**3,5-diCQA**; tr ≈ 22.7 min)
- 4,5-di-O-caffeoylquinic acid **(4,5-diCQA**; tr ≈ 24.8 min)

The identified luteolin derivatives included:

- Luteolin 7-O-glucuronide 3-O-glucoside (**Lut 7-gluc 3-glc**; tr ≈ 21.9 min)
- Luteolin 7-O-glucuronide (**Lut 7-gluc**; tr ≈ 23.5 min)
- Luteolin 7-O-glucoside (**Lut 7-glc**; tr ≈ 23.8 min)

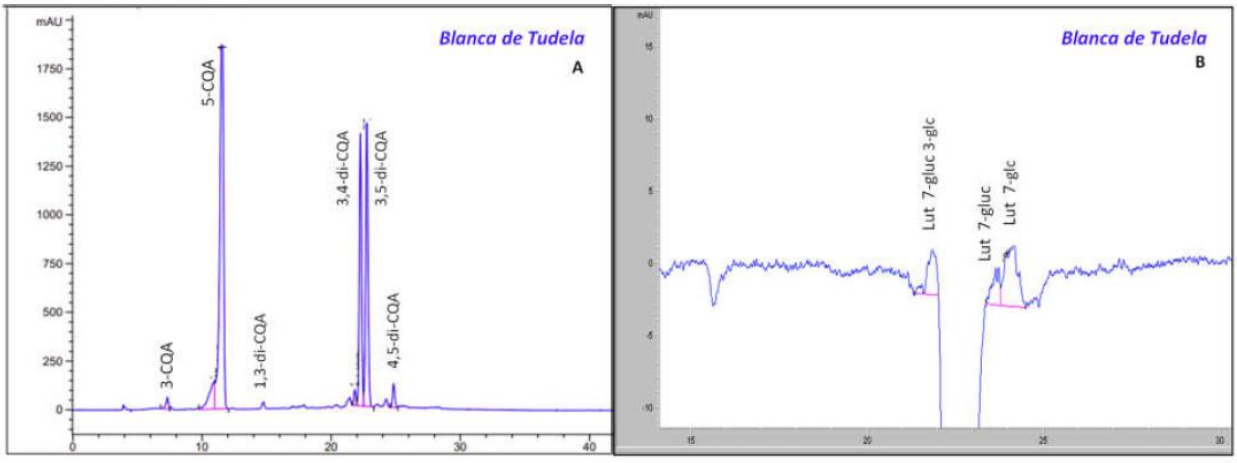

Figure 21. HPLC-DAD-ESI/MSn chromatogram of the hydroxycinnamic acids **(A)** and luteolin derivatives **(B)** identified at 320 nm and 360 nm, respectively, for 'Blanca de Tudela' cultivar.
(Giménez et al., 2021).

The concentrations of hydroxycinnamic acids and luteolin derivatives were expressed as grams per kilogram ($g \cdot kg^{-1}$) of FW. In the publications in which phenolic profile was assessed (Publication 2 and 5), the total phenolic concentration was calculated as the sum of all individual phenolic compounds and expressed as $g \cdot kg^{-1}$ FW. All results are presented as the mean ± SE.

6.13. Total antioxidant activity

The quantification of total antioxidant activity (TAA) was performed following the methodology described by Valero et al. (2011), with slight modifications. This method allows for the simultaneous extraction of hydrophilic (H-TAA) and lipophilic (L-TAA) antioxidant compounds, ensuring a robust and comprehensive evaluation of both types of antioxidant activities in artichoke tissues.

For the extraction in Publication 1, 5 g of the edible portion of each artichoke sample was homogenized in 15 mL of 50 mM sodium phosphate buffer (pH 7.8, Sigma-Aldrich, Madrid, Spain) and 10 mL of ethyl acetate using an Ultra-Turrax® (IKA TP 18, Staufen, Germany) for 2 minutes at high-speed. Alternatively, in Publication 4 and Publication 5, it is used 2 g of tissue homogenized with 10 mL of sodium phosphate buffer and 5 mL of ethyl acetate. The homogenate was then centrifuged at 10,000 × g for 15 minutes at 4 °C, resulting in two separate phases. The upper phase (ethyl acetate fraction) was used for L-TAA quantification, while the lower aqueous phase was analysed for H-TAA (Giménez et al., 2023).

The quantification of TAA was based on an enzymatic system consisting of 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horse radish peroxidase enzyme, and hydrogen peroxide as the oxidant substrate. In this reaction, $ABTS^{\cdot+}$ radicals were generated and monitored spectrophotometrically at 730 nm. The absorbance values were used to calculate TAA as the equivalent of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Results were expressed as either milligrams of Trolox equivalent per 100 g (Publication 1) or grams of Trolox equivalent per kilogram of FW (Publication 4 and 5).

For the experiments, data were presented as the mean ± SE of multiple replicates.

6.14. Polyphenol Oxidase Enzyme Activity Assay

The extraction and quantification of PPO activity were performed following the protocol described by Cabezas-Serrano et al. (2009), with slight modifications to optimize the assay. To start, 5 g of flash-frozen in nitrogen tissue samples were homogenized in 15 mL of 0.1 M phosphate buffer (pH 6.5) containing 1 g of polyvinylpyrrolidone (PVP) using an Ultra-Turrax® homogenizer (TP 18, IKA, Staufen, Germany) for 2 minutes at high-speed. The addition of PVP was critical for binding phenolic compounds and reducing their potential interference with the enzymatic activity. The homogenate was centrifuged at 10,000 × g for 15 minutes at 4 °C to remove debris. The resulting supernatant, which contained the soluble PPO enzyme, was collected for activity analysis (Giménez-Berenguer et al., 2022).

PPO activity was determined spectrophotometrically by measuring the enzymatic oxidation of catechol, a phenolic substrate, by duplicate, in a reaction mixture composed of:

- 200 µL of enzyme extract,
- 2 mL of 50 mM phosphate buffer (pH 6.5),
- 0.5 mL of 0.1 M 4-methylcatechol.

The reaction was initiated by adding the substrate (4-methylcatechol), and the increase in absorbance was monitored at 420 nm for 1 minute using a spectrophotometer that was blanked with the phosphate buffer. The rate of absorbance change (Δ 420) was used to calculate PPO activity.

The results of the enzymatic activity were expressed as units per gram of fresh weight ($U \cdot g^{-1}$ FW), where one unit (U) was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per minute under the assay conditions. Results were reported as the mean ± SE.

6.15. Ash Content Determination in Cardoon Seeds

The ash content of cardoon seeds was determined by AOAC (2005) official method 923.03. Approximately 1 g of dried and ground seed samples was placed in pre-weighed porcelain crucibles and incinerated in a muffle furnace at 550 ± 2 °C for

24 hours to ensure complete combustion of organic matter. After this initial incineration, two drops of 65% nitric acid were added to each crucible to facilitate the decomposition of any remaining residues. The samples were then subjected to an additional 24-hour drying period in an oven at the same temperature.

Once the process was completed, the crucibles were cooled in a desiccator to prevent moisture absorption and subsequently weighed to determine the residual ash content. The percentage of ash was calculated using the following equation (Equation 4):

Equation 4:

$$\%\ Ash\ Content = \left(\frac{\text{Final weight of ash}}{\text{Initial weight of sample}}\right) \times 100$$

All analyses were performed in duplicate, and the final values represent the mean of two independent determinations.

6.16. Macro- and Mico- Mineral Determination in Cardoon Seeds

N content was determined using the Kjeldahl method (Nelson and Sommers, 1980), which involves digestion, distillation, and titration to quantify total nitrogen in the samples.

The concentrations of other minerals, including K, Mg, Ca, Na, Zn, Fe, Mn, and Cu, were analysed following the AOAC (1995) official method 975.03.

Mineral quantification was performed using a Perkin Elmer AAnalyst 200 flame atomic absorption spectrometer (Norwalk, USA). Calibration curves were prepared using certified standard solutions to determine the mineral concentrations in the samples.

Macro-mineral (N, Ca, K, Mg and Na) concentrations were expressed as g kg^{-1} of dry matter (DM), while micro-element (Fe, Zn, Mn, Cu) concentrations were reported as mg kg^{-1} DM. Each analysis was conducted in duplicate, and the values presented correspond to the mean of two independent experiments to ensure

reliability. All chemical reagents used for digestion, extraction, and analysis were of analytical grade and were purchased from Sigma-Aldrich (Milan, Italy).

7. Statistical Analysis

All statistical analyses were conducted using SPSS software (versions 17.0 or 20.0, IBM Corp., Armonk, NY, USA) or STATGRAPHICS Plus software version 3.1 for Windows (Statgraphics Technologies, Inc.).

Data were expressed as the mean ± SE. For all datasets, an analysis of variance (ANOVA) was performed to assess significant differences among treatments or experimental factors. Post hoc comparisons were conducted when significant differences ($P \leq 0.05$) were detected, using appropriate multiple range tests depending on the individual needs of each experimental design. The specific statistical tests used are detailed in each individual publication. This structured approach ensured consistency across studies while allowing for the appropriate application of statistical analyses according to the characteristics of each experiment and dataset.

PUBLICATIONS

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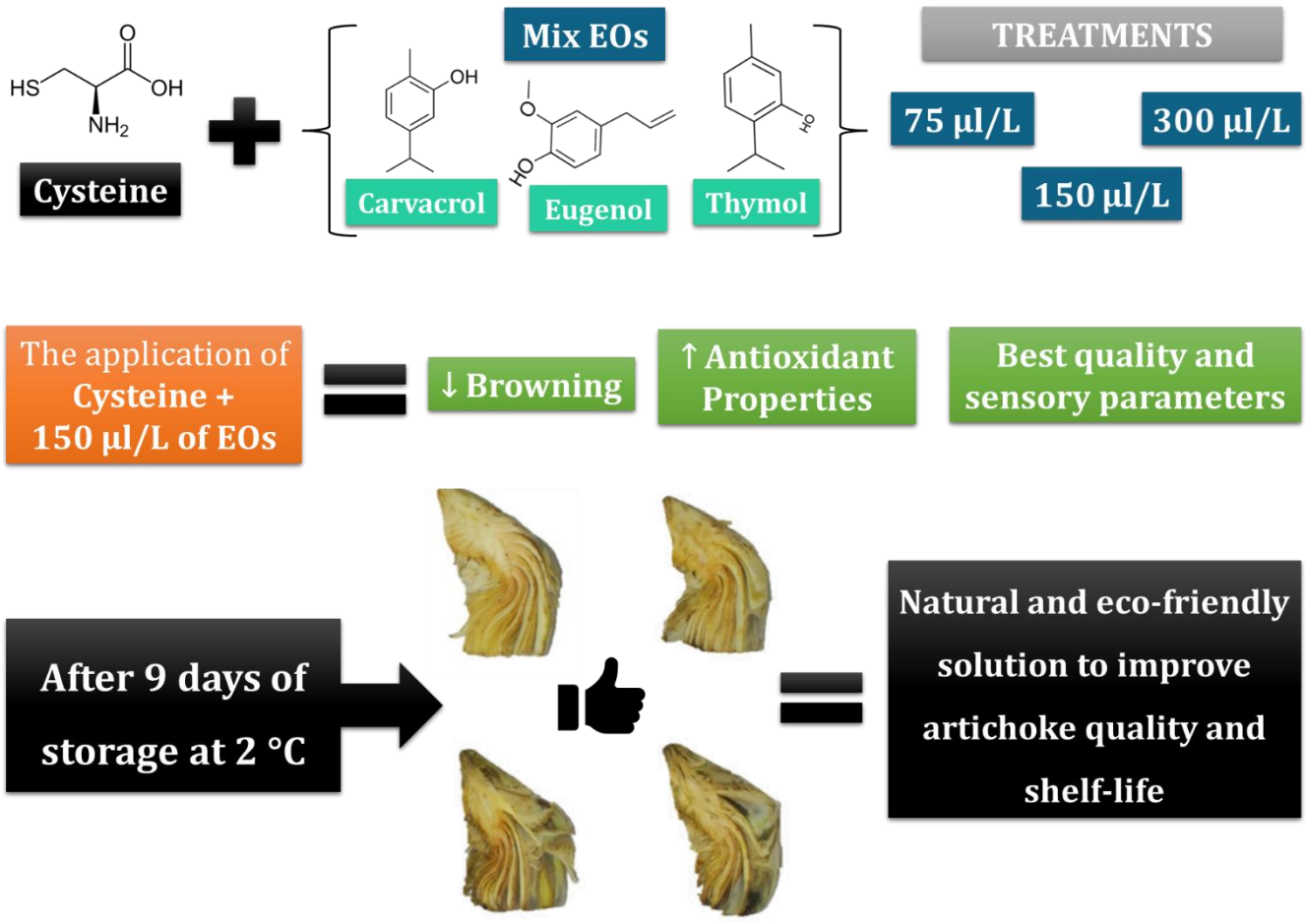
PUBLICATION 1

Effect of Cysteine with Essential Oils on Quality Attributes and Functional Properties of 'Blanca de Tudela' Fresh-Cut Artichoke.

(Literal Transcription)

María J. Giménez, **Marina Giménez-Berenguer**, Fabián Guillén, Vicente Serna-Escolano, María Gutiérrez-Pozo and Pedro J. Zapata

Foods *2023, 12 (24), 4414*



https://doi.org/10.3390/foods12244414

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Article

Effect of Cysteine with Essential Oils on Quality Attributes and Functional Properties of 'Blanca de Tudela' Fresh-Cut Artichoke

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Abstract: The commercialisation of fresh-cut artichokes with optimal quality and appearance and a maximum shelf-life is a great challenge for the artichoke market. The use of different anti-browning agents has been previously studied; however, their effect is still limited. Therefore, the objective of this study is the evaluation of the effect of L-cysteine and, in combination with a mixture of essential oils components (eugenol, thymol and carvacrol) on browning, quality and bioactive compounds of fresh-cut artichokes stored for 9 days at 2 °C. Four different treatments were applied to 'Blanca de Tudela' fresh-cut artichokes: cysteine and cysteine with 75, 150 and 300 µL of the essential oils components (EOs) mixture. After 2, 4 and 9 days of storage, physicochemical parameters (weight loss, colour, respiration rate) and functional (total phenolic content, antioxidant activity) were studied. A descriptive sensorial analysis was also carried out to evaluate sensory attributes. Results showed that the application of cysteine and 150 µL of EOs displayed the lowest browning and highest antioxidant properties, as well as the best quality and sensory parameters. The use of this post-harvest treatment on fresh-cut artichokes would result in a natural and eco-friendly solution to improve artichoke quality and shelf-life.

Keywords: artichoke; browning; sensory analysis; total phenolics; total antioxidant activity

Citation: Giménez, M.J.; Giménez-Berenguer, M.; Guillén, F.; Serna-Escolano, V.; Gutiérrez-Pozo, M.; Zapata, P.J. Effect of Cysteine with Essential Oils on Quality Attributes and Functional Properties of 'Blanca de Tudela' Fresh-Cut Artichoke. *Foods* **2023**, *12*, 4414. https://doi.org/10.3390/foods12244414

Academic Editor: Juan Luis Valenzuela

Received: 9 November 2023
Revised: 5 December 2023
Accepted: 6 December 2023
Published: 8 December 2023

1. Introduction

Artichoke (*C. scolymus* L.) is widely cultivated around the world, with Italy, Egypt, and Spain being the three main producers. Spain is in the second position in Europe, generating around 200 million tons of artichokes per year [1]. It is an essential component of the Mediterranean diet due to its high content of bioactive compounds, fibre and minerals [2]. It is considered a highly perishable commodity that suffers several physiological changes during its storage, resulting in a great inconvenience for its marketability. Nowadays, fresh-cut artichokes with pleasant appearances and organoleptic and nutritional characteristics are requested by consumers. This is a great challenge for the artichoke market, where optimal quality and a longer shelf-life are required [3].

The main causes of quality losses in fresh-cut artichokes are enzymatic browning, weight loss, dehydration and physical injuries, such as bruising or compression. Enzymatic browning is considered the main cause of quality loss in fresh-cut artichokes [3,4]. It takes place due to the high polyphenol content of artichokes, polyphenols that serve as substrate for the polyphenol oxidase (PPO). Once artichokes are subjected to wounding or cutting, a cellular disruption takes place, allowing PPOs to make contact with their substrates. These polyphenols are oxidated to quinones, resulting in dark pigment causing tissue browning on fresh-cut artichokes [5–7]. The use of low phenolic content cultivars for the minimally processed market (fresh-cut artichokes) has been previously studied [8,9]. The effect of harvest time and storage conditions (time and temperature) on the total phenolic

content of artichokes and, therefore, their effect on the enzymatic browning of fresh-cut artichokes [10–14] has been previously studied. It has been observed that the use of Modified Atmosphere Packaging (MAP) combined with low storage temperatures [15–17] and in combination with anti-browning agents, edible coatings or innovative packaging [17–22] significantly reduces enzymatic browning and increases the shelf-life of fresh-cut artichokes.

The application of anti-browning treatments, such as ascorbic acid, oxalic acid, citric acid or L-cysteine, as excellent antioxidants have been widely studied [10,23–26]. L-cysteine has been selected as the most effective anti-browning treatment for fresh-cut artichoke due to its inhibition effect on the polyphenol oxidase (PPO). When oxygen is available, PPO catalyses the oxidation of phenols, leading to the appearance of browning pigments [27,28]. Several authors have observed a reduction in browning and an extension of the shelf-life of fresh-cut artichokes when they were treated with L-cysteine. However, an increase in the yellow colour of fresh-cut artichokes was also observed as a result of the application of amino acids [23,24,29].

Therefore, research about new alternatives to L-cysteine to reduce browning and extend shelf-life in fresh-cut artichoke is required. Previous studies have elucidated the potential antioxidant effect that essential oils' major components (EOs) have when they are applied to fruits and vegetables at different stages of the food chain [30,31]. EOs contain secondary metabolites produced in different parts of the plant, known to confer disease resistance, antioxidant and antimicrobial properties [32–34]. Their use as part of edible coating has been extensively studied to reduce weight loss, respiration rate and fungal decay in citrus fruit [35–38] and table grapes [39,40], among others. The synergic effect that major components of essential oils (eugenol, thymol, and carvacrol) have on the fungal spoilage of fruit has been previously demonstrated [38,41]. Its use for the minimally processed market as an edible coating is still reduced; only a few studies have been carried out on the use of an antimicrobial agent in fresh-cut apples [42] and fresh-cut melon [43]. Rizzo et al. [44] have studied the effect of locust bean gum edible coating with added *Foeniculum vulgare* EO and its use as an active packaging-releasing system in fresh-cut artichokes as an anti-browning treatment. However, most of the studies are focused on the effect of these EOs on the sensorial and colour parameters of treated fresh-cut artichokes, while parameters such as weight loss, respiration rate and firmness are also required to achieve the highest quality in fresh-cut artichokes.

Hence, this study aimed to evaluate the effect of L-cysteine and, in combination with a mixture of essential oils components (eugenol, thymol and carvacrol) on browning, quality and bioactive properties of fresh-cut artichokes stored for 9 days at 2 °C.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Artichokes (*C. scolymus* L. cv. 'Blanca de Tudela') were harvested from a commercial plot located in Orihuela (Alicante, Spain). Heads were harvested when fully developed according to standard practices and immediately transported to the laboratory. Selection and processing were performed at 10 °C under hygienic conditions. Artichokes with defects were discarded, and heads were trimmed using a sharp stainless-steel knife to remove external greener and tougher bracts (inedible fraction) and the upper portion. After that, each artichoke heart was cut into 8 parts (1/8), mixed, and dipped in ice water containing 100 ppm of sodium hypochlorite (Sigma Aldrich, Madrid, Spain) as a disinfectant solution. The essential oils components (EOs) for this study were eugenol, thymol and carvacrol. All heart slices were drained and 72 different 1/8 parts were randomly selected for each different treatment and replicates and dipped in distilled water (Control), L-cysteine at 0.028 M at a pH of 2.2 (Cys), cysteine + 75 µL/L EOs (Cys + 75 EOs: 25 µL eugenol + 25 µL thymol + 25 µL carvacrol), cysteine + 150 µL/L EOs (Cys + 150 EOs: 50 µL eugenol + 50 µL thymol + 50 µL carvacrol), cysteine + 300 µL/L EOs (Cys + 300 EOs: 100 µL eugenol + 100 µL thymol + 100 µL carvacrol). The reagents used were L-cysteine hydrochloride monohydrate, carvacrol (>98%), eugenol (>98%) and thymol (98.5%) (Sigma Aldrich,

Madrid, Spain) at the specific concentrations diluted in distilled water. For each treatment, artichoke slices were divided into lots and dipped for 1 min in the corresponding solution and then dried at room temperature and stored in polyethylene (PE) opened trays at 2 °C and 85% RH for 9 days. Samples of eight 1/8 heart parts from each treatment in triplicate were evaluated for quality and bioactive attributes.

2.2. Artichoke Quality Parameters

Quality parameters were evaluated after 2, 4 and 9 days at 2 °C. These days represented an early, mid- and late stage of the storage of fresh-cut artichokes. Individual replicates were weighted daily, and Weight Loss (WL) was expressed as a percentage (%). The respiration rate (RR) was measured for the three replicates of each treatment. Eight 1/8 heart slices of artichokes were placed in a 1 L glass jar hermetically sealed for 30 min at room temperature, and 1 mL of the headspace was analysed and quantified in a gas chromatograph (TMGC-2010, Shimadzu Corporation, Kyoto, Japan) equipped with a thermal conductivity detector (TCD). Results were expressed as the mean ± standard error (SE) in mg of CO_2 kg^{-1} h^{-1}. Browning of artichokes was evaluated individually based on colour parameters (L*, a* and b*), using a Minolta colourimeter (CRC200; Minolta, Osaka, Japan) at three points of the external surface for each artichoke part using the CIE Lab System. Results were expressed as the mean ± SE.

2.3. Total Antioxidant Activity

Total Antioxidant Activity (TAA) was quantified according to Valero et al. [45], where both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds were determined. Eight parts (1/8) of artichoke heads for each treatment were cut into small pieces, and three replicates of five grams each were weighed for the following extraction. Artichoke pieces were homogenised in 15 mL of 50 mM Na-PO4 buffer (pH 7.8, Sigma-Aldrich, Madrid, Spain) and 10 mL of ethyl acetate and then centrifuged at 10,000 × g for 15 min at 4 °C. The L-TAA was determined from the upper fraction, while the lower fraction was used for H-TAA quantification. The quantification was based on the enzymatic system composed of ABTS, peroxidase enzyme (HRP) and its oxidant substrate, hydrogen peroxide (H_2O_2), in which ABTS+ radicals were generated and monitored at 730 nm. The reaction was carried out in duplicate following the Re et al. [46] protocol with some modifications, as presented in Giménez et al. [47]. The results were expressed as the mean ± SE of mg of Trolox equivalent per 100 g of fresh weight (FW).

2.4. Total Phenolic Content

Total Phenolic Content (TPC) was measured following Swaim and Hillis [48] protocol with slight modifications, as previously mentioned by Martínez-Esplá et al. [49]. A water: methanol (2:8) solution containing 2 mM NaF (Sigma-Aldrich, Madrid, Spain) was used, and extracts were quantified using the Folin–Ciocalteu reagent. Results (mean ± SE) were expressed as mg of gallic acid equivalent per 100 g FW.

2.5. Descriptive Sensory Analysis

The descriptive sensory analysis of fresh-cut artichokes was performed by a panel of 10 judges (5 males and 5 females) trained in sensory testing from the Department of Agri-Food Technology of Miguel Hernández University (Orihuela, Alicante, Spain). Two preliminary sessions were carried out to discuss the attributes considered by the panellist that could provide a more suitable description of the fresh-cut artichokes. The sensory attributes (visual appearance, odour, taste, texture) evaluated are presented in Table 1, according to García-Martínez et al. [16]. The panellists used a hedonic scale from 0 to 5, previously designed by Giménez et al. [19], where 1 to 2 is very poor, 3 to 4 is fair, and 5 is excellent. A score of 3 or less for any of the organoleptic characteristics evaluated indicated the end of the shelf-life.

Table 1. Organoleptic characteristics evaluated for fresh-cut artichokes.

Sensory Attribute	Description
Visual appearance	
Colour	Colours ranging from White to yellow
Browning in cut areas	Presence of black areas in cut zones
Dehydrated	High moisture loss
Odour	
General odour	Smell due to volatile compounds identified with a cardoon odour
EOs odour	Smell due to residual volatile compounds from the EOs applied
Taste	
Sweet	Primary tastes produced by substances such as sucrose (sweet), caffeine (bitter), and citric acid (sour or acid)
Bitter	
Sour, acid	
Texture	
Firmness	Force necessary to break the product and sound generated from frostbite
General acceptability	General impression of the sample

2.6. Statistical Analysis

Data sets of the different evaluated parameters (weight loss, respiration rate, colour, TPC and TAA) were subjected to an analysis of variance (ANOVA) to determine significant differences between treatments (p-value < 0.05). When significant differences were detected, a post hoc analysis was carried out using HSD Tukey's test for each pair of treatments. All statistical analyses were performed using SPSS software v. 20.0 for Windows.

3. Results and Discussion

3.1. Effect of the Application of Cysteine and EOs on Artichoke Browning

Fresh-cut artichokes are susceptible to suffering browning during cold storage, generating an undesirable appearance, causing rejection by consumers and limiting their shelf-life. Browning is caused by the oxidation of phenolic compounds catalysed by different enzymes, such as polyphenol oxidases (PPO), with the subsequent formation of dark compounds [4,8,50]. In the present work, browning was studied in non-treated and treated artichoke slices based on appearance (Figure 1) and colour parameters (Figure 2a,b). The appearance of artichokes considerably improved when they were treated with a combination of cysteine and EOs, observing the best appearance in those artichokes that were treated with cysteine and 150 µL of EOs (Cys + EOs 150). In addition, both L* (lightness) and b* (yellowness) colour parameters were studied in artichoke slices after 2, 4 and 9 days of storage at 2 °C. After four days of storage, the L* value of non-treated artichokes was 72.67 ± 0.90, while Cys, Cys+ 75 EOs, Cys + 150 EOs, and Cys + 300 EOs treated artichokes were 81.05 ± 0.45, 81.48 ± 0.42, 82.35± 0.43 and 81.39 ± 0.49, respectively (Figure 2a). Significant differences (p-value < 0.05) were detected between treated and non-treated artichokes after 2 and 4 days of storage. After 9 days, significant differences were only detected in those artichokes treated with cysteine and 150 and 300 µL of EOs. Contrary results were observed by Rizzo et al. [51], who found that the use of *Foniculum vulgare* EO did not influence the colour appearance of fresh-cut artichokes. It has been previously reported that artichokes' quality characteristics depend on cultivar, genotype, harvest, and storage time [8,9,11,50]. Furthermore, in a previous study, 'Blanca de Tudela' fresh-cut artichokes were treated with different concentrations of cysteine (10–50 mol/m^3) and packed in polypropylene films, achieving L* values of 60.17 ± 0.22 after 4 days at 5 °C [29], while in the present study, the combination with EOs resulted in a 28% higher lightness. A decrease in L* with storage time was observed in all treatments and has been previously reported [44,51]. It is associated with the PPO enzymatic reaction that occurs during the browning process [52,53]. The application of Cys + 150 EOs on artichokes slices resulted in a 5% reduction in lightness with time, while for non-treated fresh-cut artichokes, a reduction of 16.31% was observed.

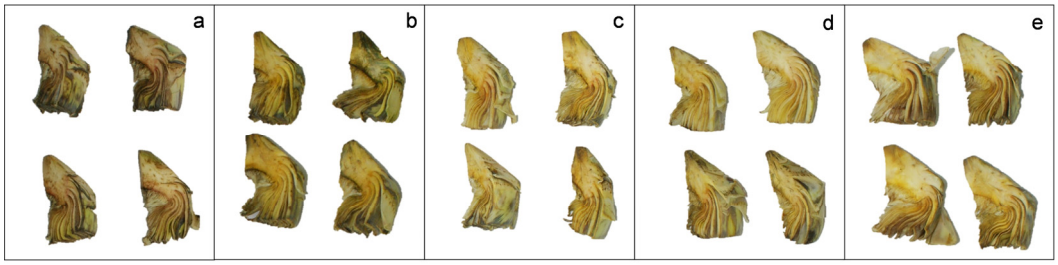

Figure 1. Browning of non-treated artichokes (**a**), and treated with Cysteine (**b**), Cysteine and EOs 75 µL (**c**), Cysteine and EOs 150 µL (**d**) and Cysteine and EOS 300 µL (**e**) after 9 days of storage at 2 °C.

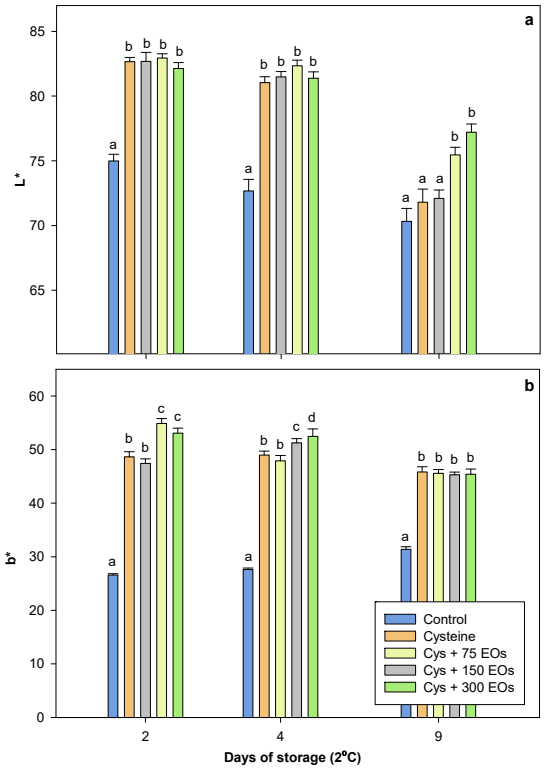

Figure 2. Colour parameter L* (**a**) and b* (**b**) of fresh-cut artichokes non-treated and treated with cysteine (Cys), cysteine and 75 µL of EOs (Cys + 75 EOs), cysteine and 150 µL of EOs (Cys + 150 EOs) and cysteine and 300 µL of EOs (Cys + 300 EOs) after 2, 4 and 9 days of storage at 2 °C. Significant differences (p-value < 0.05) between treatments are presented with different lower-case letters (Tukey's test).

On the other hand, significant differences (p-value < 0.05) were also detected in the yellowness (b*) between treated and non-treated artichokes (Figure 2b). The increase in lightness in those artichokes treated with cysteine has been previously reported by several authors [23,24,29]. Significantly higher values of b* were observed in those artichokes treated with cysteine and 150 and 300 µL of EOs after 2 and 4 days of storage, while after 9 days of storage, significant differences were detected between treated and non-treated artichokes, but no differences were observed between treatments, observing a b* of 31.38 ± 0.48 in the non-treated artichokes and values of 45.84 ± 0.97, 45.58 ± 0.97, 45.28 ± 0.51 and 45.42 ± 0.94 in Cys, Cys+ 75 EOs, Cys + 150 EOs and Cys + 300 EOs treated artichokes. In a previous study, Amodio et al. [23] treated fresh-cut Catanese cv artichokes with 0.25% and 0.5% of cysteine and stored them for 5 days at 5 °C, observing L* values of 33.4–38.4 and b* values of 36.3–38. While in the present study, the application of cysteine and EOs resulted in higher lightness and yellowness.

Therefore, the use of cysteine in combination with EOs significantly increased lightness and yellowness and reduced the browning of fresh-cut artichokes; consequently, a considerable improvement in appearance was achieved.

3.2. Effect of the Application of Cysteine and EOs on Quality Parameters of Fresh-Cut Artichokes

Weight loss (WL) during the transpiration process of artichokes is an important cause of deterioration affected by temperature and reduces product quality when artichokes are sold as processed products [54]. Weight losses increased during storage in fresh-cut artichokes, independently of treatment (Figure 3a). However, a lower increase with time was observed in those fresh-cut artichokes that were treated with the combination of cysteine and EOs. The use of cysteine and EOs at 75 and 150 µL significantly (p-value < 0.05) reduced the weight loss of fresh-cut artichokes compared to the rest of the treatments during the storage period. After 9 days of storage at 2 °C, Cys + 75 EOs and Cys + 150 EOs treated artichokes presented an 18.73 (19.41 ± 0.47%) and 20% (19.72 ± 0.75%) reduction of WL compared to the non-treated artichokes (24.26 ± 1.61%), respectively. In previous studies, the use of *Foeniculum vulgare* EOs on its own or in combination with cysteine observed a similar WL when artichokes were hermetically packed in PET trays [44,51]. In the present study, a decrease in the WL has been observed, probably due to the absence of packaging. Although it has been widely studied the use of cysteine as an anti-browning agent [18,23,24,29], in the present study, it has been observed that non-treated and cysteine-treated artichokes presented similar WL; therefore, the use of cysteine on its own did not reduce the WL of fresh-cut artichoke, while its combination with EOs did. Similar results were observed in fresh-cut potatoes treated with L-cysteine, where no effect on WL was observed [55]. However, previous studies showed that the application of EOs resulted in a reduction of WL in litchi fruit [56], apricots [57], nectarines [58], grapes [59] and pears [60]. This could be related to the barrier effect that essential oils have on the fruit, reducing moisture loss and delaying dehydration of treated fruits [56].

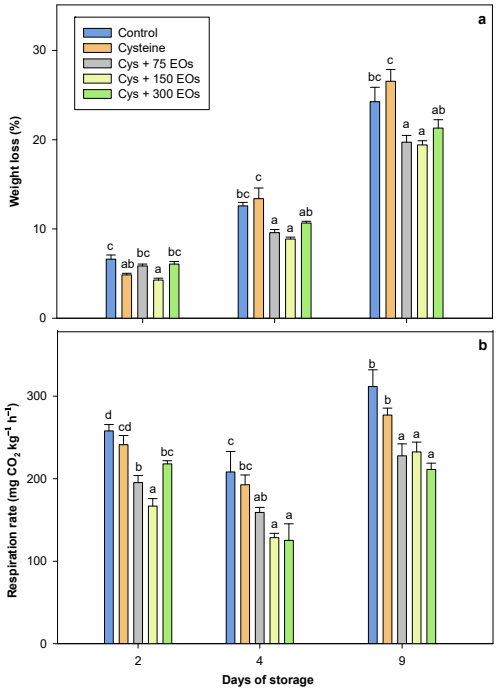

Figure 3. Weight loss (**a**) and respiration rate (**b**) of fresh-cut artichokes non-treated and treated with cysteine (Cys), cysteine and 75 µL of EOs (Cys + 75 EOs), cysteine and 150 µL of EOs (Cys + 150 EOs) and cysteine and 300 µL of EOs (Cys + 300 EOs) after 2, 4 and 9 days of storage at 2 °C. Significant differences (p-value < 0.05) between treatments are presented with different lower-case letters (Tukey's test).

'Blanca de Tudela' artichoke has been classified as a commodity with an extremely high respiration rate (RR), which is responsible for its quick deterioration and short shelf-life [61]. The RR of fresh-cut artichokes was significantly (p-value < 0.05) reduced in those artichokes that were treated with a combination of cysteine and EOs (Figure 3b). Fresh-cut artichokes treated with Cys + EOs 75, Cys + EOs 150 and Cys + EOs 300 after 9 days of storage presented significantly lower values of RR (227.75 ± 14.52, 232.45 ± 11.98, 211.27 ± 7.68 mg of CO_2 kg^{-1} h^{-1}) compared to the non-treated ones (311.83 ± 13.20 mg of CO_2 kg^{-1} h^{-1}). Although a previous study reported an increase in the CO_2 concentration of artichokes treated with cysteine and EOs sealed in a PET bag [51], it needs to be considered that the present study was carried out without packaging. Thus, the RR that has been studied was the one from artichokes after being placed on a 1 L glass jar hermetically sealed for 30 min at room temperature. A reduction in the RR was observed in those artichokes treated with cysteine and EOs, an effect that could be attributed to a lower metabolism activity during its storage that has been induced by these EOs, as it has already been reported for apples [42] and table grapes [39,62]. Overall, the application of a combination of cysteine and EOs to fresh-cut artichokes resulted in an improvement in the quality parameters studied.

3.3. Functional Parameters of Fresh-Cut Artichokes

Artichokes are a great source of bioactive compounds for human consumption. The nutritional and pharmaceutical properties of artichokes are linked to their chemical composition, which includes high levels of polyphenolic components and inulin. Derivatives of caffeic acid are the main polyphenolic compounds in artichoke heads, with chlorogenic acid (5-O-caffeoylquinic acid) as the main one, as well as dicaffeoylquinic acid derivatives and neo-chlorogenic acid [2,61,63]. An increased tendency was observed in the total phenolic content (TPC) (Figure 4) during storage, probably due to the concentration caused by the weight loss. Significant differences (p-value < 0.05) between treatments were only detected in those artichokes that were treated with Cys + 150 EOs. After 4 days of storage, a significantly higher TPC (677.46 ± 37 mg of gallic acid 100 g^{-1} FW) was observed in Cys+ 150 EOs treated fresh-cut artichokes compared to the non-treated (499.49 ± 19.25 mg of gallic acid 100 g^{-1} FW). Results were in accordance with the already mentioned study carried out by Rizzo et al. [51], which observed higher levels of TPC in the presence of the applied EOs. The fact that TPC was significantly higher in those artichokes treated with a specific concentration of EOs could be due to an increase in the phenylpropanoid pathway, as previously suggested by Gutiérrez-Pozo et al. [64]. The application of Cys + 150 EOs provided the highest value of TPC compared to the rest of the treatments. As has been previously demonstrated, the application of EOs could be dose-dependent, where an excess of EOs could be harmful to the treated product [65].

The antioxidant activity of artichokes varies among cultivars, age, plant generation, growing conditions, harvest, post-harvest handling, and storage conditions [2,63,66]. The hydrophilic antioxidant activity (H-TAA) is directly linked to the TPC, as previously reported [50,66]. A similar tendency to TPC was observed when the H-TAA was studied in fresh-cut artichokes (Figure 5). After 2 days of storage, the H-TAA of fresh-cut artichokes treated with Cys + 150 EOs was the only treatment where a significantly higher value (657.9 3± 32.07) was observed compared to the non-treated (558.17 ± 36.93). However, Cys + 75 EOs and Cys + 150 EOs H-TAA were significantly higher (p-value < 0.05) than the rest of the treatments after 4 and 9 days of storage (Figure 5a). A similar tendency was observed in the lipophilic antioxidant activity (L-AAT) (Figure 5b), where significantly higher values were observed in those fresh-cut artichokes treated with cysteine and EOs, being in this fraction both highest concentrations of EOs, the one providing the highest values of L-TAA. The H-TAA was 15–20-fold higher than L-TAA due to artichokes, the main responsible compounds for its antioxidant potential, which are of a hydrophilic nature. In addition, hydrophilic non-phenolic compounds, such as ascorbic acid, have been found at relatively high concentrations in the edible portion of the artichoke, contributing to the H-TAA [61].

Furthermore, L-TAA could be attributed to the presence of tocopherol (vitamin E) and lutein, which have been detected in artichokes [67]. Although previous studies observed that the application of cysteine to fresh-cut artichokes did not influence the antioxidant activity [24], the results of the present study are in accordance with Rizzo et al. [44,51], where the antioxidant capacity was studied in artichoke heads treated with *Foeniculum vulgaris* EOs on its own and in combination with cysteine, achieving the highest antioxidant activity after 11 days of storage. This increase in the antioxidant activity in the presence of EOs could be explained due to the high antioxidant capacity that these EOs present, as was previously reported [32,39].

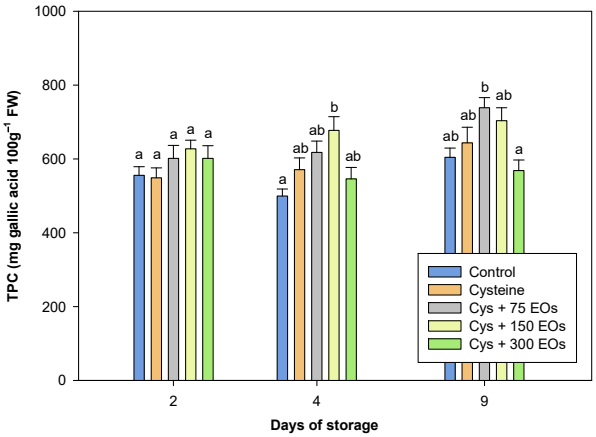

Figure 4. Total phenolic content (mg gallic acid 100 g^{-1}) of non-treated and treated with cysteine, cysteine and 75 µL of EOs (Cys + 75 EOs), cysteine and 150 µL (Cys + 150 EOs), and cysteine and 300 µL (Cys + 300 EOs) fresh-cut artichokes after 2, 4 and 9 days of storage at 2 °C. Significant differences (p-value < 0.05) are presented with different lower-case letters (Tukey's test).

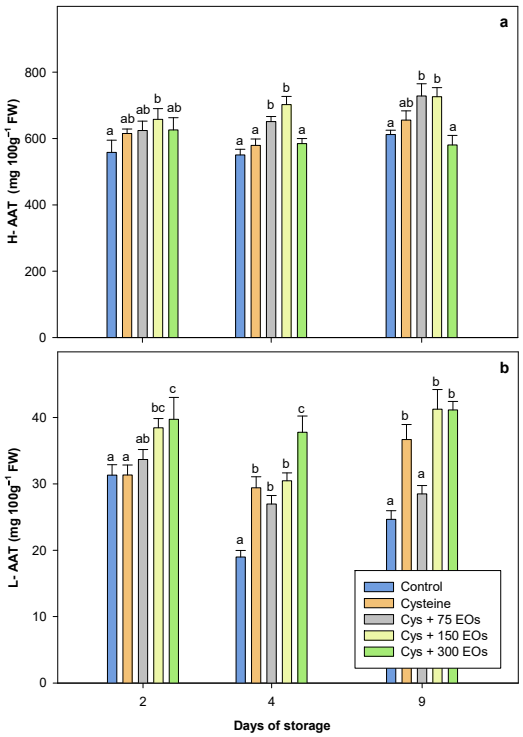

Figure 5. Total antioxidant activity (TAA) in the hydrophilic fraction (**a**) and lipophilic fraction (**b**) of non-treated and treated with cysteine, cysteine and 75 µL of EOs (Cys + 75), cysteine and 150 µL (Cys + 150), and cysteine and 300 µL (Cys + 300) fresh-cut artichokes after 2, 4 and 9 days of storage at 2 °C. Significant differences (p-value < 0.05) are presented with different lower-case letters (Tukey's test).

The increase in these functional phytochemicals as a result of the application of cysteine in combination with different concentrations of EOs also presents a health benefit that has been previously attributed to the bioactive content of artichokes [68,69]. Therefore, the potential use of an edible coating with cysteine and these EOs could result in fresh-cut artichokes with better quality after cold storage (2 °C) and present a higher benefit to human health.

3.4. Sensory Evaluation

The sensory evaluation is essential to ensure the consumer acceptability of those artichokes that were treated with cysteine and different concentrations of EOs. It could also provide key information about possible residual odour or flavour affecting fresh-cut artichokes after the post-harvest treatment with EOs. The evaluation of sensory attributes (colour, browning, dehydrated appearance, odour, sweet, bitter, firmness and general acceptability) in fresh-cut artichokes after 2, 4 and 9 days of storage was presented in Figure 6. The sensory quality, in general, presented a decreasing tendency during storage in all treatments, as has been previously reported [16,19,25]. After 2 days of storage (Figure 6a), colour and browning attributes were assessed with 3 to 5 points, where those artichokes treated with cysteine and the highest concentrations of EOs (150, 300) achieved the highest score. A similar tendency was observed after 4 and 9 days of storage at 2 °C (Figure 6b,c), observing a differentiation between both treatments with Cys + 150 EOs and Cys + 300 EOs, where colour and browning were assessed with the highest score for the intermediate concentration (Cys + 150 EOs). Similar results were observed by Rizzo et al. [51] when they treated fresh-cut artichokes with cysteine and *F. vulgare* EO.

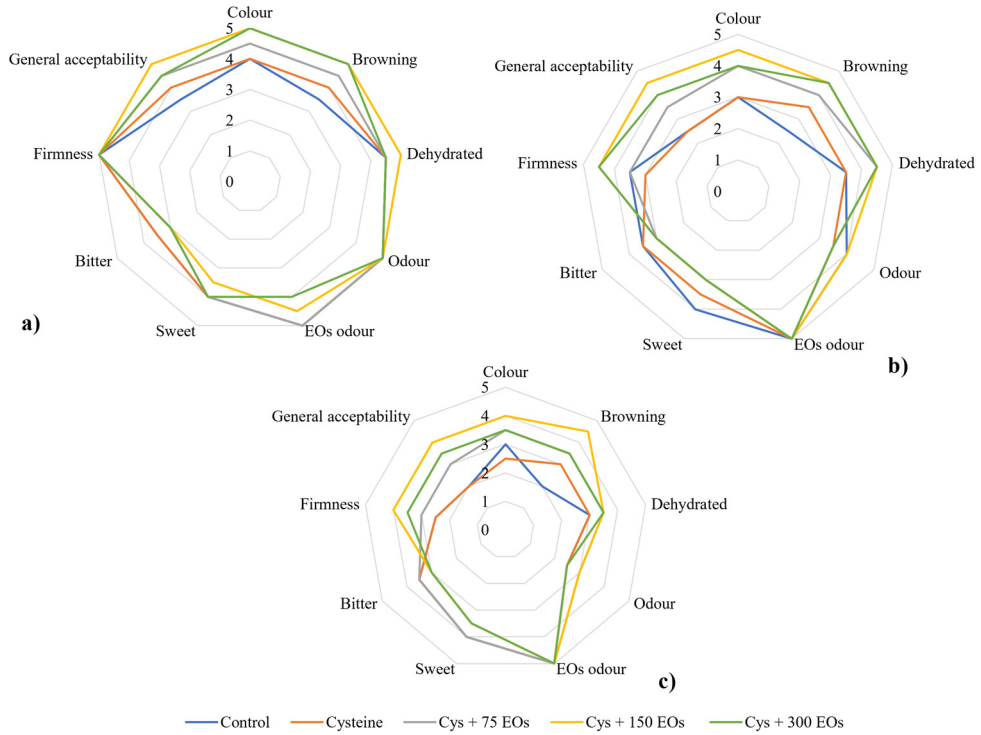

Figure 6. Descriptive sensory analysis of non-treated fresh-cut artichoke heads and treated with cysteine, cysteine and 75 µL of EOs (Cys + 75), cysteine and 150 µL (Cys + 150), and cysteine and 300 µL (Cys + 300) after 2 (**a**), 4 (**b**) and 9 (**c**) days of storage at 2 °C. Colour, browning, dehydrated appearance, odour, sweetness, bitterness, firmness and general acceptability were evaluated.

According to the panellists, no significant differences were observed in the dehydrated appearance, firmness, and odour of fresh-cut artichokes after 2 days of storage. However, after 4 days of storage, firmness and dehydration attributes significantly decreased to a score

of 3 in non-treated and cysteine-treated artichokes. At the end of the storage, Cys + 150 EOs resulted in the one presenting the highest scores of firmness and dehydration. Similar scores were attributed to odour for all the treatments, including the ones presenting the EOs. Besides, residual odour related to EOs was only detected in fresh-cut artichokes treated with the highest concentrations of EOs (150 and 300 µL) after 2 days of storage, while after 4 and 9 days of storage, no residual odour was detected due to the use of a very low concentration, a short time of treatment and their storage in open trays. Opposite results to Rizzo et al. [44], as they observed a higher intensity of fennel odour and flavour in those artichokes that were packed with a small concentration of *F. vulgare*. This difference could be attributed to the storage in hermetically sealed trays, compared to the storage in open trays in the present study. Attributes such as sweetness and bitterness of fresh-cut artichokes were also evaluated, observing similar scores between treatments, with Cys + 150 EOs the one presenting the lowest score. At last, for general acceptability, a similar tendency was observed at all the different stages of the storage period. Those fresh-cut artichokes that were treated with cysteine and 150 µL of EOs presented the highest score, followed by the highest concentration of EOs (300 µL of EOs).

4. Conclusions

The application of EOs in fresh-cut artichokes in combination with cysteine could significantly reduce browning, improve the overall quality, as well as increase the shelf-life of this ready-to-eat product. In the present study, it has been revealed that the use of cysteine in combination with 150 µL of EOs (thymol, eugenol and carvacrol) considerably reduced browning while maintaining quality parameters, increased shelf-life and resulted in excellent consumer acceptability. Considering that the present study was carried out in fresh-cut artichokes that were stored in open trays at 2 °C for 9 days, the use of cysteine and these EOs, in combination with an optimal packing system, could improve their quality and shelf-life.

Author Contributions: Conceptualisation; P.J.Z. and F.G. Data curation; P.J.Z., M.J.G. and V.S.-E. Formal analysis; M.G.-B., F.G., P.J.Z. and M.J.G. Funding acquisition; F.G. and P.J.Z. Investigation; M.J.G., M.G.-B. and M.G.-P. Methodology; M.J.G., P.J.Z. and M.G.-P. Project administration; P.J.Z. and F.G. Resources; V.S.-E. and M.G.-P. Software; M.G.-P. and M.G.-B. Supervision; M.J.G., P.J.Z. and F.G. Validation; P.J.Z. and M.J.G. Visualisation; P.J.Z. and F.G. Roles/Writing—original draft; P.J.Z. and M.G.-P. Writing—review and editing; M.J.G., F.G. and V.S.-E. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is contained within the article.

Acknowledgments: Adrian Grau and Santiago García for providing the artichokes.

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

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PUBLICATION 2

IMPACT OF FLOWER HEAD ORDER AND PHENOLIC CONTENT ON THE QUALITY OF THREE DIFFERENT ARTICHOKE CULTIVARS FOR FRESH-CUT PRODUCTS

(Literal Transcription)

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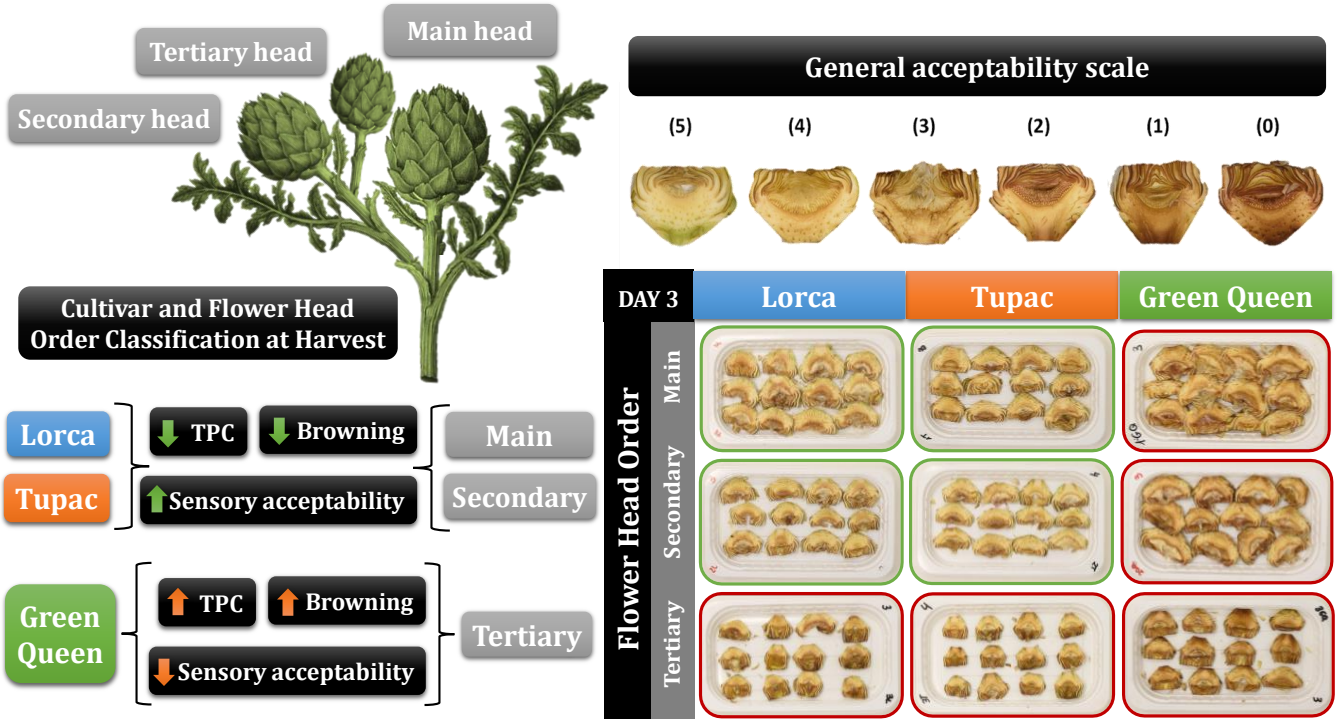
Agronomy 2025, *15*(2), 322



https://doi.org/10.3390/agronomy15020322

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Article

Impact of Flower Head Order and Phenolic Content on the Quality of Three Different Artichoke Cultivars for Fresh-Cut Products

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Abstract: The growing consumer interest in healthy and convenient food has led to an increased demand for fresh-cut vegetables, including artichokes, which are known for their bioactive compounds like antioxidants and polyphenols. However, artichokes are highly susceptible to browning, as their high phenol content complicates processing into ready-to-eat products. This study evaluated the suitability of three artichoke cultivars ('Lorca', 'Tupac', and 'Green Queen') for fresh-cut processing, focusing on flower head order (main, secondary, tertiary). Artichokes were processed as fresh-cut hearth slices, stored for 7 days at 2 °C and 85% relative humidity, and assessed for total phenolic content (day 0) and browning index (days 0, 1, 3, 5, 7). Sensory analysis was performed until day 3. Results revealed that tertiary heads, especially from the 'Green Queen' cultivar, exhibited the highest phenolic content but also greater susceptibility to browning, whereas main heads, lower in polyphenols (particularly from the 'Lorca' and 'Tupac' cultivars), showed minor browning indices and higher consumer acceptability. These parameters displayed a strong negative correlation coefficient (>−0.9), indicating that an increase in browning directly impacts sensory rejection. This study highlights the importance of cultivar and flower head order selection, providing valuable insights to enhance the quality and commercial viability of minimally processed artichoke products.

Keywords: ready-to-eat; shelf life; browning; sensory evaluation; post-harvest; polyphenols; correlation coefficient

Received: 10 December 2024
Revised: 17 January 2025
Accepted: 24 January 2025
Published: 27 January 2025

Citation: Giménez-Berenguer, M.; Giménez, M.J.; Serna-Escolano, V.; Zapata, P.J. Impact of Flower Head Order and Phenolic Content on the Quality of Three Different Artichoke Cultivars for Fresh-Cut Products. *Agronomy* **2025**, *15*, 322. https://doi.org/10.3390/agronomy15020322

1. Introduction

Artichokes (*Cynara cardunculus* L. var. *scolymus* (L.)) are a vital crop in the Mediterranean region, known not only for their culinary uses but also for their high nutritional and medicinal value [1]. They are a rich source of bioactive compounds, particularly polyphenols, flavonoids [2–5], inulin, vitamins, minerals, and fiber [6–10], which contribute to their antioxidant, anti-inflammatory, and hepatoprotective properties [11–13]. The health benefits associated with artichokes, such as promoting cardiovascular health, aiding digestion, and supporting liver function [14–16] have increased their demand in the global market, particularly in the form of fresh-cut, ready-to-eat products [17]. However, due to the morphology of artichokes, the preparation of this type of products may be quite challenging since the most used part is the heart of the artichoke. When peeling an artichoke to obtain only the heart, a significant portion of the plant (approximately 80–85% of the total harvested biomass) is discarded as waste [18,19] in the processing of fresh-cut artichoke

products. This waste typically consists of inedible components, such as the outer external bracts, stem, leaves, and fuzzy choke [18,20–22]. The larger, globe-type artichokes tend to have a slightly lower waste percentage due to their thicker, meatier hearts, while smaller or more fibrous varieties may result in higher waste. In this sense, the development of processed artichokes as fresh-cut products would serve a dual purpose. On the one hand, it would increase the convenience of using this product for consumers by reducing the waste generated from its use in the home, which, in turn, would increase its consumption by avoiding the high percentage of discarded plant waste, complexity of preparation, and trimming operations. On the other hand, these same residues generated from processing could be used as by-products in the food industry.

As previously indicated, artichokes are highly abundant in bioactive compounds. Although the health-promoting benefits mentioned earlier cannot be attributed to a single compound, several active compounds provide additive or synergistic pharmacologic effects. In this sense, polyphenols, especially hydroxycinnamic acid derivatives, represent a significant fraction of the whole secondary metabolites. As reported by several authors, 5-O-caffeoylquinic acid (5-CQA) or chlorogenic acid is the most abundant compound within the caffeoylquinic derivatives, followed by 3,5-di-O-caffeoylquinic acid (3,5-diCQA) and 3,4-di-O-caffeoylquinic acid (3,4-diCQA). Regarding the minor hydroxycinnamic acids, 3-O-caffeoylquinic acid (3-CQA) or neochlorogenic acid, 1,3-di-O-caffeoylquinic acid (1,3-diCQA) or cynarine, and 4,5-di-O-caffeoylquinic acid (4,5-diCQA) are also present in artichoke cultivars [3,10,23–25]. Furthermore, luteolin derivates are also present in artichokes, with luteolin 7-O-glucuronide (Lut 7-gluc) being a major compound identified in artichoke cultivars, followed by luteolin 7-O-glucoside (Lut 7-glc) and luteolin 7-O-glucuronide 3-O-glucoside (Lut 7-gluc 3-glc), as has been described by other authors [3,7,25].

The intricate relationship between these phenolic compounds, their biological activity, and their susceptibility to browning remains insufficiently understood due to the complexity of these interactions. Nonetheless, it is well established that despite their exceptional nutritional value, artichokes are particularly prone to browning, as their elevated phenol content complicates processing into ready-to-eat products [26–33]. Artichokes present considerable challenges in post-harvest management due to their rapid perishability and susceptibility to quality deterioration, particularly when processed for fresh-cut consumption [26–28]. One of the primary issues with fresh-cut artichokes is enzymatic browning, a reaction caused by polyphenol oxidase (PPO) that occurs when plant tissues are damaged during processing [29,30]. The exposure of polyphenolic compounds to oxygen leads to oxidation and the formation of brown pigments, significantly affecting the visual quality of the product [26,28,30–33]. This browning not only reduces the marketability of fresh-cut artichokes but also compromises their perceived freshness and nutritional value, which are key factors in consumer preferences for minimally processed products [33–36]. In addition to browning, other post-harvest challenges include microbial growth, which can be exacerbated by the high moisture content of artichokes [37]. The combination of enzymatic browning and microbial spoilage further complicates the maintenance of visual and microbiological quality during storage [38]. Furthermore, the structural integrity of artichoke tissues is compromised during cutting, leading to moisture loss, texture degradation, and an accelerated decline in sensory attributes such as taste and color [26–28,30]. These factors severely limit the shelf life of fresh-cut artichokes, making it challenging to maintain their quality over extended periods of storage.

Therefore, the increasing demand for convenient, ready-to-eat products has driven research efforts toward developing innovative post-harvest technologies to extend the shelf life of fresh-cut artichokes while preserving their sensory and nutritional qualities. Approaches such as the use of controlled atmosphere packaging, chemical treatments,

anti-browning agents, edible coatings, and innovative packaging (used separately or in combination) [26,30,34,35,37,39–42] have been explored to mitigate browning and delay the degradation processes. However, the effectiveness of these treatments may vary depending on factors such as the cultivar, the harvest date, the agronomic management, and the environmental conditions, among others [3,13,23,43–45] which determine the phenolic content, a key factor in browning. In this sense, some researchers have evaluated the genetic variation among artichoke genotypes that may affect their suitability for fresh-cut products and overall quality [26,35,38,46,47]. In addition, some studies have reported that flower head order has a profound impact on their biochemical composition, particularly phenolic compounds, which are critical determinants of post-harvest quality [3–5,28].

Understanding the physiological and biochemical changes that occur in artichokes after cutting and storage is crucial for optimizing post-harvest interventions and ensuring the successful commercialization of minimally processed artichokes. In this sense, one previous study [28] focused on the characterization of the 'Lorca' cultivar's aptitude for minimally processed products. While this earlier study identified key factors influencing phenolic content (such as flower head order and internal development stage), it only evaluated entire artichokes and measured browning over a very short timeframe (up to 180 s) using PPO activity and initial browning index values. No extended refrigerated storage or fresh-cut processing was assessed, nor was sensory analysis conducted. In the research of Giménez et al., (2021) [5], the focus shifts to the cultivar 'Blanca de Tudela.' This study evaluated whole artichokes across different flower head orders and harvest times during a full growing season, with a particular emphasis on long-term storage (up to 21 days). However, it did not involve fresh-cut processing, which limits its direct applicability to minimally processed product development. Therefore, as far as we are concerned, our study is the first to integrate these findings with sensory acceptance data for fresh-cut artichokes stored under refrigerated conditions for up to 7 days.

This study aimed to address these knowledge gaps by evaluating the performance of three different artichoke cultivars ('Lorca', 'Tupac', and 'Green Queen'), attending to their flower head order to determine the suitability of each for the potential development of a fresh-cut products, with particular attention to phenolic content, browning evolution, and overall quality preservation over time.

2. Materials and Methods

2.1. Plant Material and Experimental Design

For this experiment, three different seed-propagated white artichoke cultivars with green heads were selected: 'Green Queen', which is a hybrid cultivar, and 'Lorca' and 'Tupac', which are both open-pollinated cultivars. For the trial, the artichokes were classified for every cultivar according to the flower head order: main, secondary, and tertiary heads. Standard agronomic practices common in Southeast Spain were followed, including the use of fungicides and insecticides throughout the growing cycle, and the application of fertilizers (250 kg N, 120 kg P_2O_5, and 300 kg K_2O per ha) via drip irrigation. Gibberellic acid was not applied.

Artichokes from each cultivar were harvested when the flower heads were firm and tightly closed and reached their typical commercial size. Therefore, different harvest dates were conducted according to the availability of artichokes. Main and secondary heads of the three cultivars were harvested on 22 February 2022. Tertiary heads of the 'Lorca' and 'Tupac' cultivars were collected on 4 March 2022, and finally tertiary heads of the 'Green Queen' cultivar were collected on 14 March 2022.

2.2. Post-Harvest Processing

After harvest, artichokes were transported to the laboratory of Miguel Hernandez University and 10 artichokes of each cultivar and head order were separated to perform the total phenolic content analysis.

The rest, attending to the cultivar and the flower head order, were peeled until obtaining the artichoke hearts, which were cut into slices. Then, they were disinfected using a solution of water and food-grade bleach of sodium hypochlorite (NaClO) at a concentration of 100 ppm where they stayed for 5 min to remove any possible surface contaminants. After disinfection, the artichoke hearts were carefully rinsed with abundant water, centrifuged using a manual centrifuge to remove excess moisture, and air dried. Once dry, they were placed in polyethylene plastic trays. In this way, 10 trays of each cultivar and flower head order were filled, with 12 slices of artichoke hearts each, making a total of 90 trays. Finally, the trays were stored under controlled conditions at a temperature of 2 °C and a relative humidity of 85% for a period of 7 days (Figure 1).

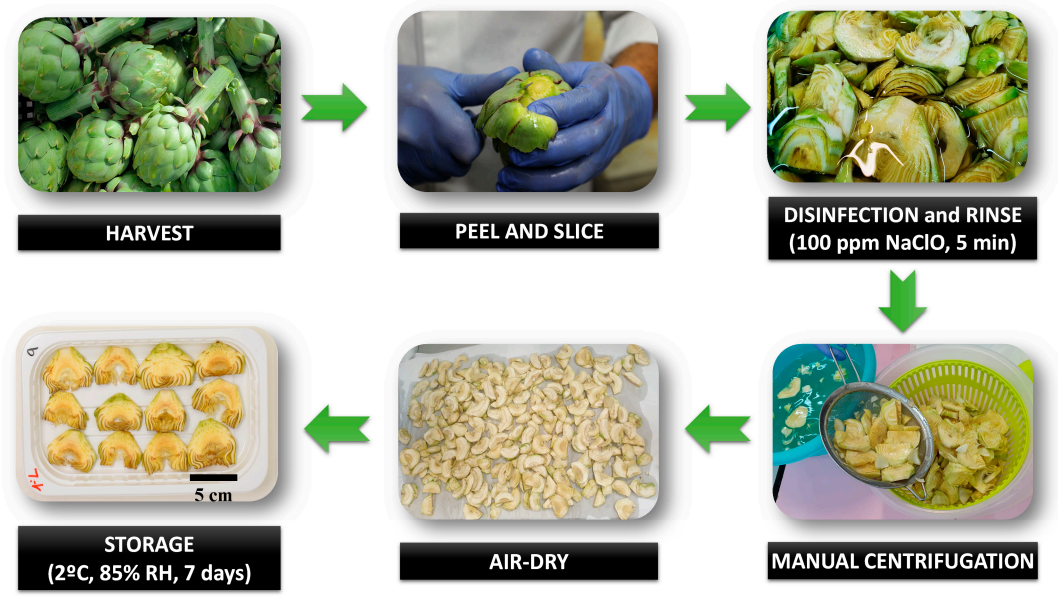

Figure 1. Flowchart of fresh-cut artichoke processing.

2.3. Extraction and Quantification of Total Phenolic Compounds

Phenolic compounds were extracted following the method described by Giménez-Berenguer et al. [28]. In short, 5 g of the edible portion of each sample (including the heart and inner bracts) were blended with 15 mL of 80% methanol solution containing 2 mM sodium fluoride (NaF) to deactivate polyphenol oxidase and prevent phenolic degradation. The homogenization was conducted for 2 min using an Ultra-Turrax® homogenizer (model TP 18, IKA, Staufen, Germany). After that, the samples were centrifuged at 10,000× *g* for 15 min at 4 °C. The resulting supernatant was used for quantification of total phenolic content using the Folin–Ciocalteu reagent according to Giménez-Berenguer et al. [28]. Briefly, duplicate replicates were prepared alongside a blank containing 500 µL of phosphate buffer. For the samples, 300 µL of phosphate buffer and 200 µL of the extract were added to each test tube. In cases where necessary, the extracts were diluted with phosphate buffer to ensure optimal absorbance readings. A 2.5 mL of a 1:10 diluted Folin–Ciocalteu reagent was then added to each tube, followed by thorough mixing. After 3 min of reaction time, 2 mL of sodium carbonate solution was introduced to the mixture. The tubes were mixed again and incubated in a water bath at 50 °C for 5 min. The absorbance of the samples was measured at 760 nm using a spectrophotometer UV-1700 PharmaSpec (Shimadzu, Kyoto,

Japan) with plastic cuvettes of 2.5 mL capacity. The blank sample was used to zero the instrument. The results (mean ± standard error) were expressed as grams of gallic acid equivalent per kilograms of fresh weight (FW).

2.4. Evaluation of Fresh-Cut Artichoke Browning

The color of each slice of artichoke was measured individually on both sides using a Minolta colorimeter (CR-400; Konic Minolta, Osaka, Japan) in triplicate. The L*, a*, and b* values that corresponded to the lightness, greenness/redness, and blueness/yellowness, respectively, of the samples were evaluated at room temperature, after calibrating the instrument using a white plate.

Browning evaluation was performed using the browning index (% BI), which was calculated with Equation (1) [48,49] presented below, and the results were expressed as mean ± SE.

$$\%\ \text{browning index (\% BI)} = \frac{\langle 100(x - 0.31)\rangle}{0.17} \quad (1)$$

where (in Equation (1)) x:

$$x = \frac{(a^* + 1.75\,L^*)}{5.645L^* + a^* - 3.012b^*}$$

2.5. Photographic Image-Based Assessment

To record the visual evolution during the conservation, images of the ready-to-eat artichoke were captured using a digital camera (Nikon D3400, Minato, Tokio, Japan) in a light box with a white background. The camera settings were as follows: light provided by two LEDs with a color temperature of 5600 K, ISO−100, speed of 1/5 s, length of 35 mm, and focal aperture (f) of 20. For each artichoke sample, images on both sides were taken post-processing and on days 1, 3, 5, and 7 of refrigerated storage.

2.6. Sensory Evaluation of General Acceptability

An evaluation of general acceptability of different artichoke head orders (main, secondary, and tertiary heads) of 'Lorca', 'Tupac', and 'Green Queen' cultivars was performed in triplicate by a trained panel of 10 judges (5 males and 5 females) from the Department of Agri-Food Technology of Miguel Hernández University (Orihuela, Alicante, Spain) on day 0, day 1, and day 3 of storage at 2 °C. The overall acceptability assessment was scored using a 6-point hedonic scale with increments of 0.5, based on a visual scale made with photos taken previously for this trial (Figure 2). The description of each sensory score is defined in Table 1. A score of 3 or less indicates the end of its shelf life, since the visual appearance does not meet the quality criteria. Results were expressed as mean ± SE.

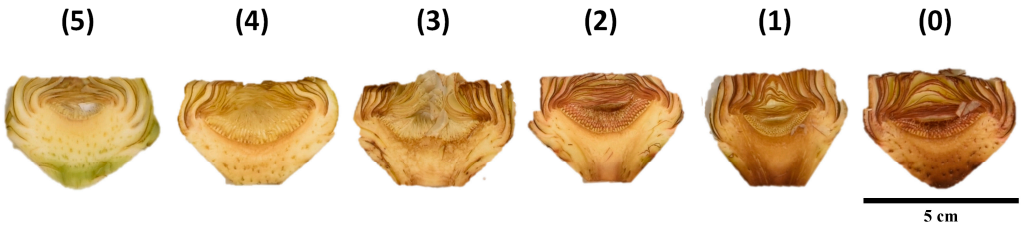

Figure 2. General acceptability scale: (**5**) optimum quality; (**4**) moderate quality; (**3**) insufficient quality; (**2**) poor quality; (**1**) very poor quality; (**0**) extremely poor quality.

Table 1. Description of the characteristics of each sensory score used to evaluate fresh-cut artichokes.

Sensory Score	Description
(5) = Optimum quality	No Browning—Excellent Freshness: Artichoke heart slice appears in perfect condition, entirely free of browning, retaining its original pale green color. There are no visible signs of dehydration, and the surface looks plump and moist. This indicates excellent freshness and quality.
(4) = Moderate quality	Slight Browning—Good Freshness: Artichoke heart slice shows a very slight brown tinge, barely noticeable. Browning is minimal, with only a few spots showing any darkening. There are no signs of severe dehydration or deterioration, and the surface still looks relatively moist.
(3) = Insufficient quality	Light Brown—Not Fresh: The artichoke heart slice displays a light brown color, but it is spread throughout all the sample. The color suggests initial quality deterioration and not freshness. A score of 3 or less indicates the end of the shelf-life.
(2) = Poor quality	Moderate Brown—Slight Deterioration: The artichoke heart slice exhibits a moderate brown color, indicating mild browning. Browning is present and extensive, with some areas showing more intense coloration than others. The surface shows slight signs of quality deterioration.
(1) = Very poor quality	Dark Brown—Moderate Deterioration: The artichoke heart slice shows a deep brown color, indicating moderate browning. Browning is noticeable across the surface, suggesting high levels of oxidation. The surface appears moderately deteriorated.
(0) = Extremely poor quality	Very Dark Brown—Severe Deterioration: The artichoke heart slice appears very dark brown, almost black, with extensive browning throughout the surface. This indicates severe oxidation and deterioration. The surface looks highly deteriorated. This suggests poor freshness and a very unappealing visual appearance.

2.7. Statistical Analysis

All data were subjected to an analysis of variance (ANOVA). For total phenolic content, a two-way factorial combination design was applied, considering cultivars (3) and flower head orders (3). For browning index and sensory evaluation data were examined using a three-way factorial combination design, incorporating cultivars (3), flower head orders (3) and days of storage (5). Results were expressed as mean square (percentage of total) per each source of variation (% MS). Significant differences were presented with F-values (** $p \leq 0.05$ and *** $p \leq 0.01$). When ANOVA assumptions were found to be valid, mean comparisons were performed using Duncan's multiple-range test to detect significant differences ($p \leq 0.05$). Pearson's test was also conducted to determine the correlation coefficient between the browning index and sensory analysis. All analyses were performed with SPSS software package v. 20 for Windows (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Total Phenolic Content at Harvest

The ANOVA results indicated that both the cultivar and the flower head order significantly influenced the total phenolic content of artichokes (Table 2). The cultivar explained 50.8% of the variation, while flower head order contributed 41.4%, both highly significant, with F-values of 89.691 and 73.053, respectively (*** $p \leq 0.01$). In contrast, the interaction between cultivar and head order contributed 7.8% to the overall variance. This suggests that the phenolic content is primarily determined by genetic factors, although the position of the flower head also plays a substantial role.

Table 2. Mean square (percentage of total) per each source of variation (% MS) resulting from analysis of variance of the total phenolic content.

Source of Variation	Total Phenolic Content		
	% MS	F-Value	Significance Level
Cultivar (C)	50.8	89.691	***
Flower Head Order (H)	41.4	73.053	***
(C) x (H)	7.8	13.824	***

Significant differences are presented, with F-values and asterisks denoting the significance level (*** $p \leq 0.01$).

As shown in Table 3, the 'Green Queen' cultivar exhibited the highest mean total phenolic content (3.73 g kg^{-1} FW), which was significantly higher than that of 'Lorca' (2.36 g kg^{-1} FW) and 'Tupac' (2.34 g kg^{-1} FW). When considering the mean values of the flower head order, regardless of the cultivar, the tertiary heads presented the highest phenolic content (3.48 g kg^{-1} FW), followed by secondary heads (2.66 g kg^{-1} FW) and main heads (2.13 g kg^{-1} FW). These results underscore the importance of selecting both cultivar and flower head order for achieving better results in the industrial process.

Table 3. Mean total phenolic content at harvest (g kg^{-1} FW) for each cultivar and mean total phenolic content for each flower head order of all three cultivars together, respectively.

	Total Phenolic Content (g kg^{-1} FW)		
CULTIVAR	Lorca	Tupac	Green Queen
	2.36 ± 0.08 a	2.34 ± 0.09 a	3.73 ± 0.16 b
FLOWER HEAD ORDER	Main	Secondary	Tertiary
	2.13 ± 0.07 a	2.66 ± 0.10 b	3.48 ± 0.16 c

Data are the mean ± SE. Different lowercase letters show significant differences in the phenolic content of each cultivar and mean flower head order respectively, according to Duncan test at $p \leq 0.05$.

The interaction between cultivar and head order (Figure 3) further highlights this trend, as tertiary heads of 'Green Queen' contained the highest phenolic levels (4.83 g kg^{-1} FW), while main heads of 'Lorca' and 'Tupac' had the lowest (1.95 and 1.93 g kg^{-1} FW, respectively). In addition, the statistical analysis reveals that the main heads of the 'Green Queen' cultivar (2.49 g kg^{-1} FW), which have the lowest phenolic content within this cultivar, were comparable to the tertiary heads of the 'Lorca' and 'Tupac' cultivars, which exhibit the highest phenolic content in their respective cultivars (2.75 and 2.63 g kg^{-1} FW, respectively). This indicates that the phenolic content of the least phenolic-rich heads of 'Green Queen' is on par with the most phenolic-rich heads of 'Lorca' and 'Tupac', highlighting a noteworthy difference in phenolic content between these cultivars despite their flower head order. Nevertheless, the significant interaction between these factors indicates that both genotype and head order should be carefully considered when selecting artichokes for their suitability as fresh-cut products.

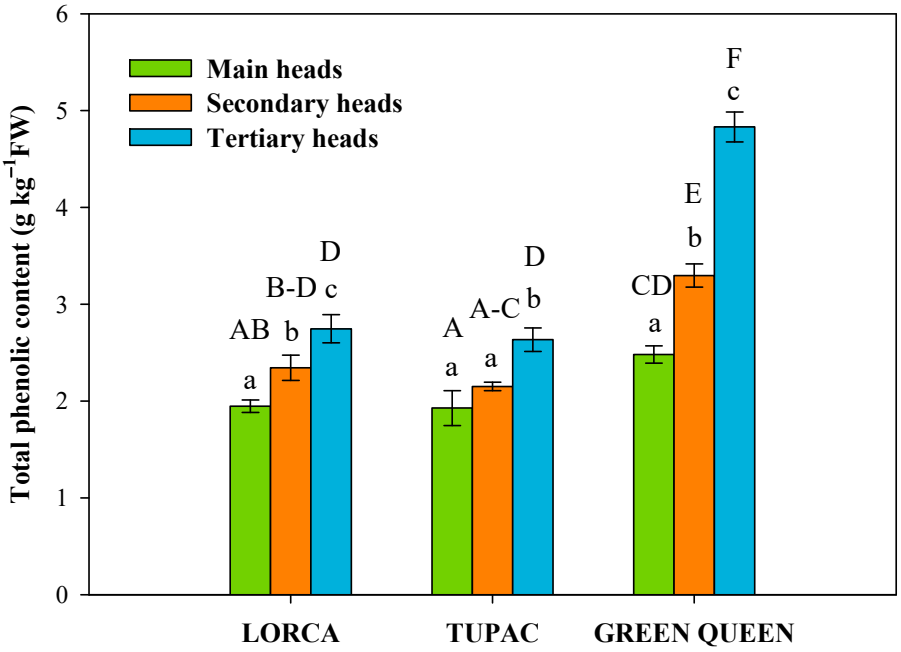

Figure 3. Total phenolic content (g kg^{-1} FW) for different artichoke head orders (main, secondary, and tertiary heads) of 'Lorca', 'Tupac', and 'Green Queen' cultivars at harvest. Different lowercase letters show significant differences between flower head orders of each cultivar individually, according to Duncan test at $p \leq 0.05$. Different capital letters show significant differences between the interaction of flower head orders and all cultivars, according to Duncan test at $p \leq 0.05$. Data are the mean ± SE.

3.2. Browning Evolution of Fresh-Cut Artichokes During Storage

The browning evolution, expressed as the browning index (%), is a critical factor that affects the visual quality and marketability of fresh-cut artichokes, limiting their shelf life. Analysis of variance shows that the browning index was significantly influenced by cultivar, flower head order, and storage duration (Table 4). The cultivar explained 35.72% of the variation, with 'Green Queen' having the highest mean browning index (52.92%), significantly greater than 'Lorca' (45.18%) and 'Tupac' (44.32%) (Table 5). Flower head order accounted for 35.24% of the variation (Table 4), with tertiary heads showing the most browning (52.82%), followed by secondary heads (45.51%) and main heads (44.36%) (Table 5). Storage time also played a significant role in browning progression, contributing 27.93% of the variation (Table 4). Browning increased progressively over 7 days of storage, with significant differences observed at each time point (Table 5). On day 0, the browning index was the lowest (40.50%), and it increased steadily to 54.29% by day 7.

Table 4. Mean square (percentage of total) per each source of variation (% MS) resulting from analysis of variance of the percentage of browning index.

Source of Variation	% Browning Index		
	% MS	F-Value	Significance Level
Cultivar (C)	35.72	393.859	***
Flower Head Order (H)	35.24	388.587	***
Days of Storage (D)	27.93	307.990	***
(C) x (H)	0.35	3.853	***
(C) x (D)	0.30	3.259	***
(H) x (D)	0.21	2.281	**
(C) x (H) x (D)	0.25	2.733	***

Significant differences are presented, with F-values and asterisks denoting the significance level (** $p \leq 0.05$ and *** $p \leq 0.01$).

Table 5. Browning evolution (% mean browning index) for different artichoke flower head orders (main, secondary, and tertiary heads), cultivars ('Lorca', 'Tupac', and 'Green Queen'), and day of storage, respectively, and for the interaction of cultivars and flower head orders.

	% Browning Index		
Cultivar	Lorca	Tupac	Green Queen
	45.18 ± 0.31 b	44.32 ± 0.35 a	52.92 ± 0.36 c
Head Order	Main	Secondary	Tertiary
	44.36 ± 0.32 a	45.51 ± 0.33 b	52.82 ± 0.38 c
Days of Storage	D0	40.50 ± 0.35 a	
	D1	44.59 ± 0.44 b	
	D3	47.31 ± 0.44 c	
	D5	51.07 ± 0.42 d	
	D7	54.29 ± 0.43 e	
Cultivar x Flower Head Order	Lorca	Tupac	Green Queen
Main	43.15 ± 0.47 a	40.20 ± 0.51 a	49.74 ± 0.52 a
Secondary	43.48 ± 0.51 a	42.35 ± 0.45 b	50.42 ± 0.55 a
Tertiary	49.31 ± 0.55 b	50.23 ± 0.59 c	58.96 ± 0.61 b

Data are the mean ± SE. Different lowercase letters show significant differences between cultivars and flower head orders, according to Duncan test at $p \leq 0.05$.

The browning evolution resulting from the interaction between cultivars ('Lorca', 'Tupac', and 'Green Queen'), head orders (main, secondary, tertiary), and storage days (D0–D7), exhibited significant differences (Table 6, Figure 4). On day 0, the main heads of 'Tupac' (33.76%) presented the lowest browning index, significantly differing from the main heads of 'Lorca' (37.81%) and 'Green Queen' (41.43%). In contrast, the tertiary heads of 'Green Queen' at D0 had the highest browning index (51.38%), indicating a much greater susceptibility to browning after processing compared to both 'Lorca' and 'Tupac' (42.73% and 40.48%, respectively).

Table 6. Browning evolution (% browning index) for the interaction of cultivars ('Lorca', 'Tupac', and 'Green Queen'), artichoke head order (main, secondary, and tertiary heads), and day of storage interrelated.

	% Browning Index								
C x H x D	Lorca			Tupac			Green Queen		
	Main	Secondary	Tertiary	Main	Secondary	Tertiary	Main	Secondary	Tertiary
D0	37.81 ± 0.52 b–d	37.42 ± 0.74 bc	42.73 ± 0.74 f–h	33.76 ± 0.78 a	36.54 ± 0.61 b	40.48 ± 1.12 d–g	41.43 ± 0.70 fg	42.19 ± 0.58 f–h	51.38 ± 0.98 p–r
D1	39.67 ± 0.80 c–f	40.05 ± 0.63 c–f	45.13 ± 0.90 h–l	35.50 ± 0.54 ab	38.06 ± 0.55 b–e	48.14 ± 0.81 l–o	46.64 ± 0.77 j–l	47.47 ± 0.77 k–n	59.58 ± 1.51 u–w
D3	40.94 ± 0.75 e–g	41.24 ± 0.90 fg	51.08 ± 0.94 o–q	39.95 ± 0.91 c–f	43.39 ± 0.89 g–i	49.91 ± 1.03 m–p	49.69 ± 0.84 m–p	49.78 ± 1.06 m–p	60.14 ± 1.33 vw
D5	47.10 ± 0.96 j–m	46.04 ± 0.86 i-l	53.61 ± 1.02 q–s	44.87 ± 0.91 h–k	44.35 ± 1.00 h–j	54.85 ± 0.90 st	54.07 ± 1.09 r–t	54.93 ± 1.09 st	61.28 ± 1.21 wx
D7	50.26 ± 0.97 n–p	52.66 ± 1.06 p–s	58.30 ± 1.08 uv	46.93 ± 1.09 j–m	47.67 ± 1.07 k–n	57.79 ± 1.12 uv	56.85 ± 0.84 tu	57.74 ± 1.05 uv	63.01 ± 1.00 x

Data are the mean ± SE. Different lowercase letters show significant differences between cultivars and flower head orders at harvest and during 7 days of storage at 2 °C, according to Duncan test at $p \leq 0.05$.

As storage progressed, browning increased significantly in all cultivars. By day 7, the tertiary heads of 'Green Queen' reached the highest browning levels (63.01%), substantially surpassing those of 'Tupac' (57.79%) and 'Lorca' (58.30%). These data show a clear trend where the tertiary heads across all cultivars consistently exhibited higher browning compared to the main and secondary heads.

Among all combinations, the main heads of 'Tupac' showed the slowest increase in browning, with values rising from 33.76% on day 0 to 46.93% on day 7, while the tertiary

heads of 'Green Queen' showed the sharpest increase, from 51.38% at harvest to 63.01% by the end of the storage period.

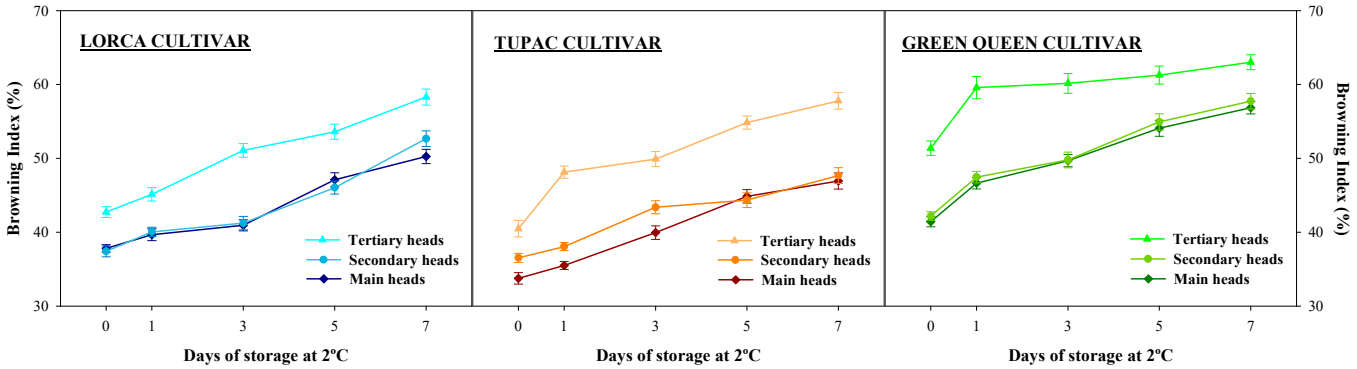

Figure 4. Browning evolution (% browning index) for different artichoke head orders (main, secondary, and tertiary heads) of 'Lorca', 'Tupac', and 'Green Queen' cultivars at harvest and during 7 days of storage at 2 C. Data are the mean ± SE.

Pictures of the progression of browning in artichoke head orders (main, secondary, and tertiary heads) from three cultivars ('Lorca', 'Tupac', and 'Green Queen') over a storage period of 7 days at 2 °C are presented in Figure 5. At the day of processing, all head orders across the three cultivars showed minimal browning, maintaining a fresh appearance (D0—green color). The main heads appeared the most vibrant, followed by secondary and tertiary heads, which were also well-preserved but slightly less uniform in color. On day one, a slight onset of browning was observed, particularly in the secondary and tertiary heads of 'Green Queen' (D1—red color). 'Lorca' and 'Tupac' maintained a relatively fresh appearance, though subtle discoloration was noticeable in tertiary heads (D1—orange color). On the third day, browning became more prominent across all cultivars, with secondary and tertiary heads showing more intense discoloration (D3—red color) than the main heads. 'Green Queen' exhibited a more advanced degree of browning (D3—red color) compared to 'Lorca' and 'Tupac', whose main heads still showed less susceptibility to the browning process (D3—orange color).

On day five, significant browning was observed in all head orders (D5—red color), with the tertiary heads of all cultivars displaying the most severe discoloration. Main heads of 'Lorca' and 'Tupac' maintained the best appearance, although the differences between head orders were becoming more pronounced. On the last day, browning was extensive in all cultivars and head orders (D7—red color). The tertiary heads had darkened considerably, indicating advanced senescence. However, the main heads of 'Lorca' and 'Tupac' still retained some visual appeal, though browning was evident. 'Green Queen' artichokes showed widespread browning, with tertiary heads almost completely brown.

This image-based assessment showed a clear trend: browning intensified with storage time, and tertiary heads were most susceptible to this deterioration across all cultivars. 'Lorca' and 'Tupac' showed the most resistance to browning, while 'Green Queen' experienced more rapid quality loss.

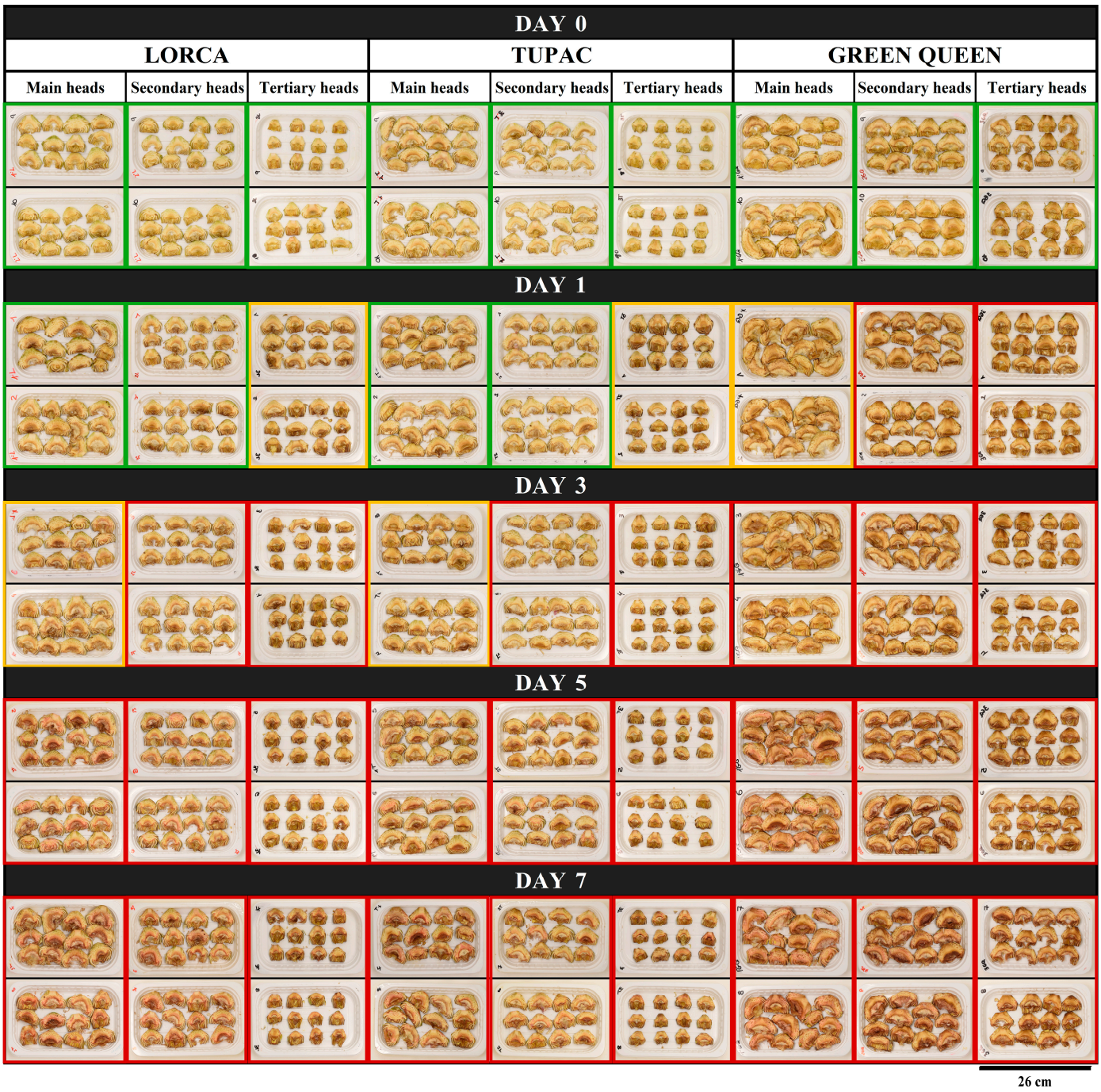

Figure 5. Images of the browning evolution for different artichoke head orders (main, secondary, and tertiary heads) of 'Lorca', 'Tupac', and 'Green Queen' cultivars at harvest and during 7 days of storage at 2 °C. Different frame colors illustrate relevant changes in the browning evolution between cultivars and flower head orders at harvest and during 7 days of storage at 2 °C.

3.3. Sensorial Evaluation of General Acceptability

Sensorial evaluation of general acceptability showed that consumer preference is significantly affected by storage duration, cultivar, and head order (Table 7). Storage days explained 77.39% of the variation, highlighting the importance of freshness in maintaining consumer satisfaction. Cultivar and head order also contributed to differences in acceptability, explaining 13.08% and 8.77% of the variation, respectively.

Table 7. Mean square (percentage of total) per each source of variation (% MS) resulting from analysis of variance of the sensorial evaluation.

Source of Variation	Sensorial Evaluation		
	% MS	F-Value	Significance Level
Cultivar (C)	13.08	1986.059	***
Flower Head Order (H)	8.77	1331.353	***
Days of Storage (D)	77.39	11,747.529	***
(C) x (H)	0.14	21.235	***
(C) x (D)	0.23	34.206	***
(H) x (D)	0.35	52.912	***
(C) x (H) x (D)	0.05	7.162	***

Significant differences are presented, with F-values and asterisks denoting the significance level (*** $p \leq 0.01$).

The sensory results highlight cultivar and head order-dependent differences in the general acceptability of fresh-cut artichokes over storage time (Figure 6), following the same tendency as the browning index, as it was a parameter highly linked to the general acceptability.

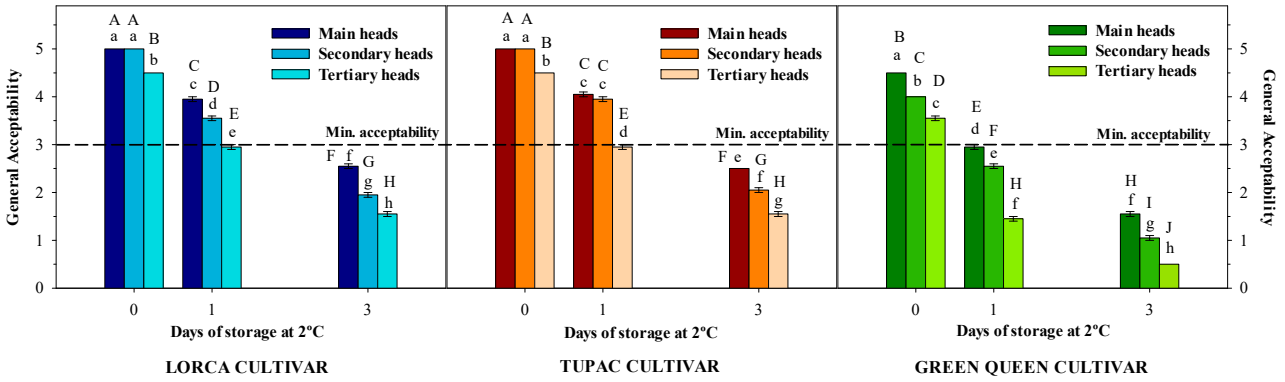

Figure 6. Sensorial evaluation of the general acceptability of different artichoke head orders (main, secondary, and tertiary heads) of 'Lorca', 'Tupac', and 'Green Queen' cultivars on day 0, day 1, and day 3 of storage at 2 °C. Different lowercase letters show significant differences between head orders of each cultivar individually during storage, according to Duncan test at $p \leq 0.05$. Different capital letters show significant differences between the interaction of head orders and all cultivars during storage, according to Duncan test at $p \leq 0.05$. Data are the mean ± SE.

At the day of processing (D0), the 'Lorca' and 'Tupac' cultivars exhibited superior sensory attributes, particularly in the main heads, which maintained the highest quality of all for up to 3 days at 2 °C, compared to the 'Green Queen' cultivar which scored lower in all the flower head orders. On the other hand, tertiary heads showed the greatest decline across all cultivars, with both 'Lorca' and 'Tupac' tertiary heads falling below the acceptable threshold by day 3.

In contrast, the 'Green Queen' cultivar performed poorly across all head orders. Its general acceptability scores for secondary and tertiary heads fell below the minimum threshold as early as day 1. In this sense, none of the samples exceeded the minimum of acceptability on day 3, since no antioxidant was added to delay the degradation process. This was due to the fact that the field of study was the intrinsic aptitude of each sample as a fresh-cut product. Within these outcomes, the 'Green Queen' cultivar obtained the worst results—well below the acceptable quality threshold—indicating significant deterioration.

3.4. Correlation Between Browning and Sensory Evaluation Acceptance

The relationship between the % BI and sensory acceptance scores was evaluated for the three artichoke cultivars ('Lorca', 'Tupac', and 'Green Queen') across all flower head orders

(main, secondary, and tertiary) at harvest and during refrigerated storage (D1 and D3). The calculated correlation coefficient (CC) between the % BI and sensory scores, presented in Table 8, reveals a strong negative correlation for all cultivars and head orders throughout the storage period.

Table 8. Correlation coefficient between % BI values and sensory acceptances evaluation scores during D0, D1, and D3 of refrigerated storage.

CC between % BI and Sensory Evaluation								
Lorca			**Tupac**			**Green Queen**		
Main	**Secondary**	**Tertiary**	**Main**	**Secondary**	**Tertiary**	**Main**	**Secondary**	**Tertiary**
−0.982	−0.971	−0.964	−0.994	−0.989	−0.950	−0.993	−0.973	−0.968

Data of the CC are the mean values of % BI and sensory evaluation scores of different artichoke head orders (main, secondary, and tertiary heads) of 'Lorca', 'Tupac', and 'Green Queen' cultivars on day 0, day 1, and day 3 of storage at 2 °C.

For 'Lorca', the CC values ranged from −0.982 to −0.964, with the main heads exhibiting the strongest correlation (−0.982), indicating that increases in browning were closely linked to declines in sensory acceptance. Similarly, for 'Tupac', CC values ranged from −0.994 to −0.950, with the main heads again showing the strongest relationship (−0.994). In 'Green Queen', the CC values followed the same trend, ranging from −0.993 to −0.968. Across all cultivars, the main heads consistently demonstrated the highest sensitivity to the relationship between browning and sensory rejection, while tertiary heads showed slightly weaker correlations. Despite this, a strong negative correlation is evident as all CC values exceeded −0.9, highlighting a significant inverse relationship between % BI and sensory acceptance across all samples.

4. Discussion

This study assessed the total phenolic content, browning evolution, and sensory acceptability of fresh-cut artichokes from three cultivars ('Lorca', 'Tupac', and 'Green Queen') in relation to flower head order (main, secondary, and tertiary heads) and storage duration. The results reveal that both cultivar and head order, as well as their interactions, significantly affect polyphenol concentration at harvest and the overall postharvest quality of the artichokes, providing essential insights into the suitability of these cultivars for fresh-cut applications.

Several studies have reported that phenolic content varies significantly among cultivars, with factors like developmental stage and agronomic and environmental conditions, among others, playing key roles in phenolic accumulation [4,13,23,28,43–45]. Moreover, artichokes are characterized by a hierarchical organization determined primarily by their growth position within the plant structure, which also influences the phenolic content [3–5,28]. This flower head order is categorized by a primary head or capitulum and multiple secondary and tertiary heads. The primary head is typically the largest and terminates the main stem, whereas the secondary and tertiary capitula are smaller and borne on lateral branches [50]. Annual production consists of a single primary head and up to a maximum of twenty secondary and tertiary heads per plant [16,18].

The findings of the present study align with prior studies by Giménez et al. [3,5] and Giménez-Berenguer et al. [4,28]. It has been corroborated that secondary and tertiary heads of artichokes frequently accumulate higher phenolic compounds than primary heads. This may be due to developmental stresses in these later-forming heads which may activate defense responses, increasing phenolic production [4,51]. Additionally, this research expands on earlier work by evaluating three distinct cultivars ('Lorca', 'Tupac', and 'Green Queen') and exploring their suitability for fresh-cut processing based on flower

head order. This combination of sensory analysis and browning assessment offers practical insights for producers aiming to optimize the development of fresh-cut artichoke products.

The phenolic variability has notable implications for the nutritional and functional quality of artichokes, including cynarine, chlorogenic acid, and flavonoids [2–4,44,52], which are bioactive compounds with several beneficial properties in human health [11,14–16,18,53]. Studies have shown that the content of 5-CQA, the primary hydroxycinnamic acid in artichokes, is significantly higher in tertiary heads, followed by secondary heads and lastly, main heads. This trend extends to both major and minor hydroxycinnamic acids, demonstrating a consistent distribution pattern among head orders [3]. Variability in caffeoylquinic acid derivative levels has also been linked to harvest timing, which aligns with the classification of heads into main, secondary, and processing categories [7]. In the case of luteolin derivatives, Lut 7-gluc content was notably higher in tertiary heads compared to secondary and main heads. Overall, tertiary flower heads have consistently exhibited significantly greater total amounts of individual luteolin derivatives than their secondary and main counterparts [3]. However, this high phenolic content also predisposes these artichokes to more rapid browning during storage, as phenolic compounds serve as substrates for PPO-catalyzed browning reactions, which compromise the visual quality of fresh-cut products [29,30,32,35].

In this study, TPC was measured on day 0 to establish its baseline correlation with browning susceptibility and flower head order. Our prior research [30] evidences no significant changes in TPC during storage under similar conditions for untreated samples up to nine days. Therefore, the measured TPC on day 0 is expected to remain stable throughout the storage period, and the differences observed between flower head orders and cultivars at the initial stage would likely persist over time. Within this investigation, tertiary heads from all cultivars displayed the highest browning indices, with 'Green Queen' showing the most pronounced darkening. Smaller and later-developing heads, such as tertiary heads, experience greater oxidative stress, which induces PPO activity and accelerates browning. This accelerated oxidation process likely due to high phenolic substrate concentration, with PPO activity further contributing to browning [26,28,31,33,34]. Conversely, the slower browning observed in 'Tupac' and 'Lorca', particularly in main heads, suggests that due to reduced phenolic substrates susceptible to oxidation, these cultivars may have lower PPO activity. This could improve the suitability of these artichokes for fresh-cut processing. Similar results in other fresh-cut produce indicate that cultivars with lower PPO activity tend to brown more slowly, potentially extending shelf life and minimizing the need for anti-browning treatments [26,28,35,38,46,47].

Sensory acceptability, a key factor in commercial viability, was highly influenced by browning rates, with panelists preferring the main heads of 'Tupac' and 'Lorca', which exhibited less browning and retained visual appeal for a longer period. In contrast, the rapid browning of 'Green Queen' reduced its sensory acceptability, especially in tertiary heads, reinforcing the challenge of balancing phenolic content with visual quality. This may indicate that visual factors, including browning, play a more significant role in consumer purchasing decisions than nutritional content alone. This tendency was also confirmed by the strong negative correlations observed, highlighting that browning is a critical determinant of sensory rejection. As the % BI values increased over time, sensory acceptance scores declined significantly, reflecting consumer aversion to visual discoloration. In this sense, Starowicz et al. [54] also reported a generally positive correlation coefficients (0.80) between the TPC and browning, proving the importance of these two parameters in determining the quality and marketability of fresh-cut products. This aligns with the findings of the present study, where samples with lower phenolic content and reduced browning susceptibility, particularly those from the main head orders of 'Lorca' and 'Tupac' cultivars, demonstrated

better sensory acceptance. These results emphasize the critical need for selecting appropriate cultivars and processing strategies to minimize browning and maintain sensory appeal, ultimately enhancing the commercial viability of minimally processed artichokes. These outcomes are supported by other studies that have identified browning as a primary factor influencing the marketability of fresh-cut artichokes and, therefore, appearance as a primary determinant of consumer preference [26,28,30,33–36,38,55].

The implications for fresh-cut applications are clear: while higher phenolic content artichokes will have a better nutritional profile, it also may necessitate careful postharvest handling to mitigate browning, especially for high-phenolic cultivars like 'Green Queen', which may require more intensive postharvest treatments to preserve visual appeal. Oppositely, cultivars with slower browning rates, such as 'Tupac' and 'Lorca', could reduce postharvest losses, lower the need for anti-browning agents, and improve marketability, lowering costs for producers and enhancing consumer satisfaction.

5. Conclusions

The challenge of developing fresh-cut artichoke products makes it necessary to define the suitability of the different cultivars and types of artichokes for fresh-cut products. It is essential to characterize and quantify their bioactive compounds, as well as study their browning evolution. The results of this study evidence for the first time that both flower head order and cultivar significantly influence the extent of browning and its impact on sensory perception.

This research underscores the importance of selecting appropriate cultivars and head orders for the development of fresh-cut artichoke products. While 'Green Queen' and tertiary heads offer potential nutritional benefits due to their high phenolic content, their rapid browning limits their applicability in fresh-cut markets and shelf-life without significant intervention.

In contrast, cultivars with lower susceptibility to browning like 'Tupac' and 'Lorca', particularly in their main and secondary heads, may present a more favorable option for extending shelf life and maintaining sensory quality in minimally processed formats. They demonstrated better commercial viability due to their lower browning rates and higher consumer acceptability, making them a more suitable candidate for fresh-cut product applications, diminishing postharvest losses, reducing dependency on anti-browning agents, and increasing market appeal, thereby cutting costs for producers and elevating consumer satisfaction. These findings suggest that choosing cultivars and head orders based on their phenolic content at harvest and browning behavior is essential to optimize the quality and shelf life of fresh-cut artichoke products. Future research should focus on exploring methods to mitigate browning and improve the stability of high-phenolic cultivars like 'Green Queen', and further studying artichokes to allow for the accurate selection of cultivars, and flower heads orders depending on their final destination.

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

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors thank Consellería de Innovación, Universidades, Ciencia y Sociedad Digital de la Generalitat Valenciana for the Ph.D. scholarship of M. Giménez-Berenguer (grant number ACIF/2021/178).

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

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PUBLICATION 3

EVALUATION OF 'LORCA' CULTIVAR APTITUDE FOR MINIMALLY PROCESSED ARTICHOKE.

(Literal Transcription)

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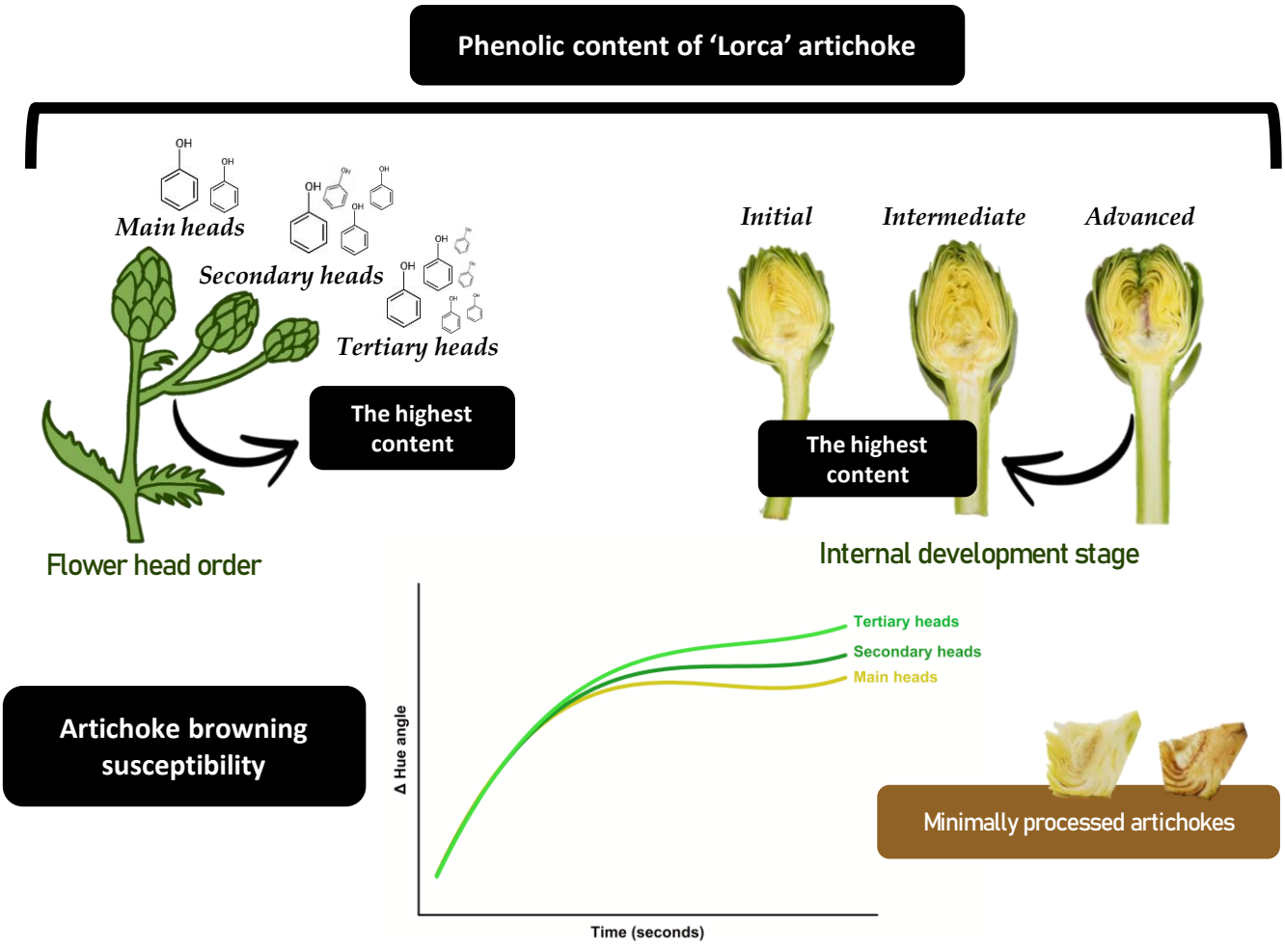
Agronomy 2022, *12*(2), 515



https://doi.org/10.3390/agronomy12020515

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Article

Evaluation of 'Lorca' Cultivar Aptitude for Minimally Processed Artichoke

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Abstract: Previous research works have reported that 'Lorca' artichoke cultivar presents a lower total phenolic content than other cultivars rich in phenolic compounds, which could show a lower susceptibility to enzymatic browning and increase its aptitude for fresh-cut processing. The aim of this study was to analyze the total phenolic content as well as browning evaluation by image analysis and polyphenol oxidase (PPO) enzyme activity in 'Lorca' cultivar in order to characterize the key factors which influence its phenolic levels for minimally processed artichokes. Thus, artichokes were harvested and classified on three head orders (main, secondary, and tertiary), as well as three development stages (initial, intermediate, and advanced). Variance components analysis was carried out for total phenolic content considering three factors: plant, flower head order, and internal development stage. For the first time, the internal development stage has been related to total phenolic content, and results showed that artichoke head order and internal development stage were responsible for a variability of 22.17% and 15.55%, respectively. Main artichoke heads and those at the advanced development stage presented the lowest phenolic concentration as well as the lowest PPO activity; therefore, they exhibit the lowest browning process, which could increase their use in ready-to-eat products at market.

Keywords: total phenol content; browning susceptibility; artichoke head order; internal development stage; postharvest

Citation: Giménez-Berenguer, M.; García-Pastor, M.E.; García-Martínez, S.; Giménez, M.J.; Zapata, P.J. Evaluation of 'Lorca' Cultivar Aptitude for Minimally Processed Artichoke. *Agronomy* **2022**, *12*, 515. https://doi.org/10.3390/agronomy12020515

Academic Editor: Sanja Cavar Zeljkovic and J. Antonio Cortinas Rguez

Received: 29 December 2021
Accepted: 17 February 2022
Published: 18 February 2022

1. Introduction

Globe artichoke, *Cynara cardunculus* L. var. *scolymus* (L.) Fiori, is an herbaceous perennial plant belonging to the family Asteraceae, and it is native from the Mediterranean region. Currently, its production and consumption have spread worldwide since it is a product highly appreciated by consumers, not only due to its flavor, but also because its intake provides benefits for human health and well-being [1]. Nowadays, changes in the society lifestyle have led to an increased demand of minimally processed products considered as added value products in terms of quality, convenience, nutritional value, and ease of preparation [2]. In this sense, processed artichokes as fresh-cut products would provide a good solution to increase their consumption due to the high percentage of discarded plant waste, complexity of preparation, and trimming operations. However, cutting operations increase respiration rate and accelerate senescence and enzymatic browning, leading to a reduction of shelf-life [3].

The nutritional importance of artichoke heads is predominantly related to its high polyphenol content [4,5], mainly hydroxycinnamic acids and flavonoids, such as luteolins [6,7]. However, quantitative and qualitative phenolic profile is influenced by different preharvest factors: genotype, harvest date, environmental conditions, and agronomic

management, among others [5,6,8]. Different authors have reported that environmental conditions influence phenol biosynthesis for artichoke genotypes and parts of the plant, as well as there being a negative correlation between phenolic content and the age of plant tissues [1,9,10]. Significant seasonal fluctuations in both phenolic content and profile were observed, which have been attributed to a combination of ambient air temperature and solar radiation [11,12]. Similarly, Lombardo et al. [13] observed that phenol concentration was higher in artichokes harvested in the spring than winter-harvested artichoke heads. Contrary, other authors observed that artichoke heads harvested in the winter season showed a better quality than artichokes harvested in the spring [14,15]. Nevertheless, minimally processed artichoke heads showed higher susceptibility to browning in the spring season than in winter, even if the polyphenol content of artichokes harvested in winter was higher compared to spring buds [16].

Phenolic compounds are highly susceptible to browning through oxidation reaction catalyzed by polyphenols oxidase (PPO) and peroxidase (POD) enzymes and the *o*-quinones formed could take part in a second non-enzymatic step, leading to the formation of dark compounds [3,17]. Enzymatic browning is influenced by many variables which are dependent on the genotype, such as the specific activity of enzymes or the quantity and nature of phenolic compounds [18]. Therefore, cultivars rich in phenolic compounds and with a high enzyme activity are unsuitable for fresh-cut processing [3,19,20]. Several studies have showed that genetic material is a key determinant of phenolics content, antioxidant enzyme activities, and thus, the suitability of artichoke cultivar for fresh-cut processing, which contributes to the overall functional quality of this crop [10,21,22]. Phenolic content was significantly lower in seed-propagated cultivars than in the vegetatively propagated ones [23]. In this sense, cultivars with a low content of total phenols could produce artichoke heads with the best quality traits for fresh-cut and agro-processing industry, reducing the susceptibility to browning and the use of antioxidant treatments in processing steps [23].

On the other hand, one study recently published has reported that individual phenolic compounds, mainly hydroxycinnamic acids and luteolin derivatives, were highly influenced by flower head order (main, secondary, and tertiary heads) in seed-propagated open-pollinated, vegetatively propagated, and seed-propagated hybrid artichoke cultivars [7]. In this work, the individual phenolic profile was similar in all cultivars studied, reporting that 5-*O*-caffeoylquinic acid was the main hydroxycinnamic acid, followed by 3,5-di-*O*-caffeoylquinic acid and 3,4-di-*O*-caffeoylquinic acid [7]. Regarding the flower head order, tertiary head orders showed the highest individual phenolic content, followed by secondary and main heads, and this effect was cultivar dependent. Among eight different artichoke cultivars studied, 'Lorca' cultivar presented a lower total phenolic content than other cultivars rich in phenolic compounds, which could increase its aptitude for fresh-cut processing [7]. Thus, the main aim of this study was to evaluate the influence of flower head order and internal development stage on phenol content as well as browning susceptibility in 'Lorca' cultivar, in order to characterize the key factors which influence its phenolic levels and determine its ready-to-eat artichoke aptitude.

2. Materials and Methods

2.1. Plant Material and Experimental Design

'Lorca' cultivar was studied throughout the developmental cycle of one season (from August 2020 to April 2021), in an experimental plot of the Miguel Hernández University (Orihuela, Southeast Spain; 38°06′63.52″ N, −0°988′09.29″ W). The growing area has a Mediterranean climate, characterized by mild and humid winters and hot and rainless summers. Maximum monthly temperature ranges between 16.2 °C (January) and 33.3 °C (July) and minimum temperature between 5 °C (January) and 21.2 °C (August) were recorded, according to historical meteorological data.

For the experiment, 40 artichoke plants of 'Lorca' cultivar, which were reproduced by seeds and open pollination, were planted in mid-August following a planting frame of 0.8 m apart within a row and 1.2 m apart among close rows. These plants were distributed

in four replicates or blocks (10 plants planted per replicate or block). Crop management was performed according to standard agronomic practices used by growers in Southeast Spain. An application of commercial sheep manure was incorporated before transplanting, at a rate of 2.5 kgm^{-2}. Fungicides and insecticides were used along the developmental cycle, and fertilizers, composed of 250 kg N, 120 kg P_2O_5, and 300 kg K_2O per ha were applied by drip irrigation system. Gibberellic acid was not applied.

All artichokes of each plant were harvested following commercial criteria, when flower heads reached their average commercial size and morphology, and they were firm and tightly closed. The first harvest date was performed on February 1st, and artichoke heads were harvested weekly. A total of 13 different harvest dates along the complete crop cycle were performed, the last one being on 21 April. For each harvest date, artichokes were classified according to the flower head order: main, secondary, and tertiary heads, and a total of 445 artichokes were harvested (Table 1).

Table 1. Number of artichokes produced in each type of flower head order for 'Lorca' cultivar.

Flower Head Order	Number of Artichokes
Main	40
Secondary	278
Tertiary	127
Total	445

After harvest, artichokes were transported to the laboratory and analyzed individually on the same day. An evaluation of the internal development stage was performed using a three-level scale (Figure 1): 1 = initial development stage (artichokes without internal hair or with a small amount of short hair as well as with a green and tender hearts); 2 = intermediate development stage (artichokes with an intermediate amount of internal hair, but still showing a green heart); 3 = advanced development stage (artichokes with a high amount of internal hair, although appreciating a change from green to purple color on the artichoke heart). For PPO analysis, a half of the artichoke heart was quickly sliced, frozen with liquid nitrogen, and stored at −80 °C.

Figure 1. Scale of internal development stage: (**a**) initial development stage; (**b**) intermediate development stage; (**c**) advanced development stage.

2.2. Extraction and Quantification of Total Phenolic Compounds

Phenolic extraction was performed according to Martínez-Esplá et al. [24]. Briefly, 5 g of the edible part of each artichoke (heart and inner bracts) was homogenized with 15 mL of methanol at 80% containing 2 mM sodium fluoride (NaF), in order to inactivate polyphenol

oxidase activity and prevent phenolic degradation, for 2 min using a homogenizer (Ultra-Turrax®, TP 18, IKA, Staufen, Germany). Subsequently, samples were centrifuged at 10,000 g for 15 min at 4 °C. The supernatant was used for total phenolic quantification in duplicate using the Folin-Ciocalteu reagent and results (mean ± SE) were expressed as grams of gallic acid equivalent per kg^{-1} of fresh weight (FW).

2.3. Browning Evaluation by Digital Image Analysis

Color was expressed by hue angle and measured with digital image analysis. Images of artichoke heads were captured using a digital camera (Nikon D3400, Minato, Tokio, Japan) in a light box with a white background. The camera setup conditions were as follows: light provided by two LEDs with a color temperature of 5600 K, speed of 1/5 s, ISO−100, focal aperture (f) 20, and length of 35 mm. For each artichoke head, a cut in half was made individually and longitudinally. An image was captured every 15 s for 3 min of the internal front side to evaluate the browning process after cutting each individual artichoke. Images were saved as JPEG files and analyzed using ImageJ v1.52a software (NIH Image, National Institutes of Health, Bethesda, USA). The CIELab model was used to calculate the hue angle (h°) with the color parameters a* and b*, as follows (1):

$$\text{hue angle} = \arctan\left(b^*/a^*\right) \quad (1)$$

2.4. PPO Enzyme Activity Analysis

The extraction and quantification of PPO enzyme activity was carried out according to Cabezas-Serrano et al. [3] with slight modifications. Briefly, 5 g of frozen tissue were homogenized (Ultra-Turrax®, TP 18, IKA, Staufen, Germany) in 15 mL of 0.1 M phosphate buffer (pH 6.5) containing 1 g of polyvinylpyrrolidone (PVP) and centrifuged at 10,000× *g* for 15 min at 4 °C. The supernatant was used for PPO activity determination which was spectrophotometrically performed using catechol as a phenolic substrate. The reaction mixture contained 200 µL of extract, 2 mL of 50 mM phosphate buffer and 0.5 mL of 0.1 M 4-methylcatechol and measured by determining the absorbance increase at 420 nm. The results were expressed as units of enzyme activity per g of FW, and one enzyme unit was defined as the amount of enzyme causing an increase in absorbance of 0.001 per min.

2.5. Statistical Analysis

An analysis of the variance components was carried out with all the phenol content values in order to estimate the amount of variability provided by each of the three factors studied in the experiment: plant, flower head order, and internal development stage. The data for the analytical determinations were subjected to analysis of variance (ANOVA), the dependent variable being the total phenolic content and the three factors indicated above. Mean comparisons were made using a multiple range test (Newman–Keuls test) to determine significant differences at $p < 0.05$ among artichoke head orders or among internal development stage in each parameter. Significant differences on Δ hue angle were performed according to *t* Student test at $p < 0.05$ among artichoke head orders. A correlation analysis was performed between phenolic content and PPO activity or hue angle increment. All analyses were performed with the STATGRAPHICS Plus software package, version 3.1 for Windows.

3. Results and Discussion

3.1. Influence of Variance Components Analysis on Phenolic Content

Table 2 shows the percentage (%) of each variance component studied after the statistical analysis for the phenolic content, showing the importance attributed to each factor. The analysis of variance divides the variance of phenolic content (dependent variable) into three components, one for each factor (Table 2). Each factor after the first one is nested in the one above. With this analysis, we estimate the amount of variability contributed by

each of the studied factors. The three factors analyzed in this study, in addition to total phenolic content, were: plant, artichoke head order, and the internal development stage.

Table 2. Analysis of variance components for phenolic content for 'Lorca' cultivar.

Source	Percentage (%)
Plant	4.55
Artichoke head order	22.17
Internal development stage	15.55
Error	57.74

Our results reported for the first time that the three variance components analyzed (plant, artichoke head order, and internal development stage) explained a 42.27% of all variances. The plant only showed a variability of 4.55%; therefore, this variance component did not have an important role on the total phenolic content since all plants studied presented similar results and only one plant of the 40 plants in total showed significant differences. This result could be related to the fact that the plants are propagated by seeds, which have been selected over time and present a high homogeneity. According to our results, the most important factors were artichoke head order and internal development stage, which presented a variability of 22.17% and 15.55%, respectively. The remaining variability of 57.74%, namely as error, could be explained by different preharvest factors. In this sense, quantitative and qualitative variability of essential nutrients and secondary metabolites in artichoke heads could depend on genetic diversity, environmental conditions, edaphic, harvest time, and climatic conditions throughout the artichoke plant growth cycle, as it was previously reported by other authors [8,11,13,25].

All artichokes were harvested in 3 months, from February to April; however, harvest time was not considered for the variance components analysis mainly due to no significant differences being found previously between winter and spring harvest season for 'Blanca de Tudela' cultivar [26]. Specifically, in 'Lorca' cultivar, the influence of gibberellic acid (GA_3) treatment on the phenolic content was evaluated, and no significant differences were observed with respect to untreated artichokes, which were harvested later [7]. On the other hand, other authors have observed that harvest date significantly influenced the total phenol content during longer developmental cycles. Specifically, Pandino et al. [11] evaluated the effect of harvest time in 'Ovoli' artichoke from November to April and reported higher phenolics concentration in artichoke heads harvested from February to April compared to previous ones. Thus, the higher phenolic level observed by other authors in those artichokes harvested in spring compared to winter season could be related to the fact that in spring, tertiary artichoke heads are mainly harvested and therefore, they have the highest phenolic content.

3.2. Influence of Artichoke Head Order and Internal Development Stage on Total Phenolic Content

The influence of artichoke head order on total phenolic content was evaluated to corroborate previous results obtained in the 2018–2019 growing season [7]. Eight white artichokes genotypes were studied, and total phenolic content showed significant differences among flower head orders and the effect was also cultivar dependent. As a general trend, all cultivars analyzed had a higher total phenolic content in tertiary flower head orders than in secondary or main ones. Specifically, 'Lorca' cultivar showed significant differences between main and tertiary heads but not between main and secondary artichokes [7]. Results of this 2020–2021 growing season showed that total phenolic content in 'Lorca' cultivar significantly varied among flower head orders (main, secondary, and tertiary). Accordingly, tertiary flower heads had significantly higher levels of phenolic compounds than secondary and main heads, the main ones being those artichokes with the lowest content (Table 3). The greater significant differences observed in the present study among the three flower head orders could be related to the higher plant number included in the present experimental design as well as the number of artichokes analyzed, which was also higher

since all flower heads produced by each plant were analyzed individually in this study. Similarly, Gagliardi et al. [27] observed that the content of both 5-*O*-caffeoylquinic acid and 1,5-*O*-dicaffeoylquinic acid were approximately 2-fold higher in processing or tertiary heads class compared to main heads class in 'Violeto di Provenza' artichoke cultivar.

Table 3. Total phenolic content (g kg^{-1}) for different artichoke head orders (main, secondary, and tertiary heads) of 'Lorca' cultivar.

Artichoke Head Order	Total Phenolic Content
Main heads	2.155 ± 0.065 c
Secondary heads	2.782 ± 0.023 b
Tertiary heads	3.221 ± 0.019 a

Data are the mean ± SE. Different lowercase letters show significant differences on total phenolic content among artichoke head orders, according to Newman-Keuls test at $p < 0.05$.

On the other hand, the internal development stage of artichoke heads for each harvest date has been evaluated and correlated to total phenolic content. Our results demonstrated for the first time that total phenolic content was significantly higher when 'Lorca' artichokes were in an initial (stage 1) or intermediate (stage 2) development stage. Contrary, the concentration of these bioactive compounds was lower at the most advanced development stage (stage 3) (Table 4). In the same way, Lattanzio et al. [28] observed that phenolics levels in artichoke heads depended on the stage of physiological organ, being higher in younger tissues. In addition, it has been observed that the development stage or the age of tissue influenced the PPO activity, found the highest activity values in the heart, which represents the younger tissue of artichoke heads [28].

Table 4. Total phenolic content (g kg^{-1}) for different internal development stages (1: initial; 2: intermediate; 3: advanced) of 'Lorca' cultivar.

Internal Development Stage	Total Phenolic Content
1 (initial)	2.889 ± 0.036 a
2 (intermediate)	2.827 ± 0.032 a
3 (advanced)	2.411 ± 0.023 b

Data are the mean ± SE. Different lowercase letters show significant differences on total phenolic content among internal development stage, according to Newman–Keuls test at $p < 0.05$.

Due to the high proportion of artichoke plant waste and the complexity of preparation and trimming operations, processing artichokes as a fresh-cut product will provide convenience. Furthermore, since receptacle and inner bracts contain high amounts of phenols, fresh-cut artichokes would also provide a high value-added product. However, the main problem of fresh-cut artichokes is the cell membranes rupture associated to cutting processes, which induces enzymatic browning from the oxidation of phenolic compounds by PPO and POD enzymes and it results in the formation of dark compounds, leading to a high browning rate of the cut surface (artichoke receptacle and bracts) [3,29].

Artichokes with high levels of total phenols can be unsuitable for ready-to-eat products, since they are associated with either enzymatically or non-enzymatically oxidative browning reactions, mainly during processing steps [19,30]. As a consequence, main artichokes and artichokes at an advanced internal development stage could be particularly suited to fresh-cut processing, since they showed the lowest total phenolic content observed. Contradictorily, artichokes with a high total phenolic level are probably the best suited for fresh consumption, since the presence of these compounds in the human diet is correlated to a protective effect against certain chronic and degenerative diseases related to oxidative stresses [8,31,32]. In this sense, tertiary and secondary artichoke heads and artichokes at initial and intermediate development stages had the highest total phenolic content and, hence, they also show potential use for fresh consumption on healthy diets [19,33].

3.3. PPO Activity for Different Artichoke Head Orders

PPO activity was analyzed in order to complete the evaluation of the artichoke orders which are the most susceptible to browning after cutting (Table 5). The results showed significant differences between main and secondary or tertiary heads; however, no significant differences were found between secondary and tertiary heads. Similar levels for PPO activity have been reported in different artichoke cultivars at harvest, with values from 400 U g^{-1} FW in 'Harmony' and 'Opal' up to 600 U g^{-1} FW in 'Concerto' and 'Symphony' cultivars. Nevertheless, PPO activity was variable during the storage period, this trend being observed cultivar dependent [8].

Table 5. Polyphenol oxidase activity (U min^{-1} g^{-1}) for different artichoke head orders (main, secondary, and tertiary heads) of 'Lorca' cultivar.

Artichoke Head Order	PPO Activity (U g^{-1} FW)
Main heads	311.30 ± 19.05 b
Secondary heads	411.90 ± 19.71 a
Tertiary heads	451.31 ± 20.17 a

Data are the mean ± SE. Different lowercase letters show significant differences on polyphenol oxidase activity among artichoke head orders, according to Newman-Keuls test at $p < 0.05$.

This is the first time that PPO enzyme activity has been analyzed according to the artichoke head order classification: main, secondary, and tertiary, in 'Lorca' cultivar. The correlation analysis between phenolic content and PPO activity was carried out and significant correlations were found for main (R^2 = 0.732), secondary (R^2 = 0.821), and tertiary (R^2 = 0.854) artichoke heads. These results agree with Cabezas-Serrano et al. [34], who found a significant coefficient of correlation between phenolic content and PPO activity, highlighting that PPO activity increased with the increment of phenolic content. The lowest phenolic content and PPO activity in main artichokes could be related with a lower increment of hue angle.

3.4. Evaluation of Artichoke Browning for Different Artichoke Head Orders

Color can be measured objectively and quickly by computerized image analysis techniques. The RGB (red, green, and blue) linear color model was used to identify the browned pixels. Starting from the RGB image by using a software that can convert the original image to non-linear coordinates L*, a*, b* (lightness, redness, and yellowness) [35]. The most critical step in image processing is to isolate the region to be studied (which can be the complete image or only a part) within the image, and this is done through an image segmentation [36].

Flower head order was the variance component with the greatest influence on phenolic content; therefore, an image analysis was carried out individually according to artichoke classification in main, secondary, and tertiary head orders. To evaluate color changes related to browning intensity of cut surface in the different artichoke orders analyzed, the increment of hue angle, such as the difference between hue angle at 180 s and at time 0, was obtained [37,38] by using ImageJ software for digital image analysis.

Total phenol content was correlated with the increase on hue angle variation. In general, artichokes underwent color changes after cutting, from yellow green to dark brown, which explained the decrease on hue angle over time [3,34]. Therefore, the increase on the hue color parameter, measured during 180 s, was higher for tertiary artichokes followed by secondary and main heads (Figure 2). For each artichoke head order, the results obtained were fitted to a third polynomial regression and a high correlation has been found between the increment of hue angle and time (R^2 = 0.9924 − 0.9975). These differences are shown by an increase on browning process in the fresh product after cutting up to 180 s for each different flower head order, with tertiary heads being those artichokes that presented the higher color changes due to their greater phenolic content (Figure 3). These results are according to those reported previously by other authors, where a high

correlation of browning score and hue angle on cut iceberg lettuce was observed [39,40]. Moreover, phenolic concentration could be directly correlated to browning susceptibility in fresh-cut artichokes since its initial high phenolic content may induce the browning reaction [41]. During the industrial processing of these vegetables, a greater impact of enzymatic browning is expected, consequently derived from mechanically damaged tissues. The browning process may be attributed to the interaction between phenols and iron due to an oxidation of iron ions and the subsequent formation of quinones catalyzed by PPO enzymes, supported by a reduction on the phenolic content [3].

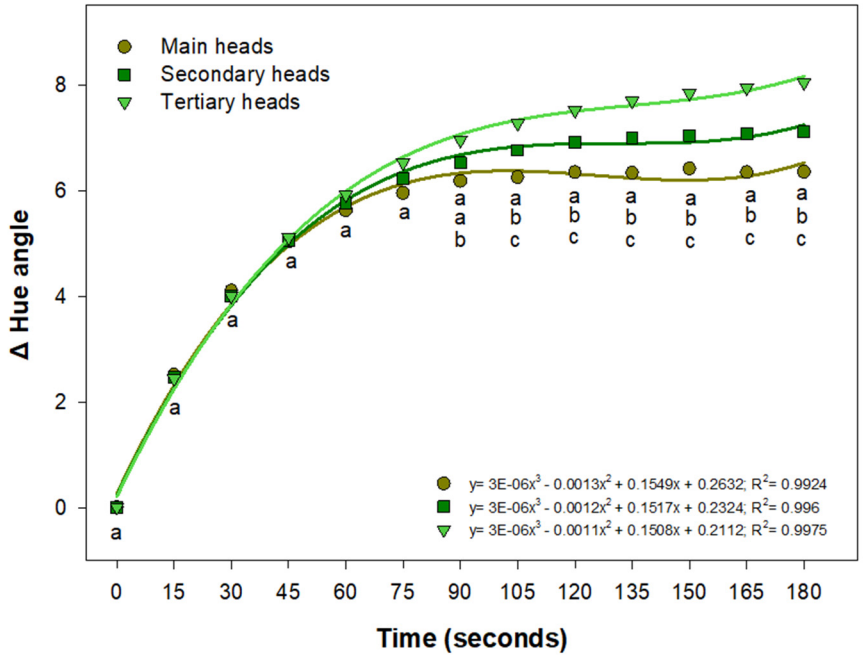

Figure 2. Effect of colour change (Δ Hue angle) of fresh-cut artichoke heads every 15 s for 3 min. Different letters show statistical differences (*t* Student test, $p < 0.05$) among artichoke head orders for 'Lorca' cultivar.

The results obtained in the present study in 'Lorca' artichoke cultivar showed differences on the susceptibility to browning attending to the flower head order. Tertiary and secondary heads showed the highest color changes and browning since they presented the highest phenolic content. On the contrary, main heads, which showed the lowest phenolic content, were the least susceptible to post-cutting browning [42]. A correlation analysis was performed between phenolic content and the increment of hue angle. Both parameters showed significant coefficients of correlation in main ($R^2 = 0.775$), secondary ($R^2 = 0.891$), and tertiary ($R^2 = 0.856$) artichoke heads. In previous works, it was observed that phenolic content was correlated with the susceptibility to browning in plant species rich in these compounds, such as artichokes, with these color changes being related to PPO enzyme activity [41]. On the other hand, browning was correlated with phenylalanine ammonia lyase (PAL) enzyme activity in species with a low phenolic content, such as lettuce [43].

In summary, our results highlight that the lowest phenolic content and PPO activity are related to the lowest increment of hue angle in main artichoke order, with the opposite effect being observed in secondary and tertiary. In general, tertiary heads showed the worst aptitude to developed fresh-cut artichoke product related to a high browning on the artichoke head (Figure 3). PPO activity is the key enzyme involved in the enzymatic browning process, which generates dark pigments called melanoidins, and are unacceptable in terms of safety as they could support microbial growth [44]. Several studies have showed that the browning process might be prevented by chemical and physical methods to inhibit PPO enzyme activity, including the reduction of temperature and/or oxygen concentration, the use of modified atmosphere packaging (MAP), and the application of anti-browning

agents, coatings, and innovative packaging [3,29,45–48]. The different methods used as PPO inhibitors could inhibit the PPO enzyme, removing its substrates or lowering pH below the optimum value for PPO [49]. For the first time, these results could be used as a tool to classify the artichoke head orders in 'Lorca' cultivar according to its ready-to-eat aptitude.

Figure 3. Enzymatic browning process of different flower head orders at time 0 s, 90 s, and 180 s for 'Lorca' cultivar.

4. Conclusions

In this study, a characterization of the total phenolic content in 'Lorca' cultivar has been performed to define their optimum commercial aptitude to minimally processed artichokes. Variance components analyses were carried out for total phenolic content considering three factors: plant, flower head order, and internal development stage. Results showed that the plant only explained a 4.55% of variability, with artichoke head order and internal development stage responsible for a variability of 22.17% and 15.55%, respectively. For the first time, the internal development stage has been related to total phenolic content. Tertiary and secondary artichokes and those at initial and intermediate development stages showed the highest phenolic content and PPO enzyme activity, showing the highest browning susceptibility after cutting. On the contrary, the opposite effect was observed in main artichokes and those at the advanced development stage; therefore, they exhibit the lowest browning process, which could increase their use in ready-to-eat products at market.

Author Contributions: Conceptualization, M.G.-B., M.J.G. and P.J.Z.; methodology, M.G.-B. and M.E.G.-P.; software, M.G.-B. and S.G.-M.; investigation validation, M.J.G. and P.J.Z.; formal analysis, M.E.G.-P.; investigation, M.G.-B.; resources, P.J.Z.; data curation, M.J.G.; writing—original draft preparation, M.G.-B.; writing—review and editing, M.G.-B., S.G.-M., M.E.G.-P., M.J.G. and P.J.Z.; visualization, M.G.-B.; supervision, M.J.G. and P.J.Z.; project administration, P.J.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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PUBLICATION 4

INFLUENCE OF FLOWER HEAD ORDER ON PHENOLIC CONTENT AND QUALITY OF GLOBE ARTICHOKE AT HARVEST AND DURING TWENTY-ONE DAYS OF COLD STORAGE.

(Literal Transcription)

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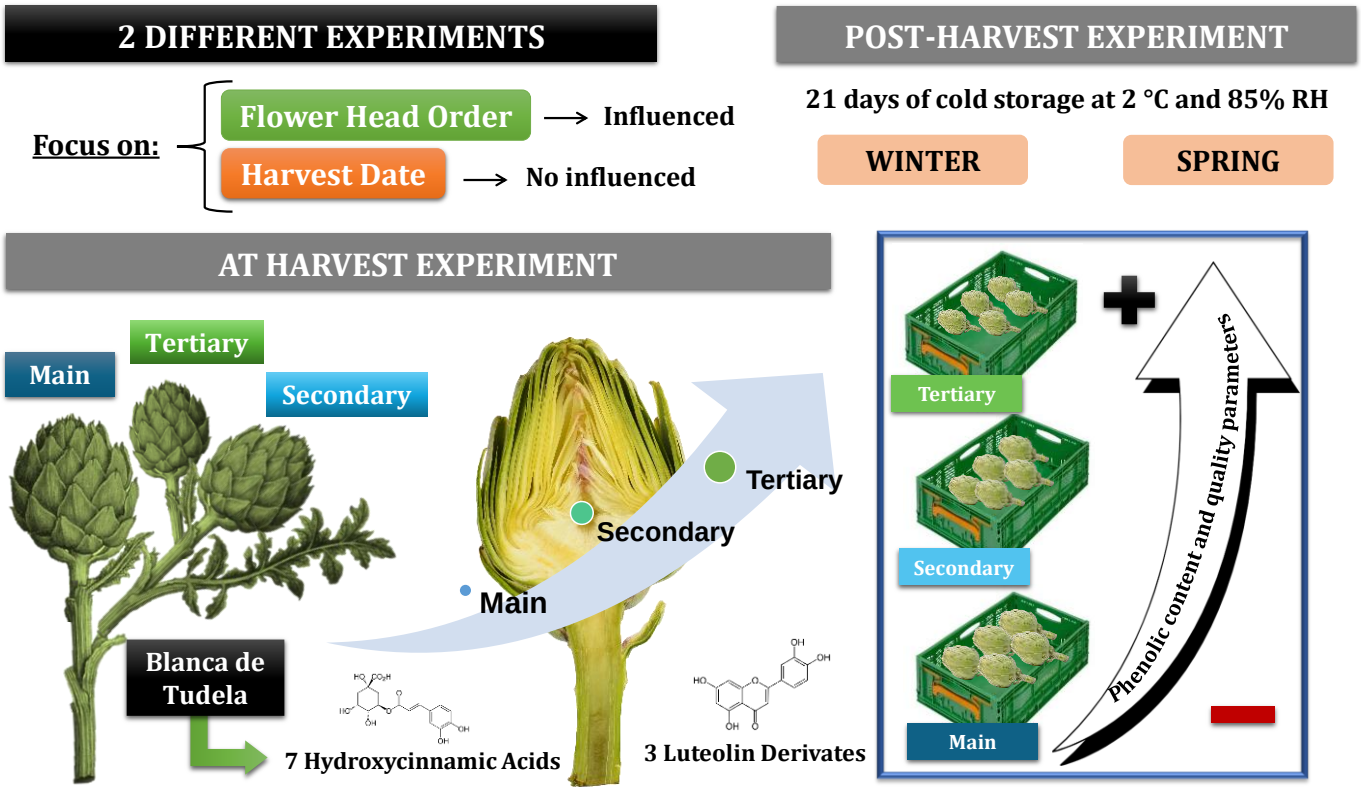
Scientia Horticulturae 2022, 295, 110846



https://doi.org/10.3390/foods12244414

Scientia Horticulturae 295 (2022) 110846

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Influence of flower head order on phenolic content and quality of globe artichoke at harvest and during twenty-one days of cold storage

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ARTICLE INFO

Keywords:
Hydroxycinnamic acid
Luteolin derivative
Antioxidant activity
Postharvest quality
Respiration rate

ABSTRACT

Artichoke is one of the vegetables with higher content in phenolic compounds, which are responsible for their taste, flavor and health beneficial effects. However, phenolic profile and concentration depends on many factors, such as genotype, harvest date, and environmental and agronomical conditions. The main aim of this study was to perform a phytochemical characterization of artichoke heads, based on their position on plant (main, secondary and tertiary head) and harvest date, during a complete growing season. Results showed that total identified polyphenol concentration was higher in tertiary heads than secondary and main heads, due to their higher concentration in hydroxycinnamic acid and luteolin derivatives. On the other hand, two postharvest storage experiments with main, secondary and tertiary artichoke heads, harvested in winter and spring, were performed. In addition, tertiary head showed the lowest weight, firmness losses and respiration rate during cold storage which could be attributed to their higher antioxidant compounds. In conclusion, tertiary heads have a greater aptitude to be stored at low temperature from harvesting to consumption since they maintained the quality properties for longer period of time and had higher content of bioactive compounds. However, main artichokes are the most appreciated by consumers due to their larger size.

1. Introduction

Globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] is a vegetable native to the Mediterranean region and its production and consumption has spread around the world in recent years. Artichoke is an immature inflorescence called *capitulum*, bud or head. Globe artichoke plant produce flower head at different order: at the apex of the central stem are formed the largest artichokes (named as primary or main head), while secondary and tertiary heads of smaller size, are developed on the central branches. The total area of artichoke cultivation in Spain is 13.862 hectares whose production is 196.965 tons (MAPA, 2020). Around 60% of production of globe artichoke heads is directed to the fresh market, and the remaining 40% is destined for the canning and freezing industries. For national market, artichokes are harvested with weight ranging from 140 to 160 g. However, largest calibers are chosen (200–500 g) when artichokes are destined for the export market (Baixauli et al., 2013). The edible fraction (receptacle and inner bracts) constitutes nearly 35–55% of the total head weight (Cecarelli et al., 2010). Vegetative propagation is the most common artichoke propagation method around the world (Grabowska et al., 2018). A common practice in Spain is planting lateral shoots with fully-developed roots that are separated from the mother plants, specifically in 'Blanca de Tudela' artichokes, which is the most cultivated cultivar in this country. 'Blanca de Tudela' is an early and re-flowering cultivar that has a large production throughout the year, which starts in autumn and continues until spring, even having two separate production peaks; the first one in winter, due to the first plant which emerges from the rhizome, and the second one in spring due to the second plant which emerges from the exhausted first plant.

Compare to other vegetables, artichokes contain the highest total polyphenol content and its intake provides benefits for human health and well-being (Brat et al., 2006). Specifically, artichoke metabolites provide hepatoprotection, cholesterol-lowering, diuretic, anti-inflammatory and antioxidative effects, and colon cancer protection (De Falco et al., 2015). Apart from having health-promoting properties, phenols are important in determining quality traits such as taste

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https://doi.org/10.1016/j.scienta.2021.110846
Received 3 March 2021; Received in revised form 4 October 2021; Accepted 18 December 2021
Available online 24 December 2021

and flavor (Lattanzio, 2003). The major phenolic compounds in artichokes are phenolic acids (mono- and di-caffeoylquinic acids) and flavonoids (luteolin and apigenin derivatives) (Schütz et al., 2004; Lattanzio et al., 2009; Pandino et al., 2011; Lombardo et al., 2018). Nevertheless, the quantitative and qualitative profile of phenolic compounds in artichoke heads is influenced by genotype, part of the plant, harvest time, agronomic management and environmental and postharvest conditions (Lombardo et al., 2009; 2010; Cecarelli et al., 2010; Pandino et al., 2013; Scavo et al., 2019). Recently, the influence of artichoke head order and the effect of gibberellic acid treatment have been described in eight different artichoke cultivars (Giménez et al., 2021). This work concluded that tertiary head artichokes showed the highest individual phenolic content, followed by secondary and main heads, and this effect was cultivar-dependent. In addition, the gibberellic acid treatment affected the content of these bioactive compounds depending on the flower head order and artichoke cultivar. However, the effect of artichoke order on plant on crop yield and quality parameters during postharvest cold storage remains unclear.

After harvest, globe artichoke heads are very perishable due to its high respiratory and transpiration rate which could lead to some physiological changes such as weight and firmness losses, bracts hardening and opening, or color changes due to chlorophyll degradation and browning of the bracts apical parts (Ruíz-Jiménez et al., 2014). Furthermore, artichokes are hihgly susceptible to cold damage and several fungal diseases during postharvest storage, mainly caused by *Sclerotinia sclerotiorum* (white mold) (Marcucci et al., 2010) and *Botrytis cinerea* (gray mold) (Larran et al., 2004). For instance, the influence of genotype and harvest date on quality traits of ready-to-use globe artichoke slices during storage has been reported (Licciardello et al., 2017; Pandino et al., 2017a). These studies showed that artichoke cultivars with lower total phenolic concentration were more suitable for minimal processing uses, mainly due to their better color parameters and reduced browning, as well as early harvested artichokes in the growing season as compared with late harvest ones.

In order to maintain artichoke quality heads and to extend their shelf-life up to 3, 4 weeks, artichokes should be cooled after harvest as soon as possible and stored at 0, 1 °C and relative humidity of 90–95% (Gil-Izquierdo et al., 2002). During cold storage of artichoke heads, some changes in secondary metabolism occur and these changes have a direct influence on their quality. On the other hand, an induction of phenylalanine ammonia lyase (PAL) activity causing a biosynthetic increase of phenolics, especially in chlorogenic acid, had been reported by Lattanzio (2003). Significant increases on chlorogenic acid concentration in artichokes stored at 0, 2, 5 and 7 °C were also reported by Gil-Izquierdo et al. (2001). Previous works have showed that preharvest treatments with oxalic acid or methyl jasmonate led to artichokes with a higher phenolic content at harvest, which was related with a delay in the postharvest senescence process, maintenance of artichoke quality traits and extension of their shelf-life (Martínez-Esplá et al., 2017a; 2017b). Thus, the main aim of this study was to characterize 'Blanca de Tudela' artichoke production cycle and its influence on total and individual phenolic content and antioxidant activity of main, secondary and tertiary artichoke heads, as well as the effect of these variables on the artichoke quality maintenance under cold storage conditions. Based on these results, it could be recommended which are the artichokes head orders with the best aptitudes for fresh market or for the industrial processing.

2. Materials and methods

2.1. Plant material and experimental design

For the experiment, 'Blanca de Tudela' cultivar was studied along the developmental cycle from autumn 2017 to spring 2018, in an experimental plot of Miguel Hernández University (Alicante, southeast Spain). The local climate of the cultivation area is Mediterranean, with mild and humid winters and hot and rainless summers. According to historical meteorological data, the minimum monthly temperature ranges between 5 °C (January) and 21.2 °C (August) and maximum temperature between 16.2 °C (January) and 33.3 °C (July).Thus, 50 lateral offshoots from first-year artichoke plants for each replicate or block, were planted at the beginning of August, 0.8 m apart within a row and 1.2 m apart among close rows.Three replicates of 50 plants were used (150 plants in total), leaving a borderline between each block and at the edge of the plot. Crop management was performed according to the conventional agronomic practices. Fungicides and insecticides were used along the growth cycle, and fertilizers, composed of 250 kg N, 120 kg P_2O_5 and 300 kg K_2O per ha, were applied by drip irrigation system.

All the artichokes of each plant were harvested at commercial developmental stage based on head size and morphology when they were firm, tightly closed and ready-to-use. A total of 17 harvesting dates were performed from November 29th to May 24th. For each harvest date, yield (number of artichoke head per plant and kg per plant) of each artichoke head order (main, secondary and tertiary) was recorded. Then, 5 artichoke heads for each order and replicate were selected to analyze phenolic content and total antioxidant activity individually in each artichoke head. In addition, artichokes harvested on February 26th (winter harvest) and April 23rd (spring harvest) were used to performed two postharvest experiments. Immediately, main, secondary and tertiary artichoke heads without visual defects were selected and transferred to the laboratory. Then, head artichokes were sorted into 4 lots of 5 artichokes for each head order and replicate and stored at 2 °C and 85% of relative humidity. After 0, 7, 14 and 21 days of storage, one lot from each head order and replicate was taken at random and used to evaluate respiration rate and quality parameters. Beside, individual phenolic compounds and total phenolic content as well as total antioxidant activity were measured at harvest and during 21 days of storage.

2.2. Artichoke plant yield

Yield was quantified as kg per plant for each replicate of 50 plants ($n = 3$). Total number of harvested artichokes was recorded and artichoke heads were classified according to the flower head order: main, secondary or tertiary, for each harvest date. Yield (kg $plant^{-1}$) was recorded as well as the average weight of artichoke heads. Results were expressed as the mean ± SE.

2.3. Respiration rate and quality parameters during postharvest storage

Respiration rate was measured individually in 5 artichoke heads of each order (main, secondary and tertiary). Each artichoke head was closed in a 3 L glass jar, hermetically sealed, for 60 min and 1 mL from the jar atmosphere was withdrawn and injected in a gas chromatograph (Shimadzu, Kyoto, Japan) with thermal conductivity detector (GC-TCD) to measure CO_2 concentration according to Martínez-Esplá et al. (2017a). Respiration rate was expressed as mg CO_2 kg^{-1} h^{-1}. Weight loss (%) was determined by weighing individual artichokes at day 0 and after each sampling date during storage period and expressed as percentage with respect to weight at day 0. Firmness was determined individually in 5 artichokes of each order and replicate, the diameter of artichoke head was measured and then a force that achieved a 5% of deformation of the head diameter was applied (TX-XT2i Texture Analyzer Stable Mycrosystems, Godalming, UK) (Martínez-Esplá et al., 2017a). Results were expressed as the force/deformation ratio (N mm^{-1}) and were the mean ± SE.

2.4. Identification and quantification of phenolic compounds

Phenolic extraction was performed by using 5 g of edible fraction (internal bracts and receptacle) from each individual artichoke head, according to the protocol described by Martínez-Esplá et al. (2017a), by homogenizing artichoke samples with 15 mL of water/ methanol (2:8)

containing 2 mM NaF. On the other hand, the identification and quantification of individual phenolics were made in the phenolic extract according to Gironés-Vilaplana et al. (2012) with slight modifications. HPLC-DAD-ESI /MSn and RP-HPLC-DAD systems were used for injection of 20 μL of the extracts, and chromatograms were recorded at 320 and 360 nm in an Agilent HPLC 1200 Infinity series, equipped with a photodiode array detector (Agilent Technologies, Waldbronn, Germany) and a mass detector in series (Bruker Daltonics Ultra HCT-ESI Ion Trap, Bremen, Germany). Mobile phases A and B were water/formic acid (99:1, v/v) and acetonitrile, respectively, with a flow rate of 1 mL per min. Compounds were identified by their mass and retention time using previous bibliography. A calibration curve of two standards, 5-*O*-caffeoylquinic acid and 3-luteolin-*O*-rutinoside (Sigma Aldrich, Germany), was used for the quantification of hydroxycinnamic acids and luteolin derivatives at 320 and 360 nm, respectively. The total identified polyphenols concentration was calculated as the sum of the individual phenolic concentrations. Results were expressed as g kg^{-1} of fresh weight (FW), and were the mean of 5 artichokes for each replicate ± SE.

2.5. Antioxidant activity

Total antioxidant activity (TAA) was quantified using the protocol described by Valero et al. (2011), which described TAA determination from both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds in the same extraction. The extraction was performed for each artichoke individually by homogenizing 2 g of the edible part of each artichoke in 10 mL of 50 mM Na-phosphate buffer at pH 7.8 and 5 mL of ethyl acetate. The upper fraction after centrifugation was used to analyze L-TAA and the lower one for H-TAA. In both cases, TAA was determined by using the enzymatic system composed of the chromophore 2,2′-azino--bis-(3- ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which $ABTS^{\cdot+}$ radicals were generated and monitored at 730 nm. Results were expressed as g equivalent of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) kg^{-1} FW and were the mean of 5 artichokes for each replicate ± SE.

2.6. Statistical analysis

Results were expressed as the mean of 5 artichokes ± SE from three replicates. Respect to field results, a *t*(MS) Student test was performed to show significant differences at $P < 0.05$ between two head artichoke orders (main and secondary heads), as well as between both harvest times (winter and spring). Data from the three head artichoke orders (main, secondary and tertiary heads) and days of storage at 2 °C were subjected to analysis of variance (ANOVA). Sources of variation were head artichoke order and storage days. Mean comparisons were performed using HSD Duncan's test to examine if differences were significant at $P < 0.05$. ANOVA assumptions were tested and found to be valid for this dataset. All analyses were performed with SPSS software package v. 17 for Windows.

3. Results and discussion

3.1. Growing season results

Yield (kg $plant^{-1}$) for each artichoke head order (main, secondary and tertiary) was evaluated for all harvest dates, from November 29th (2017) until May 24th (2018). Main heads began to be harvested at the end of November, secondary heads from December 11th, and tertiary heads at the end of February (Table 1). 'Blanca de Tudela' cultivar, the most produced one in Spain, is perfectly adapted to climate condition of Spanish southeast areas, being able to produce artichokes from November to May. Due to its earliness, the first plant to emerge from the rhizome become exhausted after 3 months and new plants develop leading to new productions. Thus, main heads showed two production peaks, in January and at the end of April, as well as the secondary heads, in February and May. Finally, the tertiary heads were harvested from February 28th to May 24th (Table 1). Taking into account the production of all the artichoke head orders along the complete growth cycle, a total yield of 4.23 kg per plant was obtained. This yield was slightly higher than the total yield obtained in other studies carried out with 'Blanca de Tudela' cultivar, in which yields between 3.14 and 3.75 kg per plant were achieved in November-April cycle (Martínez-Esplá et al., 2017b). The highest yield obtained in the present study could be related to the longest length cycle which lasted until May compared to Martínez-Esplá et al. (2017b). In a study with 'Blanca de Tudela' and others Italian and French cultivars, greater earliness and yield of 'Blanca de Tudela' was observed, due to a higher number of artichoke heads than other cultivars (Macua et al., 2005a). On the other hand, the physiological responses to differential irrigation rates in other study indicate that 'Imperial Star' artichoke plants subjected to deficit irrigation (50% ETc) exhibited a significant drought stress throughout the season, thus reducing vegetative growth and lowering yields (Shinohara et al., 2011). Others studies also showed a decrease in head number by deficit irrigation (Garnica et al., 2004; Pomares et al., 2004; Macua et al., 2005b). However, this is the first time that 'Blanca de Tudela' yield has been studied according to artichoke heads by order (main, secondary and tertiary).

3.2. Phenolic compounds at harvest

Total identified polyphenols and total antioxidant activity ranged from 5 to 12 g kg^{-1} FW and 1.5 to 3 g kg^{-1} FW, respectively, being dependent on artichoke head order. Thus, for all harvest dates, the highest total identified polyphenol content was observed for tertiary heads, followed by secondary ones, while the main artichoke heads showed the lowest total identified plyphenol concentration (Table 1). A similar trend was observed for total antioxidant activity which was significantly correlated (R^2=0.918) with total identified plyphenol content. On the other hand, an increasing general trend on total identified plyphenols among harvest dates was observed for each artichoke order (Table 1). Specifically, 1.35, 1.15 and 1.19-fold increase on this content was observed in main, secondary and tertiary heads from February 12th to May 24th.

A high phenolic content in artichoke heads has been related to their content in hydroxycinnamic acids and flavonoids (Schütz et al., 2004; Pandino et al., 2011; Martínez-Esplá et al., 2017a). The major hydroxycinnamic acids found in all artichoke heads were 5-O-caffeoylquinic acid (5-CQA, chlorogenic acid) and 3,5-di-O-caffeoylquinic acid (3, 5-diCQA), which had showed concentrations between 50 and 100-fold higher than the remaining hydroxycinnamic acids detected, leading to a total phenolic content (TPC) dependent of the concentration of these major acids. Chlorogenic acid presented an average concentration in the central flower head of 3 g kg^{-1} FW, the highest levels being observed in January. On the other hand, the secondary and tertiary heads showed an average chlorogenic acid (5-O-caffeoylquinic acid) concentration of 20.6% and 32.6%, respectively, higher than main artichoke head. Specifically, the highest content of chlorogenic acid (5 g kg^{-1} FW) was found in tertiary heads harvested at March 9th (Table 2). Respect to 3, 5-diCQA, the highest concentrations were reached in May for the central and secondary flower heads, while they were in March for the tertiary heads. Secondary heads showed an average concentration of 3, 5-diCQA ca. 3 g Kg^{-1} FW, 33% higher and 27% lower than main and tertiary flower head content, respectively (Table 2). Thus, no relationship was observed between concentration of these major hydroxycinnamic acids and harvet date, since the highest content on 5-CQA in tertiary heads was found in March 9th and in January 19th in main and secondary heads and for 3,5-diCQA the highest content was found at March 28th in main and secondary heads and at the last sampling date (May, 24th) for tertiary heads. Then, concentrations on these phenolic acids were not related with temperature changes along the growing

Table 1
Total yield (kg plant^{-1}), total number of harvested heads (n° heads in total), total identified polyphenols (g kg^{-1} FW) and total antioxidant activity (g kg^{-1} FW) in all harvest dates for different artichoke orders (main, secondary and tertiary heads). Data are the mean ± SE.

Harvest dates	Artichoke orders	Total yield	Total number of heads	Total identified polyphenols	Total antioxidant activity
2017–11–29 (1)	**Main**	0.029 ± 0.002	9.667 ± 0.333	0.066 ± 0.001	2.051 ± 0.026
	Secondary	–	–	–	–
	Tertiary	–	–	–	–
2017–12–11 (2)	**Main**	0.031 ± 0.003 a	5.667 ± 0.882 a	5.246 ± 0.090 b	1.729 ± 0.025 b
	Secondary	0.019 ± 0.002 b	5.333 ± 0.667 a	7.455 ± 0.001 a	2.152 ± 0.048 a
	Tertiary	–	–	–	–
2017–12–22 (3)	**Main**	0.053 ± 0.002 b	6.333 ± 0.186 b	6.316 ± 0.207 a	1.993 ± 0.032 b
	Secondary	0.079 ± 0.005 a	19.667 ± 0.882 a	6.723 ± 0.081 a	2.203 ± 0.042 a
	Tertiary	–	–	–	–
2018–01–08 (4)	**Main**	0.077 ± 0.006 b	13.000 ± 0.892 b	6.507 ± 0.122 b	1.976 ± 0.031 b
	Secondary	0.142 ± 0.007 a	43.333 ± 1.807 a	7.968 ± 0.216 a	2.588 ± 0.036 a
	Tertiary	–	–	–	–
2018–01–19 (5)	**Main**	0.098 ± 0.007 b	1.667 ± 0.333 b	7.581 ± 0.258 b	2.179 ± 0.025 b
	Secondary	0.198 ± 0.019 a	33.667 ± 1.207 a	8.894 ± 0.378 a	2.519 ± 0.049 a
	Tertiary	–	–	–	–
2018–01–30 (6)	**Main**	0.061 ± 0.003 b	9.667 ± 0.882 b	8.212 ± 0.346 a	2.940 ± 0.071 a
	Secondary	0.118 ± 0.005 a	50.000 ± 2.858 a	9.263 ± 0.237 a	3.147 ± 0.051 a
	Tertiary	–	–	–	–
2018–02–12 (7)	**Main**	0.038 ± 0.005 c	6.333 ± 0.602 b	5.834 ± 0.319 c	1.796 ± 0.042 c
	Secondary	0.121 ± 0.007 a	54.667 ± 2.186 a	8.299 ± 0.292 b	2.706 ± 0.062 b
	Tertiary	0.090 ± 0.006 b	0.333 ± 0.027 c	10.053 ± 0.164 a	3.085 ± 0.044 a
2018–02–26 (8)	**Main**	0.024 ± 0.002 c	64.333 ± 0.528 b	6.600 ± 0.162 c	2.315 ± 0.042 c
	Secondary	0.090 ± 0.003 b	77.333 ± 2.333 a	8.017 ± 0.184 b	2.539 ± 0.056 b
	Tertiary	0.174 ± 0.008 a	69.667 ± 2.452 ab	10.439 ± 0.223 a	3.105 ± 0.059 a
2018–03–09 (9)	**Main**	0.009 ± 0.002 c	3.667 ± 0.333 c	6.570 ± 0.204 c	2.247 ± 0.048 c
	Secondary	0.076 ± 0.005 b	52.000 ± 3.055 a	7.578 ± 0.265 b	2.522 ± 0.063 b
	Tertiary	0.290 ± 0.013 a	39.333 ± 2.372 b	12.210 ± 0.199 a	3.231 ± 0.090 a
2018–03–20 (10)	**Main**	0.008 ± 0.001 c	7.667 ± 0.728 b	6.958 ± 0.181c	2.291 ± 0.062 b
	Secondary	0.040 ± 0.003 b	33.333 ± 2.667 a	8.505 ± 0.167 b	2.407 ± 0.055 b
	Tertiary	0.335 ± 0.008 a	34.000 ± 2.215 a	10.303 ± 0.278 a	3.055 ± 0.056 a
2018–03–28 (11)	**Main**	0.016 ± 0.002 c	1.667 ± 0.333 c	8.182 ± 0.438 b	2.526 ± 0.063 b
	Secondary	0.044 ± 0.004 b	27.667 ± 0.856 a	8.923 ± 0.296 b	2.354 ± 0.071 b
	Tertiary	0.249 ± 0.009 a	20.000 ± 1.292 b	11.109 ± 0.388 a	3.001 ± 0.101 a
2018–04–11 (12)	**Main**	0.050 ± 0.004 c	2.333 ± 0.333 c	7.266 ± 0.239 c	0.224 ± 0.050 b
	Secondary	0.083 ± 0.009 b	23.667 ± 1.453 b	8.041 ± 0.154 b	2.469 ± 0.058 a
	Tertiary	0.198 ± 0.007 a	30.667 ± 2.219 a	8.998 ± 0.177 a	2.595 ± 0.056 a
2018–04–23 (13)	**Main**	0.103 ± 0.009 b	74.667 ± 1.410 a	6.665 ± 0.115 c	1.976 ± 0.053 b
	Secondary	0.171 ± 0.006 a	69.000 ± 0.786 b	8.079 ± 0.262 b	2.490 ± 0.071 a
	Tertiary	0.062 ± 0.002 c	69.333 ± 0.910 b	9.777 ± 0.193 a	2.546 ± 0.092 a
2018–05–02 (14)	**Main**	0.116 ± 0.007 b	28.667 ± 1.548 a	6.036 ± 0.248 b	1.815 ± 0.052 b
	Secondary	0.259 ± 0.008 a	22.000 ± 1.512 b	8.622 ± 0.160 a	2.487 ± 0.051 a
	Tertiary	–	–	–	–
2018–05–08 (15)	**Main**	0.070 ± 0.003 b	29.333 ± 1.667 a	7.943 ± 0.200 b	2.686 ± 0.071 b
	Secondary	0.227 ± 0.001 a	15.333 ± 0.860 b	10.214 ± 0.130 a	3.093 ± 0.065 a
	Tertiary	–	–	–	–
2018–05–15 (16)	**Main**	0.040 ± 0.004 b	17.000 ± 0.646 a	9.013 ± 0.184 a	2.536 ± 0.073 a
	Secondary	0.128 ± 0.002 a	15.333 ± 0.702 a	10.813 ± 0.717 a	3.113 ± 0.078 a
	Tertiary	–	–	–	–
2018–05–24 (17)	**Main**	0.038 ± 0.005 b	15.452 ± 0.241 a	7.877 ± 0.416 b	2.164 ± 0.053 b
	Secondary	0.079 ± 0.003 a	15.021 ± 0.104 a	9.532 ± 0.517 b	2.941 ± 0.068 a
	Tertiary	0.097 ± 0.006 a	14.984 ± 0.205 a	11.974 ± 0.337 a	3.041 ± 0.090 a

Different lowercase letters show significant differences ($P < 0.05$) among artichoke orders according to *t*(MS) Student (between main and secondary heads) or HDS Duncan's (among main, secondary and tertiary heads) tests within each harvest date and for each analyzed parameter.

cycle. Five minor hydroxycinnamic acids were quantified. 3-O-caffeoylquinic acid (3-CQA, neochlorogenic acid) showed levels around 0.04 g kg^{-1} FW, independently of head order and harvest date (Table 2). Similarly, 1,3-di-O-caffeoylquinic acid (1,3-diCQA, cynarin) concentration did not show significant differences among flower head orders (Table 3). However, 3,4-di-*O*-caffeoylquinic acid (3,4-diCQA) (Table 2), di-O-caffeoylquinic acid derivative (Table 3) and 4,5-di-*O*-caffeoylquinic acid (4,5-diCAQ) (Table 3) showed a 20% and 50% higher concentration in secondary and tertiary heads, respectively, compared to main heads, showing similar trends to those found in the major hydroxycinnamic acids.

On the other hand, the major luteolin derivatives was luteolin 7-O-glucuronide followed by luteolin 7-O-glucoside (Table 4), being luteolin 7-O-glucuronide-3-O-glucoside content 3-fold lower than the major one (Table 4). Tertiary heads showed average concentrations of 1 g kg^{-1} FW in the major luteolin derivatives; however, the main and secondary heads had 2-fold and 1.5-fold lower concentrations, respectively, than the previous ones. Finally, luteolin-7-O-glucuronide-3-O-glucoside presented an average concentration of 0.176 g kg^{-1} FW in main heads, and levels of 10 and 30% higher in secondary and tertiary heads, respectively.

Total identified polyphenols (as the sum of individual hydroxycinnamic acids and luteolin derivatives) in artichoke edible part from main, secondary and tertiary heads harvesting in winter and spring harvest times, are shown in Fig. 1. Significant differences were observed in phenolic content between head artichoke order within each harvest time. TPC was 27.6 and 22.4% higher in secondary heads for winter and spring harvest times, repectively, with respect to main heads. In the same line, tertiary heads showed an increase in TPC of 67.6 and 38.3% in winter and spring, respectively as compared with secondary heads. However, not significant differences were found between both harvest times for none of the artichoke heads.

Table 2
5-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 3-O-caffeoylquinic acid and 3,4-di-O-caffeoylquinic acid (g kg^{-1} FW) on the edible fraction of 'Blanca de Tudela' cultivar in all harvest dates for different artichoke orders (main, secondary and tertiary heads). Data are the mean ± SE.

Harvest dates	Artichoke orders	5-O-caffeoylquinic acid	3,5-di-O-caffeoylquinic acid	3-O-caffeoylquinic acid	3,4-di-O-caffeoylquinic acid
2017–11–29 (1)	**Main**	2.370 ± 0.059	1.772 ± 0.078	0.038 ± 2.70·10^{-3}	0.036 ± 9.00·10^{-4}
	Secondary	–	–	–	–
	Tertiary	–	–	–	–
2017–12–11 (2)	**Main**	2.374 ± 0.107 b	1.399 ± 0.066 b	0.009 ± 7.00·10^{-4} b	0.029 ± 6.00·10^{-4} b
	Secondary	3.332 ± 0.106 a	2.178 ± 0.049 a	0.014 ± 1.10·10^{-3} a	0.044 ± 6.00·10^{-4} a
	Tertiary	–	–	–	-
2017–12–22 (3)	**Main**	2.632 ± 0.277 a	1.484 ± 0.128 b	0.043 ± 3.70·10^{-3} a	0.030 ± 1.40·10^{-3} b
	Secondary	3.337 ± 0.116 a	1.869 ± 0.077 a	0.053 ± 5.20·10^{-3} a	0.038 ± 5.00·10^{-4} a
	Tertiary	–	–	–	–
2018–01–08 (4)	**Main**	3.317 ± 0.111 a	2.032 ± 0.109 b	0.061 ± 6.10·10^{-3} a	0.042 ± 1.10·10^{-3} b
	Secondary	3.747 ± 0.197 a	2.944 ± 0.163 a	0.035 ± 2.50·10^{-3} b	0.060 ± 1.60·10^{-3} a
	Tertiary	–	–	–	–
2018–01–19 (5)	**Main**	4.021 ± 0.339 a	2.317 ± 0.133 b	0.038 ± 5.70·10^{-3} a	0.047 ± 1.50·10^{-3} b
	Secondary	4.439 ± 0.331 a	3.186 ± 0.211 a	0.027 ± 2.60·10^{-3} a	0.065 ± 2.60·10^{-3} a
	Tertiary	–	–	–	–
2018–01–30 (6)	**Main**	4.029 ± 0.220 a	2.814 ± 0.087 a	0.058 ± 4.90·10^{-3} a	0.078 ± 2.60·10^{-3} a
	Secondary	4.476 ± 0.215 a	3.410 ± 0.183 b	0.052 ± 4.50·10^{-3} a	0.070 ± 2.30·10^{-3} a
	Tertiary	–	–	–	–
2018–02–12 (7)	**Main**	3.153 ± 0.359 a	1.710 ± 0.154 b	0.052 ± 4.70·10^{-3} a	0.035 ± 1.70·10^{-3} b
	Secondary	4.186 ± 0.298 a	2.727 ± 0.170 a	0.046 ± 2.70·10^{-3} a	0.056 ± 1.60·10^{-3} a
	Tertiary	–	–	–	–
2018–02–26 (8)	**Main**	2.987 ± 0.266 a	1.815 ± 0.095 c	0.053 ± 4.80·10^{-3} a	0.037 ± 1.02·10^{-3} c
	Secondary	3.683 ± 0.305 a	2.650 ± 0.087 b	0.041 ± 3.60·10^{-3} ab	0.054 ± 4.00·10^{-4} b
	Tertiary	3.676 ± 0.244 a	3.471 ± 0.263 a	0.036 ± 3.30·10^{-3} b	0.071 ± 3.70·10^{-3} a
2018–03–09 (9)	**Main**	3.000 ± 0.234 b	2.033 ± 0.175 c	0.047 ± 4.40·10^{-3} a	0.042 ± 1.70·10^{-3} c
	Secondary	3.589 ± 0.304 b	2.541 ± 0.086 b	0.041 ± 3.40·10^{-3} a	0.052 ± 3.10·10^{-3} b
	Tertiary	4.944 ± 0.224 a	4.465 ± 0.219 a	0.029 ± 2.20·10^{-3} b	0.091 ± 2.70·10^{-3} a
2018–03–20 (10)	**Main**	3.024 ± 0.233 b	2.284 ± 0.121 c	0.039 ± 3.75·10^{-3} a	0.047 ± 1.30·10^{-3} c
	Secondary	3.444 ± 0.206 ab	2.785 ± 0.135 b	0.042 ± 3.80·10^{-3} a	0.057 ± 1.50·10^{-3} b
	Tertiary	4.160 ± 0.270 a	3.860 ± 0.261 a	0.036 ± 2.90·10^{-3} a	0.079 ± 4.70·10^{-3} a
2018–03–28 (11)	**Main**	3.338 ± 0.320 ab	3.251 ± 0.175 b	0.045 ± 4.70·10^{-3} a	0.066 ± 3.60·10^{-3} b
	Secondary	3.178 ± 0.264 b	3.630 ± 0.126 ab	0.039 ± 3.40·10^{-3} a	0.074 ± 1.20·10^{-3} b
	Tertiary	4.538 ± 0.343 a	4.138 ± 0.261 a	0.036 ± 2.90·10^{-3} a	0.085 ± 2.50·10^{-3} a
2018–04–11 (12)	**Main**	3.090 ± 0.280 a	2.536 ± 0.120 b	0.030 ± 3.30·10^{-3} b	0.052 ± 2.20·10^{-3} c
	Secondary	2.951 ± 0.141 a	2.897 ± 0.194 ab	0.046 ± 2.90·10^{-3} a	0.059 ± 1.20·10^{-3} b
	Tertiary	3.263 ± 0.195 a	3.167 ± 0.163 a	0.039 ± 3.60·10^{-3} ab	0.065 ± 1.10·10^{-3} a
2018–04–23 (13)	**Main**	2.615 ± 0.106 b	2.481 ± 0.113 b	0.043 ± 2.80·10^{-3} a	0.051 ± 8.00·10^{-4} c
	Secondary	3.128 ± 0.308 ab	2.936 ± 0.182 b	0.051 ± 4.70·10^{-3} a	0.060 ± 1.24·10^{-3} b
	Tertiary	3.335 ± 0.214 a	3.759 ± 0.182 a	0.045 ± 3.60·10^{-3} a	0.077 ± 1.40·10^{-3} a
2018–05–02 (14)	**Main**	2.360 ± 0.206 b	2.130 ± 0.152 b	0.034 ± 3.60·10^{-3} a	0.044 ± 2.10·10^{-3} b
	Secondary	3.539 ± 0.213 a	3.123 ± 0.107 a	0.040 ± 4.00·10^{-3} a	0.064 ± 1.10·10^{-3} a
	Tertiary	–	–	–	–
2018–05–08 (15)	**Main**	2.976 ± 0.200 b	3.131 ± 0.152 b	0.049 ± 3.70·10^{-3} a	0.064 ± 1.80·10^{-3} b
	Secondary	4.099 ± 0.132 a	3.773 ± 0.115 a	0.046 ± 3.40·10^{-3} a	0.077 ± 2.60·10^{-3} a
	Tertiary	–	–	–	–
2018–05–15 (16)	**Main**	3.263 ± 0.191 a	3.497 ± 0.124 a	0.040 ± 3.90·10^{-3} b	0.071 ± 2.50·10^{-3} a
	Secondary	3.952 ± 0.363 a	4.148 ± 0.272 a	0.056 ± 4.66·10^{-3} a	0.085 ± 4.90·10^{-3} a
	Tertiary	–	–	–	–
2018–05–24 (17)	**Main**	2.730 ± 0.252 b	3.202 ± 0.154 b	0.039 ± 2.90·10^{-3} b	0.065 ± 3.35·10^{-3} b
	Secondary	3.114 ± 0.338 ab	3.813 ± 0.207 ab	0.055 ± 5.10·10^{-3} a	0.078 ± 3.90·10^{-3} ab
	Tertiary	4.084 ± 0.305 a	4.195 ± 0.256 a	0.063 ± 4.70·10^{-3} a	0.086 ± 3.10·10^{-3} a

Different lowercase letters show significant differences ($P < 0.05$) among artichoke orders according to *t*(MS) Student (between main and secondary heads) or HSD Duncan's (among main, secondary and tertiary heads) tests within each harvest date and for each analyzed compound.

In this sense, phenolic profile of artichoke has been widely studied, as well as the factors that affect the total phenolic content, such as genetic, environmental, edaphic, agronomic, among others (Schütz et al., 2004; Lombardo et al., 2018). For instance, differences on phenolic content have been reported among cultivars and harvest times, the early harvested artichoke having lower content than the latets ones (Licciardello et al., 2017; Pandino et al., 2017b). Furthermore, its correlation with total antioxidant activity has been previously confirmed (Bonasia et al., 2010; Ruíz-Jiménez et al., 2014). On the other hand, artichoke head order has been described as influencing total production, number of buds, head average weight, seed production, and phenolic content (Ortega 2002; Cappelletti et al., 2016; Gadliardi et al., 2020).

Previous works with different cultivars, including 'Blanca de Tudela', have identifed chlorogenic and 1,5-diCQA as major hydroxycinnamic acids (Pandino et al., 2011; 2017b). However, Gil-Izquierdo et al. (2002) indicated that using an HPLC-DAD-ultraviolet-visible (UV-vis), 3, 5-diCQA and 1,5-diCQA eluted together and could not be separated properly. Our results, therefore, are in agreement with recently published results in which 5-CQA and 3,5-diCQA stand out as the major hydroxycinnamic acids in several cultivars of globe and wild artichokes (Garbetta et al., 2014; Petropoulos et al., 2018). The phenolic profile obtained can be in influenced by both artichoke moisture content and extraction solvent (Martínez-Esplá et al., 2017a). Previous works carried out with purple artichoke cultivars, such as 'Violeto di Provenza', 'Romanesco C3', 'Apollo', 'Exploter' and 'Montelupone A', reported differences on phenolic content depending on flower head order (Cappelletti et al., 2016; Gagliardi et al., 2020), as also it has been observed in the present work, showing higher phenolic content in secondary and tertiary heads than in main ones. A previous research work has also reported this variability on the phenolic content and profile for white artichoke cultivar 'Blanca de Tudela' (Giménez et al., 2021), according to our results. Thus, this factor should be considered in order to

Table 3
1,3-di-O-caffeoylquinic acid, di-O-caffeoylquinic acid derivative and 4,5-di-O-caffeoylquinic acid (g kg^{-1} FW) on the edible fraction of 'Blanca de Tudela' cultivar in all harvest dates for different artichoke orders (main, secondary and tertiary heads). Data are the mean ± SE.

Harvest dates	Artichoke orders	1,3-di-O-caffeoylquinic acid	4,5-di-O-caffeoylquinic acid	di-O-caffeoylquinic acid derivative
2017–11–29 (1)	**Main**	$0.032 \pm 1.50 \cdot 10^{-3}$	$0.049 \pm 3.50 \cdot 10^{-3}$	$0.015 \pm 1.00 \cdot 10^{-3}$
	Secondary	–	–	–
	Tertiary	–	–	–
2017–12–11 (2)	**Main**	$0.018 \pm 8.00 \cdot 10^{-4}$ b	$0.016 \pm 1.40 \cdot 10^{-3}$ b	$0.013 \pm 7.00 \cdot 10^{-4}$ b
	Secondary	$0.025 \pm 1.00 \cdot 10^{-3}$ a	$0.024 \pm 1.40 \cdot 10^{-3}$ a	$0.019 \pm 8.00 \cdot 10^{-4}$ a
	Tertiary	–	–	–
2017–12–22 (3)	**Main**	$0.025 \pm 1.70 \cdot 10^{-3}$ b	$0.024 \pm 2.30 \cdot 10^{-3}$ b	$0.015 \pm 1.20 \cdot 10^{-3}$ a
	Secondary	$0.036 \pm 1.84 \cdot 10^{-3}$ a	$0.044 \pm 5.20 \cdot 10^{-3}$ a	$0.018 \pm 2.20 \cdot 10^{-3}$ a
	Tertiary	–	–	–
2018–01–08 (4)	**Main**	$0.039 \pm 1.70 \cdot 10^{-3}$ a	$0.045 \pm 3.00 \cdot 10^{-3}$ a	$0.024 \pm 1.80 \cdot 10^{-3}$ a
	Secondary	$0.037 \pm 1.60 \cdot 10^{-3}$ a	$0.046 \pm 2.80 \cdot 10^{-3}$ a	$0.026 \pm 3.10 \cdot 10^{-3}$ a
	Tertiary	–	–	–
2018–01–19 (5)	**Main**	$0.045 \pm 3.30 \cdot 10^{-3}$ a	$0.043 \pm 5.30 \cdot 10^{-3}$ a	$0.018 \pm 2.10 \cdot 10^{-3}$ a
	Secondary	$0.044 \pm 3.10 \cdot 10^{-3}$ a	$0.048 \pm 3.30 \cdot 10^{-3}$ a	$0.020 \pm 4.30 \cdot 10^{-3}$ a
	Tertiary	–	–	–
2018–01–30 (6)	**Main**	$0.037 \pm 2.80 \cdot 10^{-3}$ a	$0.042 \pm 3.10 \cdot 10^{-3}$ a	$0.033 \pm 5.20 \cdot 10^{-3}$ a
	Secondary	$0.039 \pm 3.00 \cdot 10^{-3}$ a	$0.049 \pm 3.20 \cdot 10^{-3}$ a	$0.031 \pm 4.20 \cdot 10^{-3}$ a
	Tertiary	–	–	–
2018–02–12 (7)	**Main**	$0.031 \pm 2.20 \cdot 10^{-3}$ a	$0.033 \pm 4.10 \cdot 10^{-3}$ b	$0.013 \pm 1.40 \cdot 10^{-3}$ a
	Secondary	$0.034 \pm 2.40 \cdot 10^{-3}$ a	$0.051 \pm 3.60 \cdot 10^{-3}$ a	$0.019 \pm 1.90 \cdot 10^{-3}$ a
	Tertiary	–	–	–
2018–02–26 (8)	**Main**	$0.038 \pm 3.10 \cdot 10^{-3}$ a	$0.045 \pm 6.60 \cdot 10^{-3}$ b	$0.015 \pm 1.50 \cdot 10^{-3}$ b
	Secondary	$0.046 \pm 3.40 \cdot 10^{-3}$ a	$0.055 \pm 5.30 \cdot 10^{-3}$ b	$0.020 \pm 3.00 \cdot 10^{-3}$ ab
	Tertiary	$0.037 \pm 2.50 \cdot 10^{-3}$ a	$0.074 \pm 5.06 \cdot 10^{-3}$ a	$0.026 \pm 3.40 \cdot 10^{-3}$ a
2018–03–09 (9)	**Main**	$0.034 \pm 2.30 \cdot 10^{-3}$ b	$0.049 \pm 6.60 \cdot 10^{-3}$ b	$0.014 \pm 1.70 \cdot 10^{-3}$ b
	Secondary	$0.041 \pm 3.10 \cdot 10^{-3}$ ab	$0.051 \pm 5.50 \cdot 10^{-3}$ b	$0.016 \pm 2.10 \cdot 10^{-3}$ ab
	Tertiary	$0.047 \pm 3.10 \cdot 10^{-3}$ a	$0.084 \pm 5.40 \cdot 10^{-3}$ a	$0.024 \pm 2.90 \cdot 10^{-3}$ a
2018–03–20 (10)	**Main**	$0.042 \pm 2.90 \cdot 10^{-3}$ a	$0.056 \pm 5.50 \cdot 10^{-3}$ a	$0.020 \pm 2.10 \cdot 10^{-3}$ a
	Secondary	$0.047 \pm 3.80 \cdot 10^{-3}$ a	$0.063 \pm 5.10 \cdot 10^{-3}$ a	$0.018 \pm 1.00 \cdot 10^{-3}$ a
	Tertiary	$0.048 \pm 3.10 \cdot 10^{-3}$ a	$0.069 \pm 5.10 \cdot 10^{-3}$ a	$0.023 \pm 2.10 \cdot 10^{-3}$ a
2018–03–28 (11)	**Main**	$0.051 \pm 3.40 \cdot 10^{-3}$ a	$0.062 \pm 4.80 \cdot 10^{-3}$ a	$0.027 \pm 1.60 \cdot 10^{-3}$ a
	Secondary	$0.045 \pm 2.30 \cdot 10^{-3}$ a	$0.071 \pm 5.60 \cdot 10^{-3}$ a	$0.028 \pm 2.20 \cdot 10^{-3}$ a
	Tertiary	$0.051 \pm 4.00 \cdot 10^{-3}$ a	$0.075 \pm 6.90 \cdot 10^{-3}$ a	$0.024 \pm 3.00 \cdot 10^{-3}$ a
2018–04–11 (12)	**Main**	$0.040 \pm 2.50 \cdot 10^{-3}$ a	$0.057 \pm 4.60 \cdot 10^{-3}$ b	$0.020 \pm 2.00 \cdot 10^{-3}$ a
	Secondary	$0.042 \pm 1.40 \cdot 10^{-3}$ a	$0.057 \pm 3.90 \cdot 10^{-3}$ b	$0.030 \pm 4.40 \cdot 10^{-3}$ a
	Tertiary	$0.044 \pm 3.60 \cdot 10^{-3}$ a	$0.072 \pm 3.10 \cdot 10^{-3}$ a	$0.026 \pm 2.50 \cdot 10^{-3}$ a
2018–04–23 (13)	**Main**	$0.045 \pm 3.10 \cdot 10^{-3}$ a	$0.062 \pm 5.00 \cdot 10^{-3}$ a	$0.022 \pm 2.00 \cdot 10^{-3}$ b
	Secondary	$0.044 \pm 3.10 \cdot 10^{-3}$ ab	$0.075 \pm 9.30 \cdot 10^{-3}$ a	$0.020 \pm 3.80 \cdot 10^{-3}$ b
	Tertiary	$0.034 \pm 2.40 \cdot 10^{-3}$ b	$0.076 \pm 7.00 \cdot 10^{-3}$ a	$0.035 \pm 4.10 \cdot 10^{-3}$ a
2018–05–02 (14)	**Main**	$0.035 \pm 1.40 \cdot 10^{-3}$ b	$0.053 \pm 4.10 \cdot 10^{-3}$ b	$0.022 \pm 1.30 \cdot 10^{-3}$ a
	Secondary	$0.047 \pm 3.70 \cdot 10^{-3}$ a	$0.071 \pm 4.20 \cdot 10^{-3}$ a	$0.020 \pm 2.00 \cdot 10^{-3}$ a
	Tertiary	–	–	–
2018–05–08 (15)	**Main**	$0.046 \pm 2.90 \cdot 10^{-3}$ b	$0.066 \pm 2.90 \cdot 10^{-3}$ b	$0.027 \pm 3.80 \cdot 10^{-3}$ a
	Secondary	$0.062 \pm 2.90 \cdot 10^{-3}$ a	$0.092 \pm 3.90 \cdot 10^{-3}$ a	$0.026 \pm 1.30 \cdot 10^{-3}$ a
	Tertiary	–	–	–
2018–05–15 (16)	**Main**	$0.051 \pm 3.50 \cdot 10^{-3}$ a	$0.059 \pm 4.50 \cdot 10^{-3}$ b	$0.026 \pm 2.30 \cdot 10^{-3}$ a
	Secondary	$0.061 \pm 3.90 \cdot 10^{-3}$ a	$0.109 \pm 5.10 \cdot 10^{-3}$ a	$0.024 \pm 4.60 \cdot 10^{-3}$ a
	Tertiary	–	–	–
2018–05–24 (17)	**Main**	$0.047 \pm 3.60 \cdot 10^{-3}$ b	$0.049 \pm 4.10 \cdot 10^{-3}$ b	$0.033 \pm 5.20 \cdot 10^{-3}$ a
	Secondary	$0.046 \pm 3.50 \cdot 10^{-3}$ b	$0.060 \pm 4.70 \cdot 10^{-3}$ ab	$0.042 \pm 6.20 \cdot 10^{-3}$ a
	Tertiary	$0.063 \pm 4.20 \cdot 10^{-3}$ a	$0.080 \pm 6.60 \cdot 10^{-3}$ a	$0.048 \pm 7.00 \cdot 10^{-3}$ a

Different lowercase letters show significant differences ($P < 0.05$) among artichoke orders according to *t*(MS) Student (between main and secondary heads) or HDS Duncan's (among main, secondary and tertiary heads) test within each harvest date for each analyzed compound.

improve functional quality at harvest of globe artichoke crop.

3.3. Postharvest storage

As described in the experimental design section, two postharvest experiments were carried out, one in winter and another one in spring harvest time. Respiration rate at harvest was 3-fold higher in spring (182.7 ± 9.25 mg CO_2 kg^{-1} h^{-1}) than in winter (56.45 ± 4.08 mg CO_2 kg^{-1} h^{-1}), and main head showed a higher respiration rate than secondary and tertiary heads. These differences were also maintained during cold storage, although respiration rate decreased from day 0 to day 7 and increased afterward, for all artichoke head orders and both growing seasons. In general, tertiary heads showed lower respiration rate than main heads, with values of 16.2 and 10.2% lower at the end of the experiment in winter and spring harvest times, respectively (Fig. 2). It is well-known that shelf-life of fresh products is negatively correlated with intensity of respiration rate (Kader, 2002).

Thus, artichoke could be considered as a perishable product since it has a high respiration rate even under cold storage conditions, and has a short shelf-life under 20 °C (Gil et al., 2001; Sabi et al., 2013; Ruíz-Jiménez et al., 2014). For first time, a significant reduction on respiration rate in secondary and tertiary heads at harvest in both harvest times has been reported. As far as we know, there are not studies with similar results in respiration rate. However, experiments with treatments, such as ozone, modified atmosphere and oxalic acid, showed a reduction on respiration rate of artichokes and a quality maintenance during their postharvest cold (Gil et al., 2003; Restuccia et al., 2014; Lombardo et al., 2015) or room temperature (Ruíz-Jiménez et al., 2014) storage.

Weight losses showed significant differences among head orders in both postharvest experiments, being the highest weight losses observed from main heads, 23.16 ± 0.95 and 25.64 ± 1.03%, in winter and spring harvest time, respectively, after 21 days of cold storage at 2 °C.

Table 4
Luteolin derivatives content (g kg^{-1} FW); luteolin 7-O-glucuronide, luteolin 7-O-glucoside and luteolin 7-O-glucuronide-3-O-glucoside on the edible fraction of 'Blanca de Tudela' cultivar in all harvest dates for different artichoke orders (main, secondary and tertiary heads). Data are the mean ± SE.

Harvest dates	Artichoke orders	Luteolin 7-O-glucuronide	Luteolin 7-O-glucoside	Luteolin 7-O-glucuronide-3-O-glucoside
2017–11–29 (1)	**Main**	0.415 ± 0.024	0.637 ± 0.054	0.235 ± 0.015
	Secondary	–	–	–
	Tertiary	–	–	–
2017–12–11 (2)	**Main**	0.525 ± 0.036 a	0.612 ± 0.034 b	0.251 ± 0.015 a
	Secondary	0.641 ± 0.062 a	0.903 ± 0.047 a	0.275 ± 0.017 a
	Tertiary	–	–	–
2017–12–22 (3)	**Main**	0.614 ± 0.063 b	0.518 ± 0.050 a	0.129 ± 0.018 a
	Secondary	1.016 ± 0.083 a	0.527 ± 0.034 a	0.186 ± 0.013 a
	Tertiary	–	–	–
2018–01–08 (4)	**Main**	0.251 ± 0.039 a	0.513 ± 0.046 a	0.184 ± 0.016 a
	Secondary	0.358 ± 0.039 a	0.546 ± 0.033 a	0.214 ± 0.014 a
	Tertiary	–	–	–
2018–01–19 (5)	**Main**	0.283 ± 0.022 a	0.577 ± 0.052 a	0.192 ± 0.017 a
	Secondary	0.298 ± 0.028 a	0.552 ± 0.053 a	0.215 ± 0.010 a
	Tertiary	–	–	–
2018–01–30 (6)	**Main**	0.352 ± 0.057 a	0.573 ± 0.057 a	0.196 ± 0.004 a
	Secondary	0.464 ± 0.043 a	0.568 ± 0.052 a	0.185 ± 0.007 a
	Tertiary	–	–	–
2018–02–12 (7)	**Main**	0.259 ± 0.034 b	0.399 ± 0.038 a	0.150 ± 0.017 a
	Secondary	0.444 ± 0.039 a	0.588 ± 0.068 a	0.212 ± 0.018 a
	Tertiary	–	–	–
2018–02–26 (8)	**Main**	0.474 ± 0.052 b	0.608 ± 0.069 b	0.176 ± 0.016 a
	Secondary	0.743 ± 0.062 a	0.670 ± 0.077 b	0.224 ± 0.015 a
	Tertiary	0.810 ± 0.084 a	1.074 ± 0.093 a	0.222 ± 0.016 a
2018–03–09 (9)	**Main**	0.502 ± 0.076 b	0.540 ± 0.038 b	0.170 ± 0.018 b
	Secondary	0.624 ± 0.040 b	0.588 ± 0.062 b	0.238 ± 0.018 a
	Tertiary	1.056 ± 0.078 a	1.183 ± 0.089 a	0.294 ± 0.026 a
2018–03–20 (10)	**Main**	0.541 ± 0.065 b	0.606 ± 0.048 b	0.180 ± 0.017 a
	Secondary	0.902 ± 0.078 a	0.803 ± 0.073 ab	0.185 ± 0.017 a
	Tertiary	1.063 ± 0.105 a	0.888 ± 0.072 a	0.239 ± 0.019 a
2018–03–28 (11)	**Main**	0.358 ± 0.024 c	0.737 ± 0.048 b	0.249 ± 0.023 a
	Secondary	0.719 ± 0.071 b	0.950 ± 0.082 ab	0.188 ± 0.014 a
	Tertiary	1.008 ± 0.080 a	0.966 ± 0.075 a	0.189 ± 0.018 a
2018–04–11 (12)	**Main**	0.571 ± 0.052 b	0.687 ± 0.030 b	0.151 ± 0.015 a
	Secondary	0.818 ± 0.081 b	0.995 ± 0.102 a	0.211 ± 0.026 a
	Tertiary	1.261 ± 0.100 a	0.902 ± 0.080 ab	0.159 ± 0.005 a
2018–04–23 (13)	**Main**	0.496 ± 0.047 c	0.717 ± 0.048 a	0.133 ± 0.011 b
	Secondary	0.759 ± 0.076 b	0.843 ± 0.081 a	0.163 ± 0.012 b
	Tertiary	1.268 ± 0.079 a	0.907 ± 0.090 a	0.242 ± 0.018 a
2018–05–02 (14)	**Main**	0.545 ± 0.046 a	0.597 ± 0.057 b	0.117 ± 0.011 a
	Secondary	0.730 ± 0.066 a	0.835 ± 0.033 a	0.153 ± 0.012 a
	Tertiary	–	–	–
2018–05–08 (15)	**Main**	0.597 ± 0.054 a	0.772 ± 0.045 b	0.176 ± 0.016 a
	Secondary	0.737 ± 0.062 a	1.170 ± 0.113 a	0.203 ± 0.016 a
	Tertiary	–	–	–
2018–05–15 (16)	**Main**	0.718 ± 0.112 a	0.929 ± 0.072 a	0.140 ± 0.014 a
	Secondary	0.966 ± 0.094 a	1.242 ± 0.115 a	0.172 ± 0.012 a
	Tertiary	–	–	–
2018–05–24 (17)	**Main**	0.583 ± 0.076 b	0.958 ± 0.075 b	0.170 ± 0.016 b
	Secondary	0.861 ± 0.085 b	1.259 ± 0.101 ab	0.212 ± 0.018 b
	Tertiary	1.250 ± 0.111 a	1.497 ± 0.120 a	0.309 ± 0.018 a

Different lowercase letters show significant differences ($P < 0.05$) among artichoke orders according to *t*(MS) Student (between main and secondary heads) or HDS Duncan's (among main, secondary and tertiary heads) tests within each harvest date for each analyzed compound.

Nevertheless, secondary and tertiary heads showed significant ($P < 0.05$) lower weight losses than main heads during the whole storage period, although the greatest differences were observed at the end of the experiment (Fig. 3).

Firmness, measured at the equatorial diameter of each artichoke head, showed the artichoke compactness at harvest and during postharvest cold storage. At harvest, firmness of main artichokes was 6.74 ± 0.21 and 7.28 ± 0.19 N mm^{-1} in winter and spring harvest time, respectively, and it was 37.8 and 29.1% higher in secondary heads than main ones in winter and spring harvest time, respectively. In addition, firmness values of tertiary heads were 21.7 and 105% higher than main heads for both harvest times, winter and spring, respectively. These differences were maintained during cold storage, despite of the decreasing on head firmness found in all artichokes, depending on the head order and showing a clear effect of this factor (Fig. 4).

Weight loss is an important quality parameter of artichoke heads during their postharvest storage. This quality trait is close related to the head compactness and, therefore, to the head firmness, leading to a withered appearance that could cause the non-acceptance of the product by the consumers, being the major reason of artichoke deterioration (Sabi et al., 2013). Weight losses and firmness are quality parameters directly related to respiration rate, and the effect of head order was observed at harvest and during cold storage, since tertiary followed by secondary heads maintained higher quality parameters and increased their shelf-life period as compared with main heads. On the other hand, postharvest treatments with oxalic acid or O_3-atmosphere decreased weight losses and maintained firmness levels, leading to overall quality maintenance during cold storage (Restuccia et al., 2014; Ruíz-Jiménez et al., 2014). Similar results have been reported with preharvest treatments with oxalic acid and methyl jasmonate as well as with irrigation with high quality water (Gil et al., 2001; Martínez-Esplá et al., 2017a; 2017b; Lombardo et al., 2018; Gagliardi et al., 2020). However, as far as

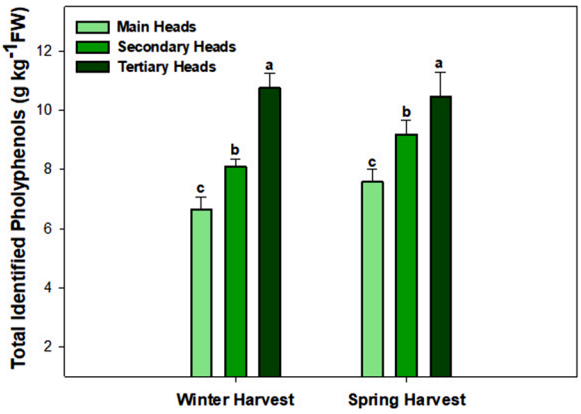

Fig. 1. Total identified polyphenols (g kg^{-1} FW) on the edible fraction of 'Blanca de Tudela' cultivar in two harvest times. Data are the mean ± SE. Different lowercase letters show significant differences at $P < 0.05$ among artichoke orders (main, secondary and tertiary heads). No asterisks found in spring harvest time bars for each artichoke order means that there were no significant differences ($P \geq 0.05$) between both harvest times.

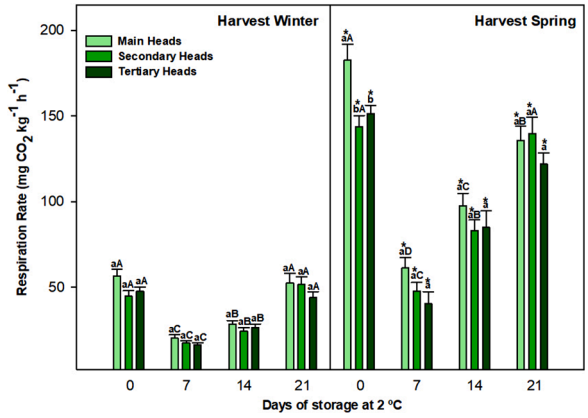

Fig. 2. Respiration rate (mg CO_2 kg^{-1} h^{-1}) of main, secondary and tertiary heads at harvest and during postharvest cold storage at 2 °C for winter and spring harvest times. Data are the mean ± SE. Different lowercase and capital letters show significant differences at $P < 0.05$ among artichoke orders (main, secondary and tertiary heads) and days of storage at 2 °C, respectively, for each harvest time. Asterisks found in spring harvest time bars for each artichoke order and storage day means that there were significant differences ($P < 0.05$) between both harvest times.

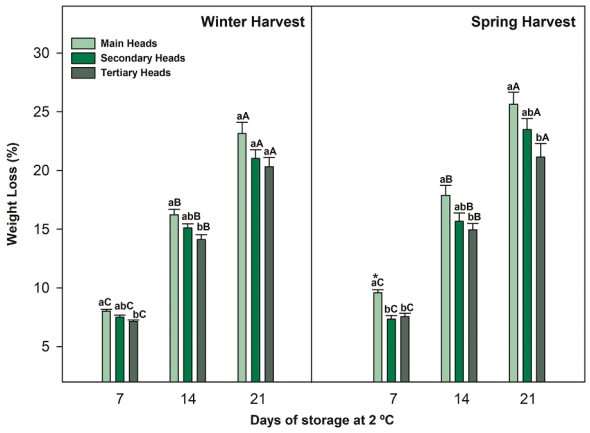

Fig. 3. Weight loss (%) of main, secondary and tertiary artichokes at harvest and during postharvest cold storage at 2 °C for winter and spring harvest times. Data are the mean ± SE. Different lowercase and capital letters show significant differences at $P < 0.05$ among artichoke orders (main, secondary and tertiary heads) and days of storage at 2 °C, respectively, for each harvest time. Asterisks found in spring harvest time bars for each artichoke order and storage day means that there were significant differences ($P < 0.05$) between both harvest times. No asterisks found in spring harvest time bars for each artichoke order means that there were no significant differences ($P \geq 0.05$) between both harvest times.

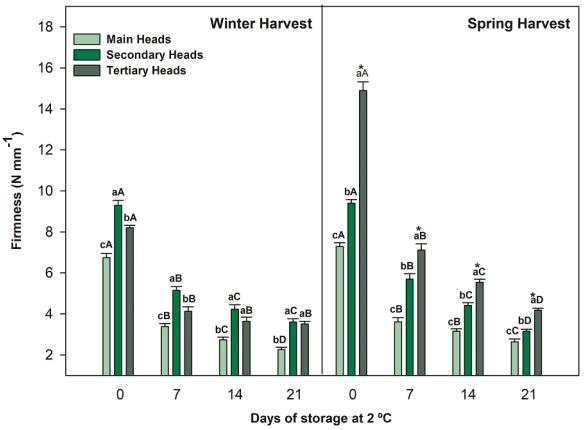

Fig. 4. Firmness (N mm^{-1}) of main, secondary and tertiary artichokes heads at harvest and during postharvest cold storage at 2 °C for winter and spring harvest times. Data are the mean ± SE. Different lowercase and capital letters show significant differences at $P < 0.05$ among artichoke orders (main, secondary and tertiary heads) and days of storage at 2 °C, respectively, for each harvest time. Asterisks found in spring harvest time bars for each artichoke order and storage day means that there were significant differences ($P < 0.05$) between both harvest times. No asterisks found in spring harvest time bars for each artichoke order means that there were no significant differences ($P \geq 0.05$) between both harvest times.

we know, this is the first report in which the effect of the artichoke head order in plant on quality traits during cold storage have been provided. Lombardo et al. (2015) concluded that the application of O_3 improved artickoke quality as a consequence of higher TPC which played a significant role in the detoxification process. In the present experiment, content of hydroxycinnamic acids and luteolin derivatives at harvest and at the end of cold storage in main, secondary and tertiary heads were analyzed (Table 5). In general, the concentration of these individual phenolics increased during cold storage, althouth it is important to note that differences among flower head orders observed at harvest were mainained during storage. Thus, chlorogenic acid content was 35.3 and 36.8% higher in secondary and tertiary artichokes, respectively, than in main heads harvested at winter time. In addition, an increase of 84.1 and 100% in secondary and tertiary heads, respectively, was observed in artichokes harvested at spring harvest time. On the other hand, 3,5-diCAQ increased in a similar trend than chlorogenic acid, which led to a 43.5 and 66.2% increase of total hydroxycinnamic acid content for secondary and tertiary heads, respectively, for artichokes harvested in winter time, and 41.9 and 73.7%, respectively, for those harvest in spring harvest time, at the end of the experiment. Luteolin derivatives showed a similar evolution during postharvest storage and especially after 21 days of storage at 2 °C, when they increased around 0.4 and 1 g kg^{-1} FW in secondary and tertiary heads, respectively, with respect to main heads in artichokes of both harvest times. This increase in phenolic content is due to the activation of phenylpropanoid biosynthetic pathways during cold storage as a defense mechanism against abiotic stresses such as high/low temperature (Saltveit, 2010; Smirnov et al., 2015; Handa et al., 2019).

Different lowercase letters show significant differences ($P < 0.05$) among artichoke orders according to Tuckey test for each analyzed compound and studied day. Significant differences between 0 and 21

Table 5
Individual phenolic compounds content (g kg^{-1} DW) on the edible fraction of different artichokes order (main, secondary and tertiary heads) at harvest and after 21 days of cold storage at 2 °C for winter and spring harvest times. Data are the mean ± SE.

		Head order	Winter Harvest		*t* (MS) Student	Spring Harvest		*t* (MS) Student
			Day 0[¥]	Day 21		Day 0[¥]	Day 21	
Hydroxycinnamic Acids	**3-*O*-CQA**	**Main**	0.441 ± 0.042	0.501 ± 0.075 a	–	0.358 ± 0.025	0.375 ± 0.025 a	–
		Secondary	0.342 ± 0.033	0.342 ± 0.017 a	–	0.425 ± 0.042	0.459 ± 0.025 a	–
		Tertiary	0.300 ± 0.025	0.342 ± 0.017 a	–	0.458 ± 0.033	0.434 ± 0.025 a	–
	5-*O*-CQA	**Main**	24.882 ± 2.216	30.199 ± 1.557 b	–	21.783 ± 0.883	18.473 ± 1.188 b	*
		Secondary	30.679 ± 2.541	41.600 ± 2.222 a	–	26.056 ± 2.566	35.409 ± 1.861 a	–
		Tertiary	30.621 ± 2.033	41.933 ± 2.331 a	–	27.781 ± 1.783	38.543 ± 2.543 a	–
	1,3-*O*-DCQA	**Main**	0.317 ± 0.025	0.242 ± 0.075 a	–	0.375 ± 0.025	0.392 ± 0.017 a	–
		Secondary	0.383 ± 0.025	0.400 ± 0.042 a	–	0.367 ± 0.025	0.334 ± 0.025 a	–
		Tertiary	0.308 ± 0.017	0.325 ± 0.033 a	–	0.283 ± 0.017	0.334 ± 0.025 a	–
	DCQA derivative	**Main**	0.125 ± 0.008	0.142 ± 0.017 b	–	0.183 ± 0.017	0.192 ± 0.017 b	–
		Secondary	0.167 ± 0.025	0.200 ± 0.017 ab	–	0.167 ± 0.033	0.225 ± 0.008 ab	–
		Tertiary	0.217 ± 0.025	0.217 ± 0.017 a	–	0.292 ± 0.033	0.275 ± 0.025 a	–
	3,4-*O*-DCQA	**Main**	0.308 ± 0.008	0.342 ± 0.008 c	–	0.425 ± 0.008	0.417 ± 0.008 c	–
		Secondary	0.450 ± 0.008	0.501 ± 0.008 b	*	0.500 ± 0.008	0.509 ± 0.017 b	–
		Tertiary	0.591 ± 0.033	0.701 ± 0.025 a	*	0.641 ± 0.008	0.751 ± 0.017 a	*
	3,5-*O*-DCQA	**Main**	15.119 ± 0.075	18.347 ± 0.626 c	–	20.667 ± 0.941	27.554 ± 0.794 c	*
		Secondary	22.075 ± 0.725	29.906 ± 0.936 b	*	24.457 ± 0.933	31.603 ± 0.526 b	*
		Tertiary	28.913 ± 1.358	41.517 ± 1.044 a	*	31.312 ± 1.516	44.575 ± 1.112 a	*
	4,5-*O*-DCQA	**Main**	0.375 ± 0.050	0.425 ± 0.067 b	–	0.516 ± 0.042	0.434 ± 0.869 a	–
		Secondary	0.458 ± 0.042	0.434 ± 0.050 b	–	0.625 ± 0.075	0.668 ± 0.058 a	–
		Tertiary	0.616 ± 0.042	0.676 ± 0.058 a	–	0.633 ± 0.058	0.601 ± 0.050 a	–
	Total HA	**Main**	41.567 ± 3.090 b	53.889 ± 2.613 b	–	44.299 ± 2.107 b	51.650 ± 0.200 c	–
		Secondary	54.562 ± 3.282 a	80.368 ± 3.604 a	–	52.596 ± 3.024 ab	76.347 ± 2.622 b	*
		Tertiary	61.592 ± 4.057 a	94.975 ± 3.246 a	*	61.400 ± 2.691 a	95.018 ± 2.028 a	*
Luteolin derivatives	**Lut 7-*O*-gluc 3-*O*-glc**	**Main**	1.466 ± 0.133	1.574 ± 0.092 a	–	1.108 ± 0.092	1.171 ± 0.108 c	–
		Secondary	1.866 ± 0.125	1.792 ± 0.108 a	–	1.358 ± 0.100	1.558 ± 0.075 b	–
		Tertiary	1.849 ± 0.133	1.674 ± 0.117 a	–	2.016 ± 0.150	1.944 ± 0.058 a	–
	Lut 7-*O*-glc	**Main**	3.948 ± 0.433	5.211 ± 0.693 b	–	4.132 ± 0.392	4.513 ± 0.836 b	–
		Secondary	6.189 ± 0.516	7.024 ± 0.643 ab	–	6.322 ± 0.633	6.719 ± 0.827 b	–
		Tertiary	6.747 ± 0.700	8.149 ± 0.576 a	–	10.562 ± 0.658	12.059 ± 0.526 a	–
	Lut 7-*O*-gluc	**Main**	5.065 ± 0.575	6.196 ± 0.359 b	–	5.973 ± 0.400	7.845 ± 0.760 a	–
		Secondary	5.581 ± 0.641	7.293 ± 0.492 b	–	7.022 ± 0.675	8.670 ± 6.571 a	–
		Tertiary	8.946 ± 0.775	11.811 ± 0.534 a	*	7.555 ± 0.750	9.383 ± 0.802 a	–
	Total Luteolins	**Main**	10.479 ± 0.392 c	13.305 ± 0.434 c	*	11.211 ± 0.183 c	13.875 ± 0.334 c	*
		Secondary	13.628 ± 0.300 b	16.550 ± 0.417 b	*	14.702 ± 0.325 b	17.479 ± 0.643 b	*
		Tertiary	17.535 ± 0.333 a	22.375 ± 0.760 a	*	20.125 ± 0.317 a	24.279 ± 0.684 a	*

[¥] Significant differences for values of day 0 have been previously included in Tables 2–4, depending on the analyzed compound on fresh weight (FW) basis, except for the total hydroxycinnamic acids and total luteolins content, which has been included in this table.

storage days for each harvest time are shown with * symbol when $P < 0.05$ performing a *t*-Student test. Not significant differences between these days are shown with – symbol.

In conclusion, our results showed that globe artichoke head order directly influences the content of individual phenolics, being higher for tertiary heads followed by secondary and main heads, respectively. These differences could lead to classify globe artichokes, not only based on their functional value, but also by their aptitude for postharvest storage, since artichokes with the highest TPC had a greater aptitude for their postharvest cold storage, extending their shelf-life period and, consequently, improving the consumer satisfaction or market value.

CRediT authorship contribution statement

María J. Giménez: Conceptualization, Methodology, Investigation, Writing – original draft. **Marina Giménez-Berenguer:** Investigation. **María E. García-Pastor:** Formal analysis, Validation. **Salvador Castillo:** Software. **Juan M. Valverde:** Methodology. **María Serrano:** Writing – review & editing. **Daniel Valero:** Writing – review & editing. **Pedro J. Zapata:** Conceptualization, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

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

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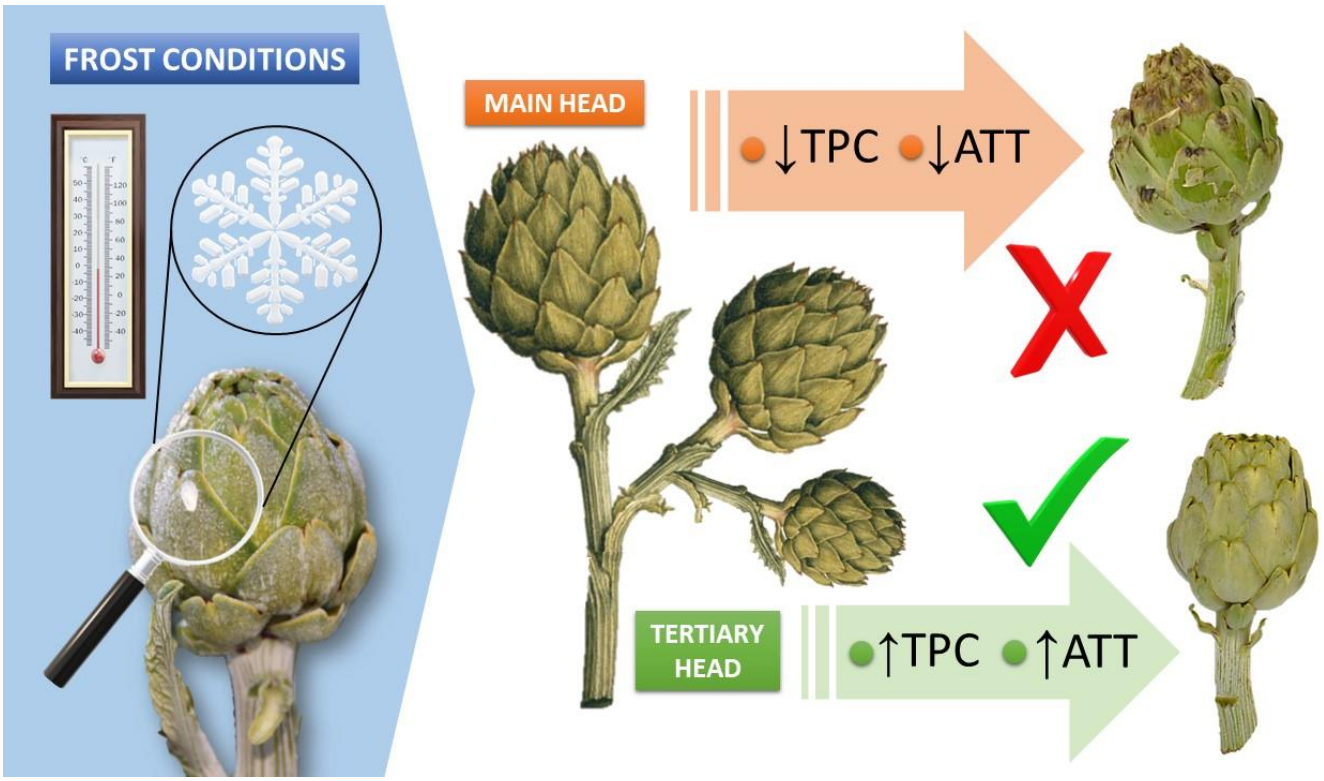
PUBLICATION 5

Influence of Artichoke Antioxidant Activity in Their Susceptibility to Suffer Frost Injury.

(Literal Transcription)

Marina Giménez-Berenguer, María Gutiérrez-Pozo, Vicente Serna-Escolano, María J. Giménez, and Pedro J. Zapata

Antioxidants 2023, *12*(11), 1960



https://doi.org/10.3390/antiox12111960

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Influence of Artichoke Antioxidant Activity in Their Susceptibility to Suffer Frost Injury

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Abstract: In the northern hemisphere countries, artichoke harvest occurs in winter months; consequently, they are exposed to cold temperatures. This can lead to frost injury, such as triggering the blistering of the cuticle and detachment of outer bracts, which eventually could display brown or black discolouration. This can cause major economic and production losses. As far as we know, no literature is available about this problem in artichokes. Thus, the main aim of this study was to evaluate the effect of total phenolic content and the antioxidant potential of 'Blanca de Tudela' artichokes in their capacity to tolerate frost injury when they are exposed to low temperatures. Several factors were analysed, including floral head order, weight and size of artichokes, total phenolic content, phenolic profile and total antioxidant activity. Results showed that tertiary heads, which are the smallest in size, exhibited a greater amount of total phenolic content and antioxidant activity. As a result, these characteristics offered enhanced protection to the artichoke against frosting temperatures. In contrast, the largest artichokes, especially the primary heads, were more susceptible to suffer frostbite. Therefore, artichokes with robust antioxidant systems, characterized by elevated phenolic content, are crucial to reduce their susceptibility to frost injury.

Keywords: artichoke head order; frostbite; total phenolic content; size; weight

Citation: Giménez-Berenguer, M.; Gutiérrez-Pozo, M.; Serna-Escolano, V.; Giménez, M.J.; Zapata, P.J. Influence of Artichoke Antioxidant Activity in Their Susceptibility to Suffer Frost Injury. *Antioxidants* **2023**, *12*, 1960. https://doi.org/10.3390/antiox12111960

Academic Editor: Claus Jacob

Received: 18 September 2023
Revised: 30 October 2023
Accepted: 1 November 2023
Published: 2 November 2023

1. Introduction

Spain is the third largest artichoke producing country in the world, only surpassed by Italy and Egypt [1]. These countries are located in the northern hemisphere and the harvesting schedule of artichokes typically goes from October to May. Additionally, the south of Spain presents a continuous production of artichokes during the winter months resulting in different harvest times, even in the coldest months of the year, January and February. In particular, the most cultivated variety in Spain, 'Blanca de Tudela', has the unique characteristic of presenting two harvest periods. The initial harvest occurs during the autumn–winter season, known as an early production, while the second harvest occurs in the spring season, referred to as a late production [2]. During the early production months, low temperatures and frost climatic conditions can occur, leading to frost injury or frostbite.

Frost stress produces plant cell injury due to the accumulation of reactive oxygen species (ROS) in plant cells with consequent oxidative damage [3]. However, the incidence of frost injury depends on different factors, including the sensitivity of the cultivar, the developmental stage, as well as the severity, duration, and frequency of frost conditions [4]. When artichoke inflorescences and leaves are exposed to temperatures below 0 °C, they can already begin to suffer physiological changes and damage. Frost injury in artichokes begins at −1.2 °C, resulting in the blistering of the cuticle, bronzing and detachment of the outer bracts. If the temperature drops further than −4 °C, the damage intensifies, causing

the bracts to become water-soaked, showing necrosis and eventually brown or black spots, particularly affecting the heart of the artichoke [5,6].

Frost is a factor that causes important economic losses due to the reduction of fruit quality and yield in different crops. Frost injury has been studied in different crops such as faba bean [7], avocados [8], kale [9], olive tree [10], wheat [11] and lettuce [12], among others. Frost damage occurred in all parts of the faba bean plant, but the tolerance was higher in pods, followed by stems, with the flowers being the most susceptible part [7]. Olive trees are susceptible to frost damage, especially in the first years of planting, being the main symptoms observed in the aerial parts of the plant and resulting in shoot tip necrosis, leaf fall and bark wounding [10]. However, frost injury has never been evaluated in artichokes.

Artichokes are rich in bioactive compounds with high antioxidant activity and are considered a functional food beneficial to consumer health [13]. Secondary metabolites, such as phenols, are produced in plants under normal environmental conditions for signalling and metabolism. They play a key role in the regulation of development and tolerance mechanisms against biotic and abiotic stresses [14]. Additionally, when plants are exposed to temperatures below 0 °C, the biosynthesis pathway of antioxidant compounds is enhanced, and these metabolites prevent plants from the phytotoxic effects of extreme temperatures. This prevents the oxidation of important proteins and lipids of the cell membrane produced by the ROS and preserves its integrity [15]. Two kinds of molecules are involved in the antioxidant system of plants known by their origin as enzymatic (catalase, ascorbate peroxidase or superoxide dismutase) and non-enzymatic (ascorbic acid, phenolic compounds or flavonoids) [16]. In this sense, phenolics and flavonoids are considered to be excellent electron donors due to the presence of hydroxyl groups, which are directly related to their antioxidant properties [17–20]. Moreover, some phenolic compounds, such as hydroxycinnamic acids and luteolin derivates, have been found to stimulate the production of endogenous antioxidant molecules within cells [21]. Several studies have reported that phenolic compounds are capable of neutralising free radicals, breaking down peroxides, deactivating metal ions, scavenging oxygen in biological systems, and preventing oxidative reactions [22–24].

The qualitative and quantitative profile of artichokes can be influenced by several preharvest factors such as harvest date, genotype, agronomic management and environmental conditions [13,25–29]. Moreover, previous studies have reported that total phenolic content in different artichoke cultivars are highly influenced by the flower head order. Tertiary head orders presented the highest individual phenolic content, followed by the secondary and main heads, with this effect being cultivar-dependent [2,30]. Therefore, the aim of this research was to evaluate the effect of total phenolic content, and, therefore, the antioxidant potential of 'Blanca de Tudela' artichokes in their capacity to tolerate frost injury when they are exposed to low temperatures.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiment was carried out in an experimental plot of Miguel Hernández University (Orihuela, Southeast Spain; 38°06′63.52″ N, −0°988′09.29″ W). 'Blanca de Tudela' cultivar was studied along the developmental cycle (autumn 2022 to spring 2023). Thereby, 300 offshoots from first-year artichoke plants were planted in a planting frame of 0.8 m × 1.2 m at the beginning of August. Crop management was performed according to standard commercial agronomic practices. Along the developmental cycle, insecticides, fungicides and fertilizers composed of 250 kg N, 120 kg P_2O_5, and 300 kg K_2O per ha were applied using a drip irrigation system.

Even though the growing area has a Mediterranean climate, characterized by mild and humid winters and hot and rainless summers, January, the month before harvest, was characterized by having an absolute maximum and minimum temperature of 23.4 °C and −1.3 °C, respectively, and an average relative humidity of 57% (weather station

7244X, Orihuela-Desamparados, 38°04′04″ N, 00°58′53″ W). Considering that the minimum temperature dates when frost injury occurred (≤0.0 °C) were 29 and 30 January, the weather conditions by hours are presented in detail in Table S1. The average temperature and relative humidity were 2 and 68% on day 29, and 8 and 67% on day 30, respectively. Only one day of rainfall was recorded in January, with values of total precipitation of 0.2 mm.

All artichokes were harvested with a 15–20 cm floral stem on the 1 February 2023 according to the commercial criteria, based on head size and morphology, when they were tightly closed and firm. During harvest, they were classified according to the flower head order: main, secondary and tertiary heads. Main artichokes emerge from the central stem of the plant and are the ones that reach the lowest height. Its ramifications give rise to the secondary artichokes with medium height. Tertiary heads grow up from the stem of the secondary ones and are the highest ones (Figure 1) [31].

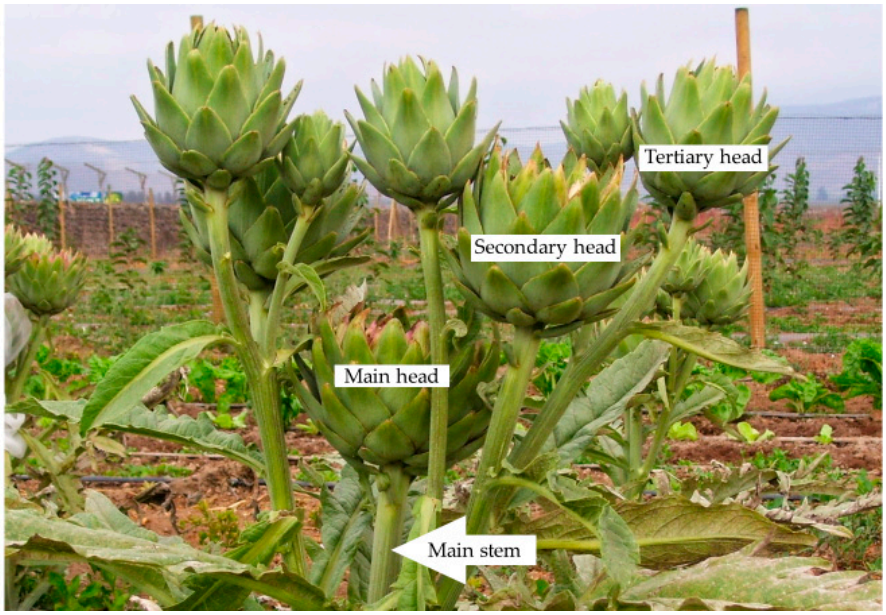

Figure 1. Flower head order disposal in artichoke plant.

2.2. Frost Injury Evaluation

A total of 330 artichokes were harvested and separated according to their flower head order (main, secondary and tertiary). Then, artichokes were transported to the laboratory where analytical determinations and an evaluation of the frost injury were performed. Each artichoke of these three groups was classified individually depending on the grade of frostbite presented (from 0—no frost injury to 5—severe frost injury) using a six-grade hedonic scale based on the severity of frost injury symptoms (Figure 2): 0 = no appreciable frost injury; 1 = slight frost injury; 2 = light frost injury with the extern bracts turning slightly brown in the ends; 3 = moderated frost injury with browner bracts ends; 4 = severe frost injury with greater brown spots; 5 = severe frost injury with greater brown spots and the presence of dehydration in the external bracts. The hedonic scale was performed with photographs taken by the authors.

2.3. Analytical Determinations

2.3.1. Average Weight of Artichokes

All artichokes were weighed with 15–20 cm of the floral stem using a Radwag WLC 2/A2 Precision Balance (Radwag Wagi Elektroniczne, Radom, Poland) with accuracy to two decimal places. The average flower head weight was expressed as the mean ± standard error (SE) in grams (g).

2.3.2. Size of Artichokes

Artichokes length, diameter and steam thickness were also measured with a precision digital calliper (Digimatic Caliper CD-P15K, Mitutoyo Corporation, Kanagawa, Japan) with accuracy to two decimal places. The average flower head size was expressed as the mean ± SE in millimetres (mm).

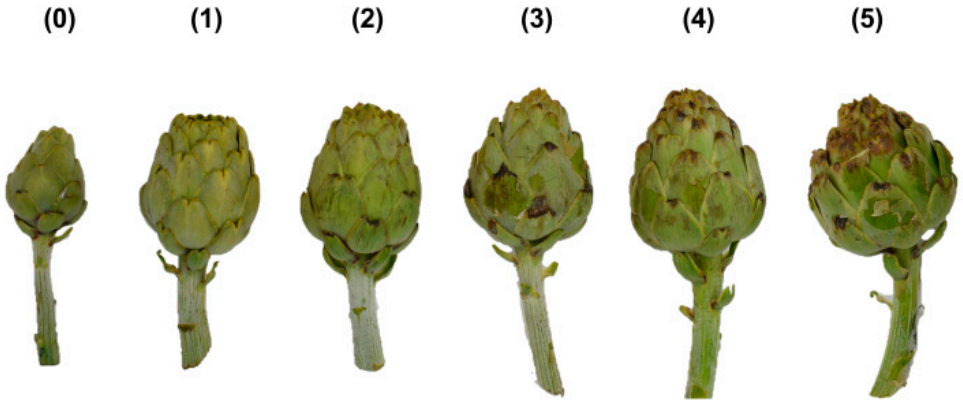

Figure 2. Frost injury scale: (**0**) no appreciable frost injury; (**1**) slight frost injury; (**2**) light frost injury; (**3**) moderated frost injury; (**4**) severe frost injury; (**5**) severe frost injury with dehydrations in external bracts.

2.3.3. Identification and Quantification of Individual Phenolic Compounds

Phenolic extraction was performed according to Giménez et al. [30]. Briefly, 5 g of the edible fraction (internal bracts and receptacle) was ground with 15 mL of a water/methanol solution (2:8) containing 2 mM NaF. This mixture was processed using an Ultra-Turrax® (IKA TP 18, Staufen, Germany) for 2 min, followed by centrifugation at 10,000× *g* for 15 min at 4 °C. The identification and quantification of individual phenolics was carried out using both DAD-ESI/MSn (Bruker Daltonics Ultra HCT-ESI Ion Trap, Bremen, Germany) and RP-HPLC-DAD (Agilent HPLC 1200 Infinity series, Agilent Technologies, Waldbronn, Germany) systems. The hydroxycinnamic acids and luteolin derivatives were quantified at 320 and 360 nm using a calibration curve of two standards, 5-*O*-caffeoylquinic acid and 3-luteolin-*O*-rutinoside (Sigma Aldrich, Darmstadt, Germany), respectively [30]. The total phenolic content (TPC) was calculated by summing up the concentrations of individual polyphenols. The results were expressed as g kg^{-1} of fresh weight (FW) and represented the average ± SE of 10 frosted (affected by severe frost injury: grades 4–5) artichokes and 10 non-frosted (grade 0) artichokes, respectively.

2.3.4. Total Antioxidant Activity

The quantification of total antioxidant activity (TAA) was conducted following the protocol described by Giménez et al. [30]. Briefly, for each individual artichoke, 2 g of the edible portion was homogenized with 10 mL of Na-phosphate buffer (50 mM, pH 7.8) and 5 mL of ethyl acetate for 2 min using an Ultra-Turrax® (IKA TP 18, Staufen, Germany). Subsequently, the homogenate was centrifuged at 10,000× *g* for 15 min at 4 °C. The quantification of TAA was carried out from the sum of both hydrophilic and lipophilic compounds in the same extraction. The determination was performed by monitoring the reaction of ABTS+ radicals generated from the enzymatic system consisting of 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horse radish peroxidase enzyme (HRP), and the oxidant substrate (hydrogen peroxide). The absorbance was measured at 730 nm. Results were expressed as the equivalent of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per kilogram of fresh weight (g equivalent of Trolox kg^{-1} FW). The values were the mean ± SE of 10 frosted (affected by severe frost injury: grades 4–5) artichokes and 10 non-frosted (grade 0) artichokes, respectively.

2.4. Statistical Analysis

Data was subjected to the analysis of variance (ANOVA). Mean comparisons were performed using a multiple-range test (HSD Duncan's test) to detect significant differences ($p < 0.05$). ANOVA assumptions were tested and found to be valid for this dataset. All analyses were performed with SPSS software package v. 20 for Windows (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Influence of Artichoke Flower Head Order, Weight and Size of Artichokes on Frost Injury

Artichokes were separated according to their flower head order (main, secondary and tertiary) at harvest, and then, in the laboratory, each artichoke of these three groups was classified individually depending on the grade of frostbite presented (from 0—no frost injury to 5—severe frost injury). The percentages of main, secondary and tertiary artichoke heads in each frost injury grade are presented in Figure 3.

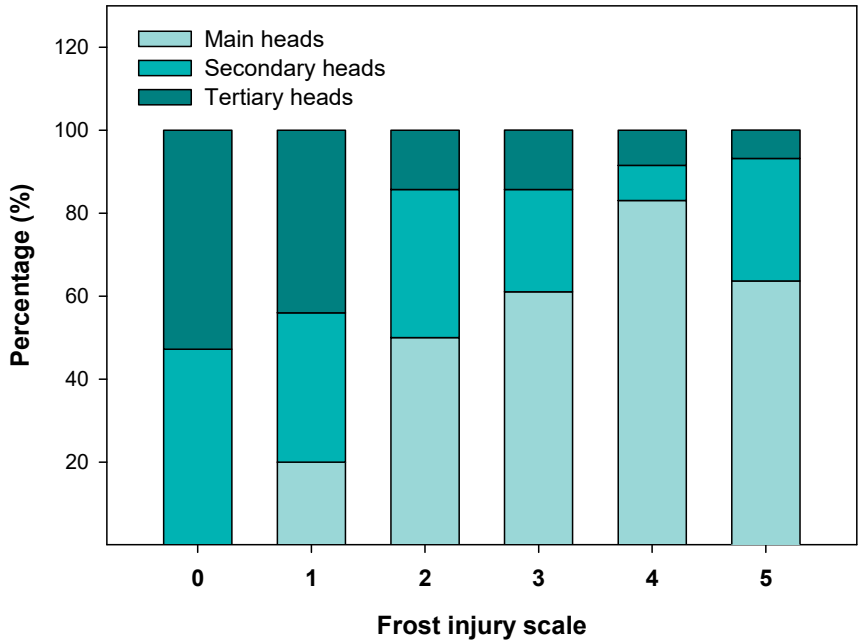

Figure 3. Percentage of artichokes affected by frost injury attending to the flower head order in every frost injury grade.

Artichokes that did not have any frost injury (grade 0) were mainly tertiary heads, (52.78%), followed by secondary ones with 47.22%. Nevertheless, no main artichokes were found without frost injury. Additionally, main artichokes were the most affected with a higher frost injury (grades 4 and 5) with an average percentage of 73.34%, after a 19.01% of secondary heads and 7.65% of tertiary heads (Figure 3).

Furthermore, the correlation between the weight and size of artichoke heads and their susceptibility to be affected with frost injury was studied (Figure 4). The smallest artichokes, also classified as tertiary heads, were found on the lowest frost injury scales (0 and 1). Medium artichokes (secondary heads) presented lower frost injury (grades 2 and 3) than the bigger ones (main heads), which showed the highest frost injury (grades 4 and 5).

In grade 0, artichokes with no frost injury had an average weight, artichoke length, equatorial diameter and stem thickness of 72.40 ± 2.96 g, 68.98 ± 1.13 mm, 51.17 ± 0.85 mm and 11.02 ± 0.30 mm, respectively. However, the most affected artichokes, grades 4 and 5, presented significant differences ($p < 0.05$) compared to artichokes with lower frost grades, with the average weight, artichoke length, equatorial diameter and stem thickness of 126.11 ± 4.95 g, 79.18 ± 1.31 mm, 64.10 ± 1.05 mm and 13.63 ± 0. 31 mm, respectively (Figure 4).

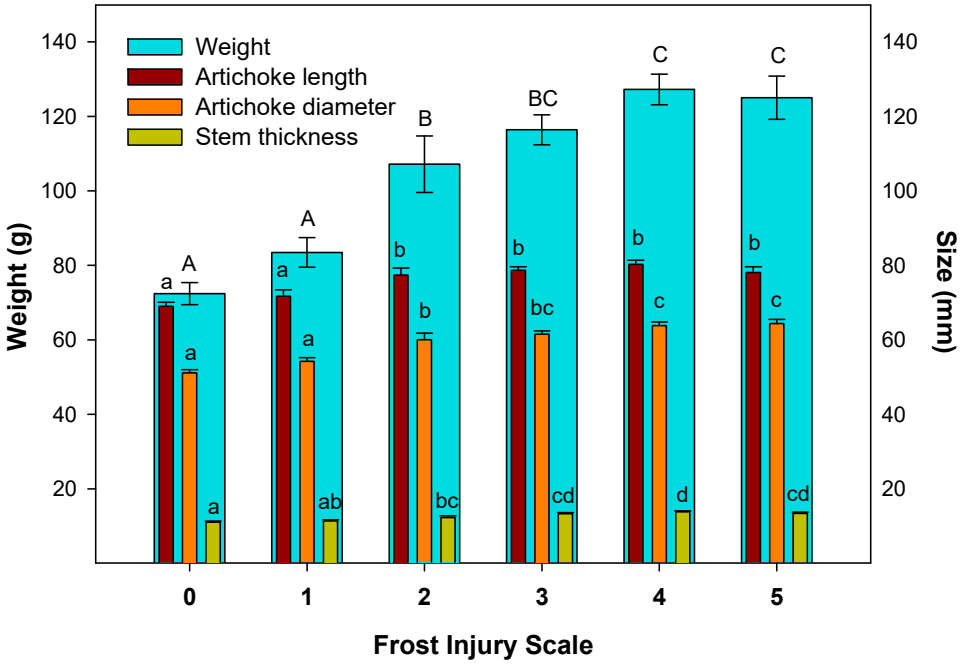

Figure 4. Average weight (g) and size (mm) of 'Blanca de Tudela' artichokes in each frost injury grade. Results were expressed as mean values ± SE. Different letters show significant differences ($p < 0.05$ according to HSD Duncan's test) in weight (capital letters) and size (lowercase letters) between frost injury grades.

Therefore, there was a relationship between the susceptibility to frost injury and the size of the flower head order. The difference involving the weight of grade 0 and grades 4 and 5 artichokes was almost a two-fold increase. Meanwhile, parameters such as length, equatorial diameter and stem thickness increased by 12.88%, 20.16% and 19.14%, respectively. Thus, there was a close relation between the weight and size of artichokes and the flower head's order.

3.2. Influence of Phenolic Content and Different Phenolic Profiles on Frost Injury

The phenolic profile was evaluated for frosted (grades 4 and 5) and non-frosted artichokes (grade 0). Seven hydroxycinnamic acids were identified in both types of artichokes, being 5-*O*-caffeoylquinic acid and 3,5-*O*-dicaffeoylquinic acid the major ones (Figure 5a). The five minor hydroxycinnamic acids found, attending to their concentration, were 3,4-*O*-dicaffeoylquinic acid, 1-*O*-caffeoqylquinic acid, 4,5-*O*-dicaffeoylquinic acid, 3-*O*-caffeoylquinic acid and 1,3-*O*-dicaffeoylquinic acid (Figure 5b).

Results showed that all hydroxycinnamic acids were detected in higher concentrations in non-frosted artichokes than in frosted ones. Particularly, a two-fold increase was observed on almost every compound identified, increasing the total hydroxycinnamic acid content by 51.58% (Figure 5a,b).

The concentration of 5-*O*-caffeoylquinic acid, the major phenolic compound, was 1.908 ± 0.211 g kg^{-1} FW for frosted artichokes and 4.239 ± 0.217 g kg^{-1} FW for non-frosted artichokes, showing an increase of 55%. Furthermore, for the second major phenolic compound, 3,5-*O*-dicaffeoylquinic, an increase of 48.18% (1.379 ± 0.136 and 2.658 ± 0.211 g kg^{-1} FW for frosted and non-frosted artichokes, respectively) was found (Figure 5a).

A similar tendency was observed for the minor hydroxycinnamic acids; in general, their concentration was significantly higher in non-frosted artichokes than the frosted ones (Figure 5b). The concentration of 1-*O*-caffeoqylquinic acid observed, which almost triplicated its content, had values of 0.011 ± 0.002 g kg^{-1} FW in frosted artichokes and 0.039 ± 0.008 g kg^{-1} FW in non-frosted ones (Figure 5b). This was the minor hydrox-

ycinnamic acid which showed the highest increment, changing the importance of 1-*O*-caffeoqylquinic acid in non-frosted artichokes compared to frosted ones.

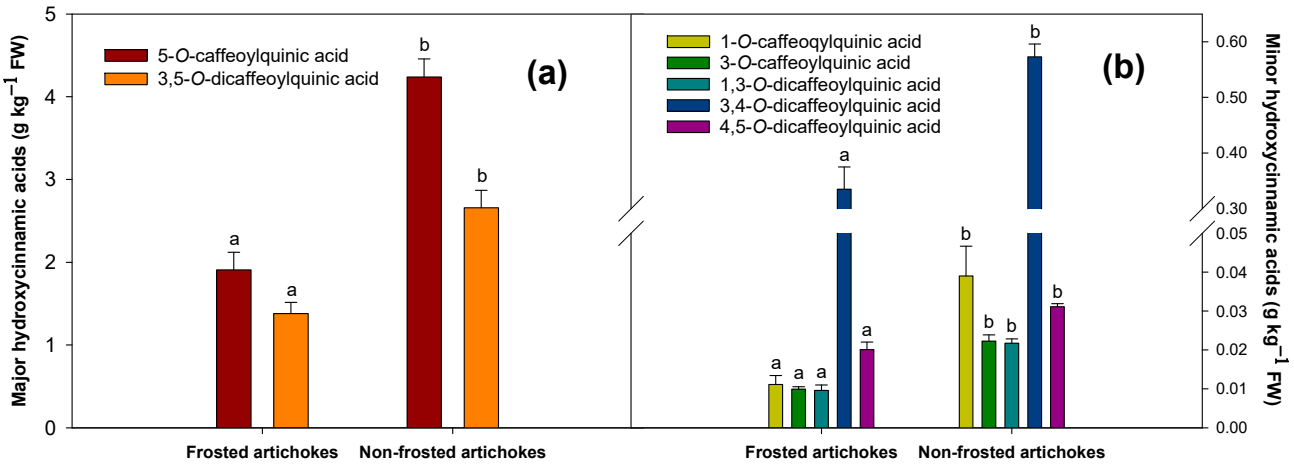

Figure 5. Hydroxycinnamic profile: (**a**) Major hydroxycinnamic acids; (**b**) Minor hydroxycinnamic acids) and content (g kg^{-1} FW) for 'Blanca de Tudela' frosted (grades 4 and 5) and non-frosted artichokes (grade 0). Results were expressed as mean values ± SE. Different letters show significant differences ($p < 0.05$ according to HSD Duncan's test) between frosted and non-frosted artichokes for each individual phenolic compound.

Moreover, three different luteolins were identified: Luteolin 7-*O*-glucoside was the major one, followed by Luteolin 7-*O*-glucuronide, and Luteolin 7-*O*-glucuronide 3-*O*-glucoside (Figure 6). Luteolin's derivatives content showed a similar tendency as the hydroxycinnamic acids, being 22.15% higher in non-frosted artichokes than in frosted ones, except for Luteolin 7-*O*-glucuronide, which did not present any significant differences ($p < 0.05$).

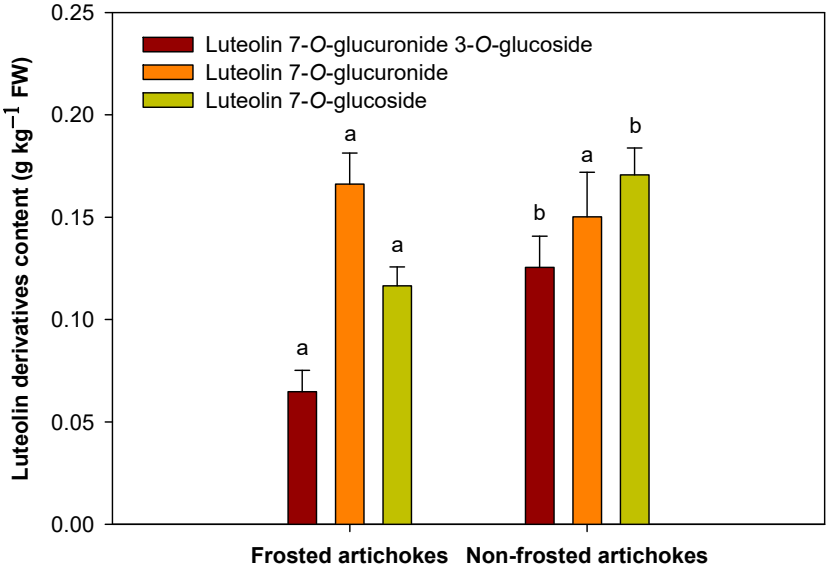

Figure 6. Luteolin profile and content (g kg^{-1} FW) for 'Blanca de Tudela' frosted (grades 4 and 5) and non-frosted artichokes (grade 0). Results were expressed as mean values ± SE. Different letters show significant differences ($p < 0.05$ according to HSD Duncan's test) between frosted and non-frosted artichokes for luteolin derivatives content.

Luteolin 7-*O*-glucoside was the major compound for the non-frosted artichokes, being 31.73% higher than in frosted ones, with a concentration of 0.116 ± 0.009 g and

0.170 ± 0.013 g kg^{-1} FW for frosted and non-frosted, respectively. Furthermore, Luteolin 7-*O*-glucuronide 3-*O*-glucoside showed the highest increase (48.40%) in non-frosted artichokes compared to frosted ones, despite being the minor luteolin (Figure 6).

3.3. Influence of Total Phenolic Content and Total Antioxidant Activity on Frost Injury

The TAA and TPC, calculated as the sum of the individual phenolic compounds, were evaluated for frosted and non-frosted artichokes (Figure 7a,b). Results showed that the TPC was significantly higher ($p < 0.05$) in non-frosted artichokes compared to frosted ones, with an increase of 49.94% (4.020 ± 0.379 g and 8.031 ± 0.402 g kg^{-1} FW for frosted and not-frosted, respectively) (Figure 7a). Regarding TAA, it was 25.09% higher in non-frosted artichokes than in frosted ones with values of 2.644 ± 0.038 g kg^{-1} FW and 3.529 ± 0.085 g kg^{-1} FW, respectively (Figure 7b).

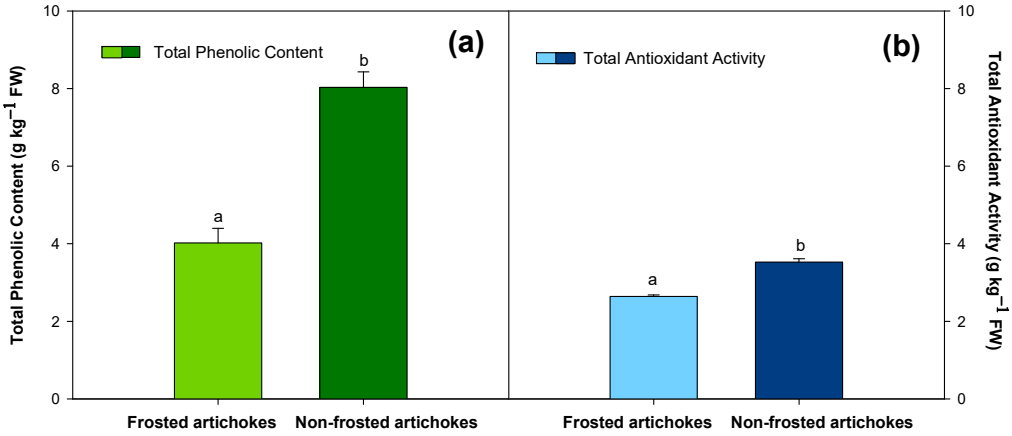

Figure 7. (**a**) Total phenolic content (g kg^{-1} FW) and (**b**) total antioxidant activity (g kg^{-1} FW) for 'Blanca de Tudela' frosted (grades 4 and 5) and non-frosted artichokes (grade 0). Results were expressed as mean values ± SE. Different letters show significant differences ($p < 0.05$ according to HSD Duncan's test) between frosted and non-frosted artichokes for total antioxidant activity and total phenolic content.

4. Discussion

The aim of this study was to understand the effect of the total phenolic and antioxidant content of 'Blanca de Tudela' artichokes in their susceptibility to suffer frost injury, helping to mitigate the consequent production and economic losses involved. Low temperatures can cause damage to all plants, but the mechanisms and type of injury could vary considerably [4]. In the present study, the percentage of artichokes affected by frost injury was evaluated. A high percentage of tertiary heads presented a frost injury grade of 0 and 1, showing less susceptibility to be affected by frost injury followed by the secondary heads. In contrast, main artichokes were more affected by frostbite, since a higher number of artichokes were classified with a higher injury grade. Even though grades 4 and 5 were both associated with the highest frost injury, differences between them were detected, and higher dehydration symptoms were observed in the last grade. Frost injury produced by ice is mainly related to the physical disruption of cellular structures and subsequent dehydration due to the increased water potential in the interior of the cells compared to the space between them where ice crystals are formed [32,33].

The flower order in plants determines the characteristics or physical parameters of artichokes [2]. The main artichokes tend to be the biggest, followed by the secondary heads, and tertiary artichokes being the smallest [2,25,30], in accordance with the results achieved in the present study. In this sense, artichoke is not the only vegetable where there is a differentiation based on its time of formation and position on the stem. A study on faba beans found that the weight of seeds in the lower position was higher than in the middle

and upper positions [34]. Another study on pigeon peas showed that the early forming pods were usually heavier than those forming later [35].

The susceptibility of artichokes to suffer frost injury could be determined by their position on the plant. As previously mentioned, main artichokes are those that grow from the central stem of the plant and therefore could be more protected by the leaves. Lateral ramifications of the central stem led to secondary artichokes and from the stem of the secondary ones, tertiary heads were produced. Due to the morphology of the artichoke plant, secondary, and especially tertiary heads, were developed higher in the plant (Figure 1) [31], and they could be more exposed to climatic conditions, and therefore, may suffer more from frost injury. However, in this study, the opposite effect was observed, being that the main artichokes presented the highest grade of frost injury, followed by secondary and tertiary ones. Thus, the position of the artichoke in the plant did not present any effect on their susceptibility and grade of frost injury. The differences observed could be due to the role that bioactive content plays during biotic and abiotic stresses [14].

Artichoke heads are known to have high nutritional value, mainly due to their rich polyphenol content [26,36], including hydroxycinnamic acids and flavonoids, such as luteolins [2,27]. In the present study, individual phenolic content results showed that the seven hydroxycinnamic acids and the three luteolin derivatives analysed were higher in non-frosted artichokes compared to the frosted ones. This is the first study that describes that these changes in phenolic content influence the susceptibility of artichokes to suffer frost damage. The most abundant hydroxycinnamic acids quantified in 'Blanca de Tudela' cultivar were 5-*O*-caffeoylquinic acid (chlorogenic acid), followed by 3,5-di-*O*-caffeoylquinic acid, in agreement with previous studies performed on different artichoke cultivars [2,27,37–40]. In general, the hydroxycinnamic acids quantified were at least two-fold higher in non-frosted than in frosted artichokes. A similar trend was observed for hydroxycinnamic acids and for two of the three luteolins quantified. Luteolin 7-*O*-glucoside and luteolin 7-*O*-glucuronide 3-*O*-glucoside presented higher concentration for non-frosted artichokes compared to frosted ones, but no differences were found for Luteolin 7-*O*-glucuronide. However, there are few studies that have reported changes in the hydroxycinnamic acid and luteolin profile. The influence of artichoke head order (main, secondary and tertiary) on the phenolic content (hydroxycinnamic acid and luteolin content) has been previously described in different artichoke cultivars [2,25]. In general, hydroxycinnamic acid content varied among flower head orders, showing that tertiary heads had higher levels than the main or secondary heads for major and minor hydroxycinnamic acids; however, this tendency was cultivar-dependent. The total amount of each individual luteolin was significantly higher in tertiary flower head orders than secondary or main heads. In particular, luteolin-7-*O*-glucuronide was the major luteolin identified in all artichoke cultivars [2]. These results are in agreement with the results obtained for frosted artichokes; however, for non-frosted artichokes it was lut-7-*O*-glucoside the one presenting the greatest concentration.

The sum of individual phenolic content was represented as total phenolic content (TPC). Results showed that frosted artichokes presented lower TPC than non-frosted ones. A similar tendency was observed in the total antioxidant activity (TAA). Different authors have found a direct correlation between antioxidant capacity and total phenolic content [17,19,20,22]. The susceptibility of artichokes to suffer frost injury may be related to the differences in the TPC and TAA, which were also related to the flower head order. These findings suggest that artichokes with a higher phenolic content show better frost tolerance by enhancing their antioxidant defence systems. In this sense, tertiary artichokes presented the best aptitude to resist frostbite and frost conditions as their cryopathy incidence was lower. Although no studies have been carried out on the effect of frost injury in artichokes, these changes in the bioactive compounds according to climatic conditions have been previously studied in different crops such as avocado, kale leaves and lettuce plants [8,9,12]. On the one hand, it was reported that the antioxidant activity, mainly from a non-enzymatic source, such as the accumulation of phenolic compounds, was responsible for the increased

tolerance to frost injury in avocados [8]. In that study, two different avocado cultivars were evaluated, 'Ettinger' and 'Hass'. TPC and TAA were significantly higher in 'Ettinger' cultivar than in 'Hass', suggesting that the greater frost tolerance of the first cultivar was due to its greater content of antioxidant compounds [8]. The relation between greater TAA and better frost tolerance has also been previously described in pines in which it was observed that high levels of ROS elimination activities were associated with greater frost tolerance [41]. Another study found that the treatment of olive plants with salicylic acid (SA) increased their tolerance to freezing by increasing antioxidant enzyme activities and total phenolic content [42]. The defence of plants against stress is linked to the antioxidant capacity and higher levels of phenols play a protective role against stress damage [10], as the antioxidant potential often depends on the content of bioactive compounds [22].

It is well known that when plants are exposed to unfavourable conditions, an increase in ROS-scavenging activities is generally observed as a protection mechanism. In particular, low temperatures can induce the accumulation of antioxidant compounds in order to protect the cell membrane from breakdown and peroxidation. Redox reactions and turnover of ROS in cells can be carried out through non-enzymatic and enzymatic antioxidants that maintain ROS at a sub-lethal level [10]. The present study showed that the highest total phenolic content and total antioxidant activity of tertiary head artichokes increased their resistance to suffer frost injury or minimise its effects. Up to date, no literature is available related the susceptibility to frostbite in artichokes. This research is the first study where a relation between susceptibility to frost injury and total phenolic content, and the consequent total antioxidant activity attending to the flower head order, has been observed.

5. Conclusions

For the first time, the effect of total phenolic compounds on the susceptibility of 'Blanca de Tudela' artichoke to frost injury when they are exposed to low temperatures has been studied. Flower head order, along with weight, size, total phenolic content (TPC) and total antioxidant activity (TAA) played an important role in the susceptibility of artichokes to suffer frost injury. Tertiary head artichokes, also the smallest characterized, presented the highest TPC and TAA, which increased their resistance against frost temperatures and reduced their susceptibility to frost injury. On the contrary, the biggest artichokes, main heads, were more affected by adverse frost temperatures, presenting a higher frostbite incidence. In conclusion, artichokes with higher antioxidant systems related to their higher phenolic compounds would be less susceptible to suffer frost injury. Consequently, it is plausible that the application of elicitors, bio-stimulants, or other compounds capable of stimulating the antioxidant systems of the plant, could be used as a preventive measure against frost injury when extreme environmental conditions occur.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox12111960/s1, Table S1: Climatic conditions recorded in the artichoke field on 29 and 30 January.

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

Funding: This work has been funded by the Ministry of Agriculture, Rural Development, Climate Emergency and Green Transition of the Valencian Community (2021/VALORIZA/VSC/003).

Institutional Review Board Statement: Not applicable, as no animal or human were involved in this study.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used to support the findings of this study are included within the article.

Acknowledgments: The authors thank Consellería de Innovación, Universidades, Ciencia y Sociedad Digital de la Generalitat Valenciana for Ph.D. scholarship of M.G-B. (grant number ACIF/2021/178).

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

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Influence of artichoke antioxidant activity in their susceptibility to suffer frost injury

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Supplementary Material

Table S1. Climatic conditions recorded in the artichoke field on the 29th and 30th of January.

DAY	29th				30th			
HOUR	Temperature (ºC)	Relative Humidity (%)	WIND		Temperature (ºC)	Relative Humidity (%)	WIND	
			Direction (tens of degrees)	Speed (km/h)			Direction (tens of degrees)	Speed (km/h)
0	2.2	82	-	0	6.4	76	19	2
1	1.9	85	-	0	4.8	78	-	0
2	1.3	81	-	0	4.1	83	23	2
3	2	83	-	0	3.1	83	-	0
4	0.9	84	25	2	2.1	85	-	0
5	0.9	79	24	3	1.3	85	-	0
6	-0.1	79	-	0	0.6	87	-	0
7	-1.3	83	-	0	-0.2	90	-	0
8	0.8	75	22	3	0.7	91	-	0
9	3.5	72	24	5	4.8	84	27	4
10	7.1	52	23	9	8	67	22	4
11	9.3	47	21	6	10.6	56	22	5
12	11.2	46	22	6	12.4	47	22	8
13	12.7	44	22	4	13.9	43	14	4
14	13.1	42	9	8	13.9	41	15	5
15	11.8	56	7	13	13.4	37	21	4

16	10.7	59	7	14	13.9	36	26	5
17	9.7	63	7	13	13.9	43	17	3
18	9.1	67	5	11	10.2	54	-	0
19	8.5	68	6	10	8.5	60	-	0
20	7.7	75	5	8	6.1	68	-	0
21	7.9	73	7	8	4.7	74	-	0
22	8.3	68	8	6	3.8	77	22	4
23	7.3	72	23	2	2.4	77	24	3

PUBLICATION 6

SEEDS MINERAL PROFILE AND ASH CONTENT OF THIRTEEN DIFFERENT GENOTYPES OF CULTIVATED AND WILD CARDOON OVER THREE GROWING SEASONS.

(Manuscript submitted)

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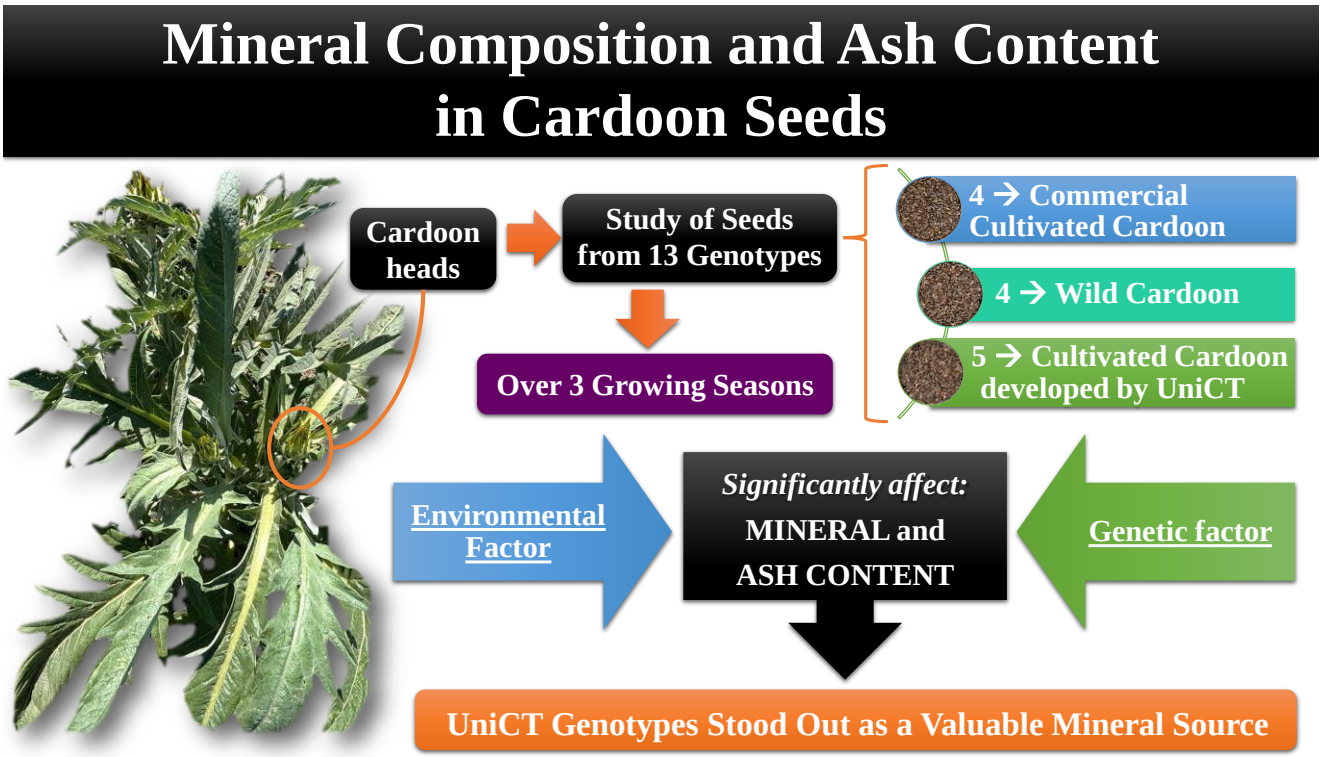
Agriculture 2025, under review



agriculture

MDPI

Type of the Paper (Article)

Seeds mineral profile and ash content of thirteen different genotypes of cultivated and wild cardoon over three growing seasons

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Abstract: Cultivated and wild cardoons are versatile plants with significant economic and bioactive potential. They have gained attention in the recent years for their nutritional value and potential health benefits due to their high mineral content and unique composition. The aim of this study was to investigate the variations in mineral composition and ash content of thirteen distinct genotypes (4 commercial, 4 wild and 5 self-developed by Catania University) of cultivated and wild cardoon seeds over three consecutive growing seasons. Results showed that ash content and macro and micro-elements are significantly influenced by environmental conditions, genetic factors and the interaction between both. For example, ash content showed notable fluctuations over the three seasons, with the lowest value recorded in season 2, probably linked the higher rainfall level respect to season 1 and 3. The genotypes self-developed showed the highest mean content on all micro-mineral elements under study, with Zn and Cu peaked in Linea 7. In general, it was reported that cardoon seeds are a valuable source of macro and micro-elements, highlighting, in particular, the potential of the genotypes developed by Catania University. This research provides, for the first time, valuable insights into the long-term consistency and variability of mineral content and ash composition in cardoon seeds, contributing to a more comprehensive understanding of their nutritional value and potential applications.

Keywords: cardoon, genotype, season, macroelements, microelements seed, *Cynara cardunculus*

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

Citation: To be added by editorial staff during production.

1. Introduction

Cynara cardunculus L. is a perennial plant native of the Mediterranean region. These herbaceous species belong to the Asteraceae family and comprises three botanical varieties, the ancestor, so-called wild cardoon or wild artichoke (*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori), and his two descendants: cultivated cardoon (*C. cardunculus* L. var. *altilis* DC.) and globe artichoke (*C. cardunculus* L. var. *scolymus* L. Fiori) [1,2].

With a rich history, dating back to ancient civilizations such as the Egyptians, Greeks, and Romans, cardoon has been cherished for both its culinary and medicinal properties [3,4]. Throughout the Mediterranean region, it has played a prominent role in traditional recipes, as their immature inflorescences are consumed fresh, canned or frozen [5] As mentioned, it also has been used in traditional medicine as it aids in the treatment of liver diseases, diabetes, and heart conditions, and acts as a choleretic and antihemorrhoidal agent [6–8]. Thus, in the recent years, some research has revealed its rich content of bioactive compounds, making it appealing for various applications[9,10]. This plant is a valuable source of dietary fibers, minerals, inulin, phenolic and antioxidant compounds [11–14].

Cardoon cultivation is concentrated in southern Europe, particularly in Italy, Spain, Portugal, Greece, and France [15,16]. Well adapted to Mediterranean climates, where it thrives under conditions of hot, dry summers and mild, humid winters [17], this species tolerates abiotic stress and contributes to soil health by improving fertility and preventing degradation [18]. Its cultivation enhances soil microbial activity and nutrient content, including organic matter, nitrogen, potassium, and phosphorus [16,19,20].

Due to its diverse applications, cardoon has garnered growing industrial interest. It is consumed as a traditional and functional food [21–24], and its flowers are used as vegetable rennet in PDO cheeses from Portugal, Spain, and Italy [21,23,25]. Cardoon by-products serve as sustainable animal feed [26–29], and the plant is a promising bioenergy crop owing to its high biomass and low input needs [30–32]. It also finds uses in the paper, pharmaceutical, and cosmetic industries [21,26], and is increasingly cultivated for ornamental purposes [33].

Regarding the specific industrial uses of cardoon seeds, they are mostly used for to obtain oil for human consumption or biodiesel [34–37]. Thus, the solid residues after oil extraction are also considered suitable for animal feed due to their high protein, fiber, and energy content, as well as their high digestibility [23,26,29,38]. Additionally, cardoon oil cakes are being explored for biodegradable and edible plastics, contributing to the circular economy and developing eco-friendly technologies [23,26,39]. Furthermore, cardoon seed also have been proven with several pharmacological and nutraceutical properties [40,41].

Previous studies on cardoon seeds have investigated various aspects, including their chemical composition and biological activity. Mandim et al. [14] evaluated these factors across four maturity stages of a commercial cultivated cardoon genotype. Additionally, Mandim et al. [42] explored the chemical composition and 'in vitro' biological activities of cardoon seeds from another commercial cultivated cardoon genotype. Petropoulos et al. [14,28] conducted a series of studies investigating the chemical composition, including mineral compounds, and potential applications of cardoon. In 2018, they analyzed a commercial cultivated cardoon genotype, evaluating various extraction methods [14,28]. Subsequently, in 2019, they expanded their research to examine the bioactivities, chemical composition, and nutritional value of seeds from both wild and commercial cardoon genotypes [43]. Likewise, Angelova et al. [44] also evaluated these parameters of another commercial cultivated cardoon, while Piluzza et al. [45] evaluated only the phenolic compounds content and antioxidant capacity in cardoon seed from different head orders.

Thus, as far as we are concerned, no research has explored the influence of the genotype over multiple growing seasons on the mineral composition and ash content of different cardoon seeds. To address this research gap, this study analyses thirteen distinct genotypes of cardoon seeds across three consecutive growing seasons, providing insights into their nutritional value and stability. Understanding the variations in mineral profiles across different genotypes and growing seasons is crucial for evaluating the consistency and potential uses of cardoon seeds.

2. Materials and Methods

2.1. Field research site, crop management and plant materials

Plants were grown during three seasons (2017-2018; 2018-2019; 2019-2020; hereafter referred to as S_1, S_2 and S_3, respectively) into an experimental field at the Catania plain, Sicily (south Italy, 37°30′ N. 15° 4′ E, 10 m a.s.l). The local climate is typically Mediterranean with mild winters and hot dry summers. The soil characteristics and meteorological variables are reported in table 1. Sowing was carried out on greenhouse at the beginning of August 2017 and seedlings, at the stage of three-four leaves, were transplanted in the field at mild September 2017. Spacing was 1.25 m between rows and 0.8 m along the row, with a planting density of 1 plant m^{-2}. The plant material was arranged in a randomized block design with four replicates, with an experimental unit of 10 plants. Crop was grown for three consecutive seasons, of which season cycle (early August) the epigeal biomass was cut down. Crop regrowth was naturally carried out by September fellow rains. Each season, the crop received about 50 mm of supplier water, 80 kg ha^{-1} of nitrogen and a manual weeding. The harvest of epigeal biomass, heads enclosed, was performed each season at the end of July, when the plants were completely dried up. From the harvested heads were collected the achenes (seeds).

Table 1. Meteorological variables and soil characteristics at the experimental field.

Parameter	
Metereological variables	
Temperature min (°C) 2017[a]	13.2
Temperature min (°C) 2018[b]	13.1
Temperature min (°C) 2019[c]	13.4
Temperature max (°C) 2017[a]	23.8
Temperature max (°C) 2018[b]	23.4
Temperature max (°C) 2019[c]	24.0
Rainfall (mm) 2017[d]	448.2
Rainfall (mm) 2018[e]	758.1
Rainfall (mm) 2019[d]	571.3
Soil characteristics	
Clay (< 0.002 mm) (%)	46
Silt (0.02 – 0.002 mm) (%)	36
Sand (2 – 0.02 mm) (%)	18
Total N (%)	0.1
Organic Matter (%)	1.1
P_2O_5 available (ppm)	11
K_2O (exchangeable) (ppm)	704
Electrical conductivity (mS cm^{-1})	0.2
pH	7.2
Cation exchange capacity (meq $100g^{-1}$)	32.4
Ca (ppm)	5435
Mg (ppm)	308
Na (ppm)	216
K (ppm)	577
Fe (ppm)	118
Zn (ppm)	3.0

Mn (ppm)	162
Cu (ppm)	4.0

[a]Average from August 2017 to July 2018; [b]Average from August 2018 to July 2019; [c]Average from August 2019 to July 2020; [d]Total from August 2017 to July 2018; [e]Total from August 2018 to July 2019; [f]Total from August 2018 to July 2019.

The study involved thirteen genotypes of a germplasm collection (Table 2): four commercial cultivate cardoon ('Cardo Avorio', 'Cardo Gobbo', 'Gigante Inerme', 'Verde di Peralta'); four wild cardoon, ('Creta', 'Kamarina', 'Marsala', 'Valparaiso') and five selected lines from cultivated cardoon crossed program ('Altilis 41', 'Linea 1', 'Linea 7', 'Linea 25', 'Linea 32'). The cultivated cardoon are commercial varieties traditionally cultivated as vegetable in Mediterranean countries for the enlarged blenched petiole. The wild cardoon are landraces genotypes collected from population in Creta ('Creta'), coastal of Sicily ('Marsala', 'Kamarina') and central of Chile ('Valparaiso'). The cardoon lines are developed by University of Catania (UniCT) from progenies obtained by a controlled clones of commercial varieties of cardoon and selected on the basis of their high biomass and phytochemicals yield production [46].

Table 2. List of genotype seeds selected, and acronyms used for this experiment attending to their plant material.

PLANT MATERIAL		GENOTYPE	ACRONYMS
***C. cardunculus* L. var. *altilis* DC.**	**Commercial CC**	Gigante Inerme	GI
		Verde di Peralta	VdP
		Cardo Gobbo	CG
		Cardo Avorio	CA
	CC developed by Catania University (UniCt)	Altilis 41	A41
		Linea 1	L1
		Linea 7	L7
		Linea 25	L25
		Linea 32	L32
***C. cardunculus* L. var. *sylvestris* (Lamk) Fiori**	**Wild Cardoon**	Marsala	MA
		Val Paraiso	VP
		Kamarina	KA
		Creta	CR

CC: cultivated cardoon.

2.2. Sampling and determination of seeds ash and minerals content

At each growing season, about 1000 seeds, per each genotype and replicate, were oven-dried at 65 °C (Binder, Milan, Italy), until a constant weight. The dry material was ground and used for the ash, macro-mineral (N, K, Mg, Ca, and Na) and micro-mineral (Zn, Fe, Mn, and Cu) determination. The ash content was determined by AOAC official method 923.03 (2005), which involved dry ashing in muffle furnace at 550±2 °C until greyish white ash was obtained. N determination was performed by using Kjeldhal method. The other minerals were determined following the AOAC official method (1995), by using a Perkin Elmer AAnalyst 200 flame atomic absorption spectrometer (Norwalk, USA). The quantification of the mineral content in the sample was performed by calibration curves. All data presented are mean values of two independent experiments and expressed as g kg^{-1} and mg kg^{-1} of dry matter (DM), respectively, for macro

and micro-element minerals. All the chemical products adopted for analyses were purchased from Sigma-Aldrich (Milan, Italy) and were of analytical grade.

2.3. Statistical Analysis

Bartlett's test was adopted to test for homoscedasticity, and then the data were subjected to a two-way analysis of variance (ANOVA), based on a factorial combination of "genotype (13) × growing season (3)". Means were separated by Duncan test, when the F-test was significant. Statistical analysis was performed using SPSS software package v. 20 for Windows (IBM Corp., Armonk, NY, USA).

3. Results

The ANOVA (Table 3) clearly indicate that both genotypes, growing season and their interaction significantly affected the ash and mineral elements content of cardoon seeds.

Table 3. Mean square per each source of variation (percentage of total) resulting from analysis of variance.

Source of variation	Degree of freedom	Ash content	Mineral element								
			N	Ca	K	Mg	Na	Fe	Zn	Mn	Cu
Genotype (G)	12	11.4***	58.7***	19.1***	23.0***	14.0***	23.1***	16.5***	50.5***	57.6***	54.1***
Growing season (S)	2	81.5***	11.1*	72.5***	73.0***	68.0***	23.1***	66.4***	38.7***	16.9***	33.9***
(G) x (S)	24	7.1***	30.1***	8.2***	4.1***	18.0***	53.8***	17.1***	10.8***	25.4***	12.0***

*** and * indicate significant at $P \leq 0.001$ and $P \leq 0.05$.

3.1. Influence of genotype on ash content and macro-elements content of cardoon seeds

The genotype significantly influenced ash and macro-elements. The most notable contribution was observed in N at 58.7% (Table 3).

On average, the commercial cultivated cardoon genotypes accumulated less N (27.7 g kg^{-1} DM) than both, cultivated cardoon developed by UniCT and wild cardoon, respectively (28.8 g kg^{-1} DM) (Table 4). On the contrary, the commercial cultivated cardoon recorded the highest mean ash content (38.1 g kg^{-1} DM) respect to the other genotypes groups (approximately 3.9% more on average). Moving on the individual genotypes, it is worth to note that CR showed the highest N content (31.0 g kg^{-1} DM) and lower ash content (32.8 g kg^{-1} DM) respect to others genotype under study. Regarding Ca content, wild genotypes demonstrated a unique profile with the highest mean level (3.12 g kg^{-1} DM). Controversy, the genotypes developed by UniCT recorded the lowest mean Ca content (2.26 g kg^{-1} DM). Within the wild genotypes, MA revelled the highest amount followed by KA (3.73 and 3.55 g kg^{-1} DM, respectively). MA also stood out for the lowest K content among all genotypes under study (1.18 g kg^{-1} DM). Genotypes developed by Catania University showed the highest mean K content (1.82 g kg^{-1} DM), with A41 having a notable content of 2.18 g kg^{-1} DM, which was the highest among all genotypes. Furthermore, commercial genotypes exhibit the highest Mg and Na mean content among the three groups (1.43 and 0.21 g kg^{-1} DM, respectively). In contrast, genotypes developed by UniCT and wild genotypes presented average reductions of approximately 5.6% and

7.0% in Mg content, respectively, and about 9.5% in Na content, compared to commercial genotypes.

However, both groups showed a high variability, since within them were presented the highs and lowest genotypes in the study for both macro-minerals. In wild cardoon, CR presented the lowest Mg level and KA the highest (1.17 and 1.51 g kg^{-1} DM, respectively). Within cultivated cardoon developed by UniCT, A41 obtained the lowest Na level and L25 the highest among all genotypes studied (0.16 and 0.24 g kg^{-1} DM, respectively).

Table 4. Ash content and macro-elements content (g kg^{-1} DM) for different *Cynara cardunculus* genotype seeds.

PLANT MATERIAL	*GENOTYPE*	**Ash Content**	**Macro-elements**				
			N	**Ca**	**K**	**Mg**	**Na**
Commercial CC	Gigante Inerme	35.36±2.18 cd	27.13±0.67 ab	2.41±0.20 c	1.91±0.08 f	1.45±0.02 fg	0.19±0.01 c
	Verde di Peralta	33.60±1.18 ab	28.58±0.92 de	3.00±0.33 g	1.68±0.02 c	1.47±0.10 fg	0.23±0.03 e
	Cardo Gobbo	37.78±3.30 e	26.90±0.17 a	3.08±0.33 g	1.79±0.03 e	1.43±0.05 ef	0.21±0.02 d
	Cardo Avorio	45.78±6.44 h	28.39±0.32 c-e	3.00±0.35 g	1.65±0.15 c	1.39±0.08 de	0.20±0.02 d
Mean ± SE		**38.13±2.03**	**27.75±0.32**	**2.87± 0.15**	**1.76±0.04**	**1.43±0.08**	**0.21±0.01**
CC developed by Catania University	Altilis 41	41.65±0.89 g	29.51±0.25 fg	1.72±0.17 a	2.18±0.10 g	1.34±0.03 cd	0.16±0.02 a
	Linea 1	34.19±2.66 bc	29.65±0.38 g	2.08±0.05 b	1.75±0.13 de	1.22±0.05 a	0.18±0.00 c
	Linea 7	36.60±1.26 de	27.88±0.97 cd	2.29±0.15 c	1.65±0.08 c	1.30±0.08 bc	0.18±0.02 b
	Linea 25	35.38±2.05 cd	28.49±0.39 de	2.34±0.39 c	1.70±0.11 cd	1.38±0.13 de	0.24±0.04 f
	Linea 32	35.19±2.53 c	28.39±0.28 c-e	2.85±0.41 f	1.78±0.10 e	1.50±0.16 g	0.21±0.02 d
Mean ± SE		**36.60±0.96**	**28.78±0.25**	**2.26±0.13**	**1.82±0.06**	**1.35±0.05**	**0.19±0.01**
Wild Cardoon	Marsala	33.44±2.15 ab	27.64±0.67 bc	3.73±0.46 i	1.18±0.10 a	1.36±0.10 d	0.21±0.02 d
	Val Paraiso	39.44±0.62 f	28.90±0.22 ef	2.54±0.32 d	1.82±0.13 e	1.28±0.09 b	0.21±0.04 d
	Kamarina	41.46±0.75 g	27.68±0.20 bc	3.55±0.31 h	1.96±0.05 f	1.51±0.07 g	0.18±0.01 c
	Creta	32.77±2.73 a	30.96±0.47 h	2.67±0.09 e	1.46±0.08 b	1.17±0.06 a	0.17±0.02 b
Mean ± SE		**36.78±1.15**	**28.79±0.35**	**3.12±0.19**	**1.61±0.08**	**1.33±0.05**	**0.19±0.01**

CC: cultivated cardoon. Data are the mean ± standard error (SE). Different letters within each column show significant differences on ash content and macro-minerals content among all genotype seeds respectively, according to Duncan test at $P \leq 0.05$. The highest means values per trait are colored in green, while the lowest averages are colored in red.

3.2. Influence of genotype on micro-elements content of cardoon seeds

The results of the analysis of variance ANOVA regarding the genotype showed a significant influence in all micro-elements, but especially in Mn at 57.6%, Cu at 54.1% and Zn at 50.5% (Table 3). This indicates a strong genetic control over these minerals.

As a general trend, genotypes developed by UniCT showed the highest mean content on all micro- elements under study and commercial cultivated cardoon presented intermediate contents (Table 5). On the contrary, wild genotypes exhibit the lowest mean. Fe content for cultivated cardoon developed by UniCT was slightly higher than that obtained by commercial cultivated cardoon (26.8 and 26.0 mg kg^{-1} DM, respectively). However, it is worth mentioning that the genotype that obtained the highest Fe level among all, CA, was within commercial cultivated cardoon with a content of 42.6 mg kg^{-1} DM. Regarding wild genotypes, MA and CR stood out for the lowest content on Fe (18.1 and 16. 8 mg kg^{-1} DM, respectively) and Zn (4.38 and 4.42 mg kg^{-1} DM, respectively). This group exhibited an average Zn content of 5.89 mg kg^{-1} DM, displaying a reduction of 58.2% and 61.2% compared to commercial cultivated cardoon and university-developed genotypes, respectively. Moving on the individual genotypes within cultivated cardoon developed by UniCT, the highest Zn level was displayed for genotype L7 (20.0 mg kg^{-1} DM), which, in turn, also obtained the highest content of Cu (6.52 mg kg^{-1} DM). In contrast, L1 exhibit the lowest Cu and Mg level (4.03 and 8.09 mg kg^{-1} DM, respectively) respect to others genotype under study. Despite this, cultivated cardoons developed by UniCT, exhibit, yet again, the highest content of Cu and Mg content (5.07 and 9.70 mg kg^{-1} DM, respectively).

Table 5. Micro-elements content (mg kg^{-1} DM) for different *Cynara cardunculus* genotype seeds.

PLANT MATERIAL	*GENOTYPE*	**Micro-elements**			
		Fe	**Zn**	**Mn**	**Cu**
Commercial CC	Gigante Inerme	16.25±3.16 a	9.51±1.48 c	8.92±0.17 c	5.10±0.38 ef
	Verde di Peralta	21.09±2.28 b	15.01±1.67 e	10.43±0.67 e	5.71±0.09 g
	Cardo Gobbo	23.96±1.02 d	15.18±1.65 e	8.82±0.46 bc	4.07±0.20 a
	Cardo Avorio	42.65±10.31 h	16.61±2.41 f	10.25±0.31 e	4.60±0.36 b-d
Mean ± SE		**25.99±3.32**	**14.08±1.03**	**9.60±0.26**	**4.87±0.18**
CC developed by Catania University	Altilis 41	27.23±9.36 e	16.69±2.95 f	8.78±0.35 bc	4.49±0.11 bc
	Linea 1	23.18±2.13 cd	9.01±0.23 c	8.09±0.54 a	4.03±0.23 a
	Linea 7	21.87±3.74 bc	20.00±2.10 h	10.43±0.22 e	6.52±0.17 h
	Linea 25	35.72±7.44 g	12.52±2.32 d	11.53±0.72 f	5.45±0.31 fg
	Linea 32	26.18±1.47 e	17.61±1.52 g	9.65±0.60 d	4.87±0.21 de
Mean ± SE		**26.84±2.54**	**15.17±1.12**	**9.70±0.31**	**5.07±0.18**
Wild Cardoon	Marsala	18.08±2.25 a	4.38±1.15 a	8.41±0.44 ab	4.83±0.28 c-e
	Val Paraiso	35.20±9.39 g	7.31±0.93 b	8.96±0.10 c	5.37±0.19 fg
	Kamarina	29.71±4.16 f	7.46±0.72 b	10.66±0.39 e	4.83±0.17 c-e
	Creta	16.78±5.49 a	4.42±0.47 a	9.01±0.31 c	4.26±0.25 ab
Mean ± SE		**24.94±3.20**	**5.89±0.51**	**9.26±0.24**	**4.82±0.13**

CC: cultivated cardoon. Data are the mean ± standard error (SE). Different letters within each column show significant differences on micro-minerals content among all genotype seeds respectively, according to Duncan test at $P \leq 0.05$. The highest means values per trait are colored in green, while the lowest averages are colored in red.

3.3. Influence of different growing season on ash content and mineral content

The ANOVA outcomes showed that the growing season had a pronounced effect on ash content (81.5%) and some mineral elements, particularly K (73.0%), Ca (72.5%), Mg (68.0%) and Fe (66.4%) (Table 3).

The influence of different growing seasons on the ash content, macro- and micro-elements in cardoon seeds was investigated over three consecutive growing season (Table 6). Ash content showed notable fluctuations over the three seasons, with the low-

est value recorded in S_2 (35.7 g kg^{-1} DM), followed by S_3 (37.9 g kg^{-1} DM) and the highest in S_1 (41.7 g kg^{-1} DM). The concentration of macro-elements in cardoon seeds also varied significantly across the three growing seasons: S_1 likewise exhibit the highest Na level (0.21 g kg^{-1} DM). Quite the opposite for S_2 that showed the lowest Na content along with S_3 (on average 0.19 g kg^{-1} DM). Regarding the N content, it remained relatively stable between S_1 and S_2 (on average 28.3 g kg^{-1} DM), with a slight increase observed in S_3 (28.7 g kg^{-1} DM). The S_3 also recorded the highest level of both, K and Mg (1.90 and 1.50 g kg^{-1} DM, respectively). Mg content decreased progressively from S_3 to S_1 (-14.7%). Additionally, in S1 was observed the lowest Ca content (2.16 g kg^{-1} DM). This macro-mineral level peaked in S_2 (3.22 g kg^{-1} DM), as well as for the micro-minerals Fe and Zn (32.4 and 14.5 mg kg^{-1} DM, respectively). The minimum level for Fe and Zn was recorded for S_3 and S_1, respectively (17.4 and 10.3 mg kg^{-1} DM). Nevertheless, S_1 and S_2 exhibit the highest Mn content (on average 9.69 mg kg^{-1} DM) and S_3 the lowest (9.23 mg kg^{-1} DM). Conversely, that last season along with S_2, recorded the highest Cu level (on average 5.08 mg kg^{-1} DM) and S_1 the lowest with a content of 4.62 mg kg^{-1} DM.

Table 6. Ash content, macro-elements content and micro-elements content of *Cynara cardunculus* seeds for different consecutive growing seasons.

		GROWING SEASON		
		Season 1	**Season 2**	**Season 3**
Ash Content (g kg^{-1} DM)		41.70±1.47 c	35.73±1.18 a	37.95±0.59 b
Macro-elements (g kg^{-1} DM)	**N**	28.33±0.27 a	28.34±0.27 a	28.74±0.38 b
	Ca	2.16±0.11 a	3.22±0.20 c	2.75±0.13 b
	K	1.79±0.05 b	1.51±0.06 a	1.90±0.05 c
	Mg	1.28±0.02 a	1.32±0.05 b	1.50±0.04 c
	Na	0.21±0.01 b	0.19±0.01 a	0.19±0.01 a
Micro-elements (mg kg^{-1} DM)	**Fe**	28.21±3.12 b	32.36±3.30 c	17.41±1.16 a
	Zn	10.26±1.01 a	14.47±1.41 c	11.20±1.18 b
	Mn	9.73±0.24 b	9.65±0.33 b	9.23±0.26 a
	Cu	4.62±0.18 a	5.10±0.17 b	5.07±0.17 b

Data are the mean of all genotypes ± standard error (SE). Different letters show significant differences on ash content and minerals content among growing seasons, according to Duncan test at $P \leq 0.05$. The highest means values per trait are colored in green, while the lowest averages are colored in red.

3.4. Influence of the 'genotype x growing season' interaction on ash and macro-elements content of cardoon seeds

The results of the analysis of variances (ANOVA) indicate that the interaction between genotype and growing season contributed significantly, albeit to a lesser extent than the other factors (Table 3). The interaction effects were particularly prominent for macro-elements Na (53.8%), N (30.1%) and Mg (18.0%).

The influence on the ash content and macro-elements content (N, Ca, K, Mg, Na) resulting from the interaction of cardoon seed genotypes across three consecutive growing seasons (S_1, S_2 and S_3) is represented in Figure 1. The ash content of cardoon seeds exhibits significant variability both among different genotypes and across different growing seasons. It can be observed a remarkably general season trend: S_2 was characterized by a reduction of 23.9% and 16.4% respect to S_1 and S_3. Besides, all genotypes in S_2 showed the lowest ash content, except for A41 and KA. This tendency was particularly

noticeable for commercial cultivated cardoon, which showed the lowest average ash content in S_2 compared to the other groups and growing seasons (29.5 g kg^{-1} DM). The lowest values were detected in CR followed by L1 (24.2 and 25.8 g kg^{-1} DM, respectively). In contrast, genotype CA consistently shows the highest ash level across all growing seasons, with a marked peak in S_1 (65.6 g kg^{-1} DM).

Figure 1. Ash and macro-mineral content of *Cynara cardunculus* seeds in relation to 'genotype x growing season' interaction. Different letters show significant differences, according to Duncan test at $P \leq 0.05$. Data are the mean ± standard error. CC: cultivated cardoon. For acronyms see Table 2.

A similar season prone was found in K content, S_2 also showed the lowest level, with a reduction of 15.6% and 20.5% compared to S_1 and S_3 respectively. However, in this case, the lowest mean values were found in wild genotypes (1.38 g kg^{-1} DM), especially in MA and CR (0.88 and 1.22 g kg^{-1} DM, respectively). The highest K level was recorded in S_3 for cultivated cardoon developed by UniCT, with an average content of 2.01 g kg^{-1} DM. Within this group, A41 consistently showed the highest K content, peaking in that season at 2.34 g kg^{-1} DM. In the same way, the highest N and Mg content was also recorded in S_3 for university-developed genotypes, with an average level of 29.4 and 1.60 g kg^{-1} DM, respectively. Genotypes like VdP and CR highlights for the highest N content (31.2 and 31.3 g kg^{-1} DM, respectively). In turn, genotypes such as L32 and L25 stood out with the highest Mg content (1.98 and 1.80 g kg^{-1} DM, respectively). On the contrary, the lowest amount among groups was likewise found within university-developed genotypes in S_2 for Mg and Na elements (on average 1.14 and 0.14 g kg^{-1} DM, respectively). That season also showed the lowest Ca level, in particular cultivated cardoon developed by UniCT (on average 1.71 g kg^{-1} DM). Conversely, all commercial genotypes accumulated the highest content in Ca in Season 2 (on average 3.82 g kg^{-1} DM, respectively). A similar trend was found in wild cardoon for Ca content (on average 3.81 g kg^{-1} DM) with MA and KA genotypes peaking in that season (5.15 and 4.50 g kg^{-1} DM, respectively). Quite the reverse for the Na level, which had its highest mean content in S_1, like VP, and his lowest in S_2, with the exception of some commercial genotypes, such as VdP.

3.5. Influence of the 'genotype x growing season' interaction on micro-elements content of cardoon seeds

Following the same tendency as in macro-element profile, the ANOVA analysis of the interaction between genotype and growing season revealed significant differences. However, as before, these differences were less pronounced than those between the individual factors. Nonetheless, there were still significant differences. The maximum percentage of interaction was found for Mn (25.4%), followed by Fe (17.1%), Cu (12.0%) and, lastly, Zn (10.8%).

The results of the interaction between cardoon genotype and growing season on micro-mineral content (Fe, Zn, Mn and Cu) over three consecutive growing seasons (S1, S2 and S3) is presented in Figure 2.

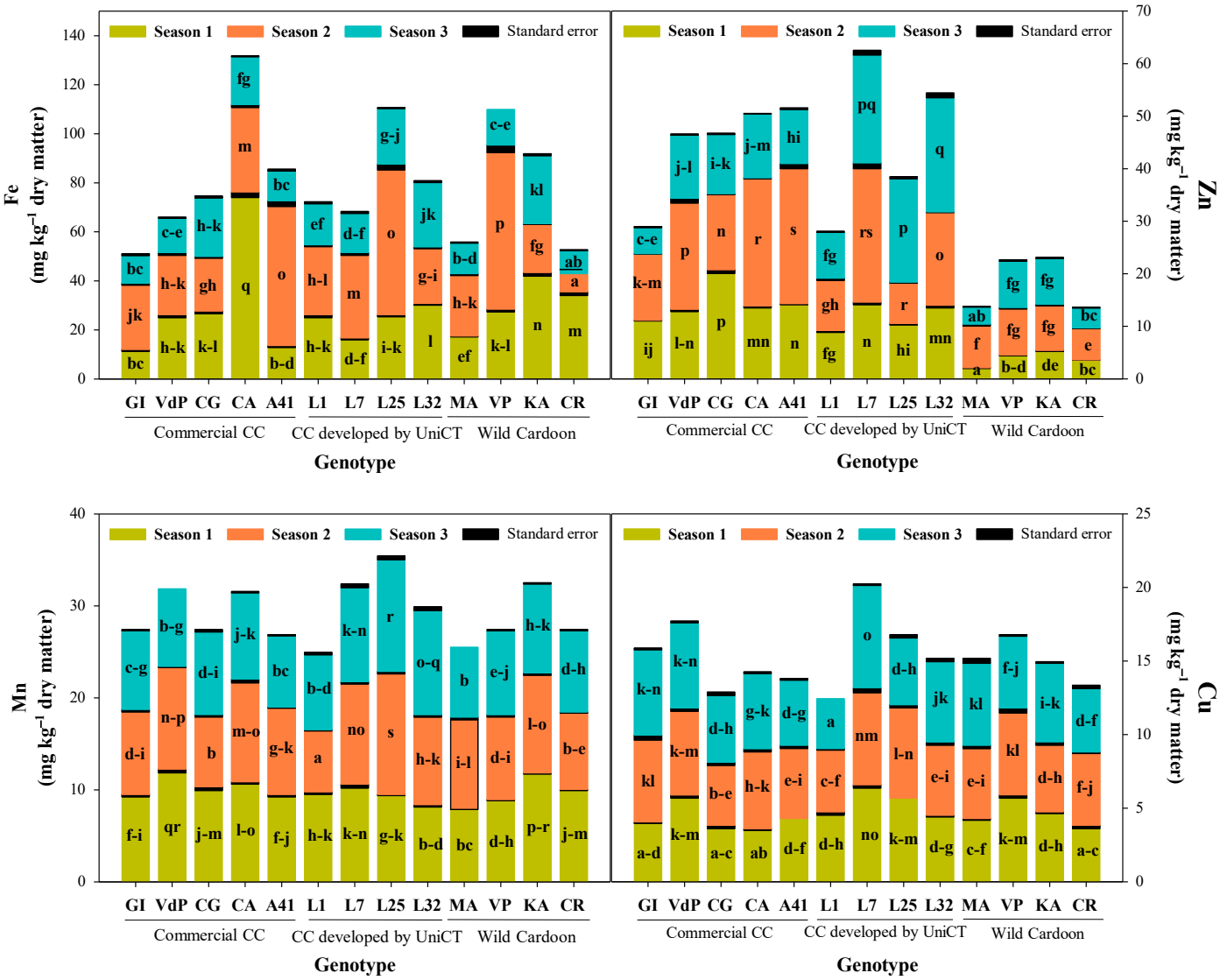

Figure 2. Micro-mineral content of *Cynara cardunculus* seeds in relation to 'genotype x growing season' interaction. Different letters show significant differences, according to Duncan test at $P \leq 0.05$. Data are the mean ± standard error. CC: cultivated cardoon. For acronyms see Table 2.

As a general season trend, it could be observed that for Fe most genotypes showed a peak in its content in either S_1 or S_2, with a decrease in S_3. This was the case of the commercial genotype CA and the wild genotype VP, that exhibited the highest Fe contents among all genotypes under study in S_1 and S_2, respectively (74.0 and 63.8 mg kg^{-1} DM). Thus, cultivated cardoon developed by UniCT exhibit the highest Fe mean content in S_2 (30.9 mg kg^{-1} DM). On the contrary, S_3 recorded the lowest values regardless of the group analyzed. Nevertheless, the lowest genotype values were observed by CR in Season 2 followed by S_3 (6.98 and 9.32 mg kg^{-1} DM, respectively). Following the previous season's tendency, most genotypes showed an increase in Zn content in S_2, followed by a decrease in S_3 (on average, -22.6%), except for L25 and L32, which peaked in S_3 (+157% and +23%). Commercial cultivated cardoon and university-developed genotypes, as observed for Fe, showed the highest Zn content (on average 17.5 mg kg^{-1} DM) and within this last group, genotypes like A41 and L7 in Season 2 exhibit the highest Zn content among all genotypes under study (25.7 and 25.4 mg kg^{-1} DM, respectively). However, the most important thing to note is that regardless of the growing season, wild cardoon genotypes

obtained the lowest Zn content among the three groups, displaying a mean reduction of 59.7%, respect to commercial cultivated cardoon and genotypes developed by UniCT. This trend also maintained for Mn, in the same way, wild genotypes generally exhibited lower Mn content compared to the other two groups (on average, -4.0%), with notable variability season-to-season. Besides, on S_3 it was recorded the lowest mean level of Mn for wild genotypes and commercial cultivated cardoon (8.79 and 8.82 mg kg^{-1} DM, respectively). Conversely, commercial cultivated cardoon showed the highest mean content of this micro-element on S_1 (10.4 mg kg^{-1} DM). Regarding the individual genotypes, L25 exhibit the highest Mn content among all genotypes under study, especially for S_2 and S_3 (on average 12.6 mg kg^{-1} DM). The lowest values were recorded by L1 with 6.62 mg kg^{-1} DM, and CG and MA, both at 7.58 mg kg^{-1} DM. Genotypes like VP maintained the most consistent level of Mn among the three-growing seasons. Likewise, VdP also showed this stable trend in the Cu content. Though, most commercial genotypes showed an increasing trend in Cu content over the three growing seasons, from the minimum amount of Cu in S_1 (4.18 mg kg^{-1} DM) to the maximum achieved in S_3 (5.30 mg kg^{-1} DM). Despite this, L7 (university-developed genotype) showed the highest and most consistent content of Cu, slightly higher peaking in S_3 with 6.94 mg kg^{-1} DM compared to the others.

4. Discussion

Cardoon seeds are very important from a nutritional point of view as, among other compound, are a proven source of various minerals [40,43,47]. In this sense, mineral elements play critical roles in both, human health and animal and livestock health. In human well-being, minerals performance diverse and vital functions in numerous biochemical processes, including the regulation of blood pressure and the reduction of cardiovascular disease risk, structural tasks in bones and teeth, regulatory roles in metabolism and immune defence, among others [48]. Ensuring an adequate intake of these essential nutrients through diet or supplementation is crucial for maintaining overall health and preventing deficiency-related diseases [40].

Concerning the livestock, in recent decades, there has been a significant increase in its production, driven by a rising demand for animal-source foods, particularly in developing countries [49]. Globally, livestock production has shifted from extensive, small-scale, subsistence systems to more intensive, large-scale, commercially oriented, specialized units [50]. To sustain these high production standards, animal nutrition has become highly specialized, relying almost exclusively on concentrate feed sourced from the international market. Consequently, correct mineral supplementation in concentrated feed, which not only is the primary or only feed for intensive systems but also a complementary feed for many traditional farms, is crucial. It is well known that mineral micronutrients are required for the normal functioning of all biochemical processes in the animal organism. They are part of numerous enzymes and coordinate a great number of biological processes, and consequently they are essential to maintain animal health and productivity.

Overall, our results show that, both genotype and growing season significantly affect the mineral content and ash composition of cardoon seeds, with substantial interaction effects. According to the literature, the concentration and availability of chemical compounds are subject to a complex interplay of factors, such as environmental conditions (associated with different growing seasons such as temperature, precipitation and soil conditions), genetic background and maturation stage [8,42,43,51–55], underscoring the importance of considering both genetic and environmental factors in the optimization of cardoon seed production for improved nutritional quality.

In relation to the genotype, the wild cardoon presented lower ash content than the commercial and developed by UniCT cultivated cardoon. Opposite results were observed in the study by Petropoulos et al. [43], where wild cardoon genotypes showed a higher ash content (between 26.7 and 27.7 g kg^{-1} DM) compared to commercial genotypes (20 g kg^{-1} DM). However, in the present study, the average ash content across all genotypes analysed was approximately 49% higher than those reported by Petropoulos et al. [43].

Among the nine mineral elements studied, the one that was presented in a greater mean quantity was the macro-element N, followed by Ca, K, Mg and Na. As for the micro-minerals, Fe exhibits the highest average content with Zn, Mn and Cu showing progressively lower levels. The same trend in the order of quantity of mineral content was also observed in other studies [28,43]. The N content was also evaluated in the study of Petropoulos et al. [43] in wild and commercial cardoon with an average content of 41.4 and 46.6 g kg^{-1} DM, respectively. In our study, although the content of this macro-element in general in all genotypes was approximately 34% lower, the reverse trend was found. The highest average content was found in wild genotypes followed by those developed by UniCT and, in the last place, commercial ones. However, our findings on N content align with those reported by Cajarville et al. [47], who investigated the use of cardoon seeds as animal feed for ruminants. Same inverse trend occurred in the content of Ca. Petropoulos et al. [43] reported a mean content in wild cardoon that varied between 10.47 and 13.53 g kg^{-1} DM. Our results also correlated these differences depending on the genotype. In commercial cardoon, the average Ca content was 7.35 g kg^{-1} DM, however, for the same genotype (CA) and plant but the previous season a content of 11.97 g kg^{-1} DM was reported [28]. However, in our case the average Ca content was higher in wild genotypes than in the commercial and developed by the UniCt cultivated cardoon. Besides, the quantity reported for genotype CA in our research and, in general, in all genotypes was approximately 70% lower, around 3 g kg^{-1} DM. This quantity is more aligned with the results obtained by Angelova et al. [44], who reported a Ca content in seeds of commercial cardoon of 2.83 g kg^{-1} DM. Lower contents in K macro-mineral were also found in our research compared with Angelova et al. [44] and Petropoulos et al. [28, 43] as it ranges between 5.93 and 6.68 g kg^{-1} DM. Thus, differences were also found depending on the growing season. These variations may be due to growing conditions as it was reflected in our results (Table 3). Same happens in the Mg content, as the quantity reported in this study is approximately 65% lower. However, similar Na values to those obtained in this research have been reported in other studies, ranging from 0.18 to 0.24 g kg^{-1} DM [28,43]. The uniform Na content suggests minimal variability in this element and this consistency indicates that the seeds maintain a balance in this essential electrolyte.

The greatest differences in terms of micro-mineral content can be observed for Fe, as the results obtained in this assay differ greatly from others. Petropoulos et al. [28,43], reported a content that ranges between 116–160 mg kg^{-1} DM, this means an increase of more than 400% respect our results. On the other hand, Angelova et al. [44] described a Fe content of 51.1 mg kg^{-1} DM in cardoon seeds, which despite doubling our values is more aligned with the results obtained in this study. Same case happens for Mn, since the content obtained by Petropoulos et al. [28,43] varies between 46–60 mg kg^{-1} DM, being approximately 480% higher than the quantity found in our study, while the one reported by Angelova et al. [44] is 18 mg kg^{-1} DM, duplicating our results. In that mentioned study it is also reported an amount of Cu of 11.4 mg kg^{-1} DM, also doubling the values obtained in this research.

To our knowledge, no literature has been found that specifically addresses the influence of the growing season on ash and mineral elements of cardoon seeds. However, there are studies in different plant parts on related species, such as globe artichoke, which

provide insights into similar phenomena. For example, Elia and Conversa [56] reported that the mineral element content in globe artichoke capitula was significantly affected by soil type and crop management practices. Others also highlighted that various factor, including seasonal variations, influenced the mineral composition in globe artichoke [1,51]. These findings suggest that, although specific research on cardoon seeds is lacking, it is reasonable to infer that similar seasonal effects might influence cardoon as well. Just as with globe artichoke, seasonal changes could alter the availability of nutrients in the soil, which in turn affects the mineral content in cardoon seeds. The fluctuations in climatic conditions throughout the growing season could lead to variations in nutrient uptake and accumulation, reflecting broader trends observed in related species. Ash content fluctuated considerably between seasons, with the lowest amount recorded in S_2 and the highest in S_1. This pattern could have been associated with variations in rainfall level throughout the growing seasons. The S_2 recorded a rainfall level of about 69% higher than S_1 and S_3. This could have leached and diluted the soil mineral concentration and, consequently, the minerals uptake by plant. The S_2 reduced the K level in seeds. The role of K in plant drought resistance has well reported [57]. Probably, the major rainfall observed in S_2 could explain the lowest K content found in seeds. On the contrary, the S_2 favoured the Ca accumulation in seeds. This is aligned with the findings of Sorooshzadeh et al. [58], which highlighted a reduced Ca concentration in soybean plant tissues under water stress condition. On the other hand, it is known how K^+ and Ca^{2+} compete with each other [59]. Similar trend was observed for both Fe and Zn. Also, Choukri et al. [60] documented a significant reduction in Fe and Zn content in lentil genotypes under drought stress.

5. Conclusions

This research not only contributes to the understanding of cardoon seed composition but also unveils the assessment of element mineral content variation among different seed genotypes and growing seasons, reflecting the complex interplay between genetics and environmental conditions, affecting nutrient and mineral accumulation.

The results of the present study demonstrated that, in general, cardoon seeds are a good source of macro and micro-elements, such as N, K, Mg, and Fe, while they also contain a low amount of Na and Cu. Commercial genotypes generally show a higher amount of ash and macro-minerals such as Mg and Na, in particular Verde di Peralta, whereas university self-developed genotypes excel in N, K, Zn, Fe, Mn and Cu content. For example, Linea 7 was characterized by the highest level of both Zn and Cu. Wild genotypes, while variable, stand out for Ca quantity, such as Kamarina. Overall, of all the 13 genotypes studied in this research, those that provide a higher or more stable content of the minerals under study are the cultivated cardoon developed by Catania University, providing a valuable genetic reservoir for future breeding programs aimed at enhancing macro and micro-mineral content in cultivated genotypes.

Future research should focus on understanding the specific environmental conditions that promote macro and micro-mineral accumulation and the genetic mechanisms underlying these traits. Such knowledge will be crucial for breeders and farmers in selecting suitable genotypes for specific purposes or target markets.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, G.M. S.L. and G.P.; methodology, M.G-B, SA.S. and C.F.; software, G.P.; validation, M.G-B, PJ.Z. and G.M.; formal analysis, M.G-B., SA.S. and C.F.; investigation, G.P.; resources, S.L.;

data curation, M.G-B., SA.S. and C.F.; writing—original draft preparation, M.G-B and G.P.; writing—review and editing, M.G-B, MJ.G., PJ.Z., G.M., S.L. and G.P.; visualization, MJ.G.; supervision, MJ.G., PJ.Z., G.M. and S.L.; funding acquisition, PJ.Z. and S.L. All authors have read and agreed to the published version of the manuscript."

Institutional Review Board Statement: Not applicable.

Funding: This research was funded by Conselleria de Educación, Cultura, Universidades y Empleo of the Generalitat Valenciana for Ph.D. scholarship (grant number ACIF/2021/178) and mobility scholarship (grant number CIBEFP/2023/86) of M.G-B.

Data Availability Statement: All data are available via an email request to the authors..

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

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RESULTS AND DISCUSSION

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RESULTS AND DISCUSSION

In recent years, the demand for fresh-cut and ready-to-eat products have significantly increased due to their convenience, time-saving benefits, and adaptability to modern consumer lifestyles. These products offer a practical solution for individuals seeking healthy and high-quality food options with minimal preparation time, aligning with evolving dietary preferences and trends. In this context, fresh-cut artichokes have garnered considerable attention in the food industry for their convenience and nutritional benefits, but they also face challenges related to quality maintenance. Enzymatic browning is a major challenge in the preservation of fresh-cut artichokes, particularly during cold storage. This process occurs due to the enzymatic oxidation of phenolic compounds, primarily catalysed by the PPO enzyme. The oxidation of these compounds results in the formation of quinones, which then polymerize to form brown pigments, significantly altering the visual appearance of the artichokes (Cefola et al., 2012; Constabel and Barbehenn, 2008; Görgüç et al., 2024; Pompili et al., 2023; Ricceri and Barbagallo, 2016). This browning not only reduces the visual quality of the product but also affects its overall consumer acceptance, leading to potential rejection. Such deterioration can limit the shelf-life of fresh-cut artichokes, as it indicates lack of freshness. As a result, browning can negatively impact the marketability of the product, particularly in the ready-to-eat or minimally processed food sector (Del Nobile et al., 2009; Giménez et al., 2003; Pandino et al., 2017a; Ricceri and Barbagallo, 2016; Rizzo et al., 2021, 2019). The susceptibility of artichokes to enzymatic browning has been well-documented and is considered one of the primary factors limiting their commercialization and extended storage (Cabezas-Serrano et al., 2009; Cefola et al., 2012; Del Nobile et al., 2009; Ghidelli et al., 2013; Giménez et al., 2003; Licciardello et al., 2017; Pandino et al., 2017a; Ricceri and Barbagallo, 2016). Consequently, effective strategies to control enzymatic browning and improve shelf-life are critical for meeting consumer expectations and enhancing the commercial viability of fresh-cut artichokes. Thus, maintaining quality attributes such as weight, firmness, and colour is essential for ensuring the marketability of fresh-cut artichokes and their acceptance in the ready-to-eat product category (Cabezas-Serrano et al., 2009; Cefola et al., 2012; Tortosa-Díaz et al., 2025). Pursuing this objective, studies focused

for the development of fresh-cut artichokes (Publications 1, 2, and 3) revealed critical factors affecting quality during postharvest storage.

The first research (**Publication 1**) (Giménez et al., 2023), aimed to evaluate the effect of the application of cysteine, alone or in combination with different concentration (75, 150 and 300 µL/L) of an equal mix of EOs (thymol, eugenol and carvacrol), on the quality attributes and functional properties of fresh-cut 'Blanca de Tudela' artichokes hearth quarters during storage. Colour parameters (L* and b*) were used to evaluate the visual quality of the treated samples. Treated samples, particularly those receiving moderate to high concentrations (150 and 300 µL) of EOs, maintained higher lightness (L*) and yellowness (b*) parameters compared to untreated samples, slowing the decline in colour quality typically associated with enzymatic browning (Arias et al., 2009; Chung et al., 2015; Rizzo et al., 2021, 2019). This improvement may be attributed to the synergistic effects of cysteine and EOs. The inhibitory effect of cysteine on PPO activity is attributed to its ability to form stable complexes with copper ions in the enzyme's active site, thereby preventing the oxidation of phenolic substrates (Amodio et al., 2011; Cabezas-Serrano et al., 2013; Ghidelli et al., 2015, 2013). The additional contribution of EOs in reducing browning could be linked to their antioxidant properties, which scavenge ROS, reducing oxidative stress and further preventing browning (Babbar et al., 2015; Côté et al., 2010; Janda et al., 2003; Toscano et al., 2019). These results contrast with previous findings where the use of *Foeniculum vulgare* EO did not significantly affect the colour quality of fresh-cut artichokes (Rizzo et al., 2019). This discrepancy could be attributed to differences in cultivar, genotype, harvest conditions or storage parameters, factors known to influence the post-harvest quality of artichokes (Cabezas-Serrano et al., 2009; Cefola et al., 2012; Giménez et al., 2021; Licciardello et al., 2017; Pandino et al., 2017a). Further, the application of cysteine and EOs (specially 75 and 150 µL) to fresh-cut artichokes effectively reduced WL and RR during storage, which is a major factor in product deterioration (Gil-Izquierdo et al., 2002; Leroy et al., 2010; Rojas-Graü et al., 2007; Sánchez-González et al., 2011; Valverde et al., 2005). The barrier effect of EOs likely contributed to reduced moisture loss and delay dehydration (Ali et al., 2016), while the decrease in RR suggests a lower metabolic activity during storage (Rojas-Graü et al., 2007; Sánchez-González et al., 2011; Valverde et al., 2005). These findings align with previous

research demonstrating the efficacy of EOs in preserving the quality of perishable fruits, which enhances their shelf-life and marketability (Abd El Wahab, 2015; Ali et al., 2016; Rizzo et al., 2021, 2019; Saleh et al., 2019; Salimi et al., 2013; Shemy, 2020). Other important indicators of the functional quality of artichokes are TPC and TAA. The combined treatments of cysteine and EOs (75 and 150 µL) preserved higher these parameters compared to controls, indicating better retention of bioactive compounds with antioxidative potential (Bakkali et al., 2008; Sánchez-González et al., 2011). This preservation is crucial not only for extending shelf-life but also for maintaining the health-promoting properties of artichokes, which are valued for their phenolic compounds and associated antioxidant capacity (Del Rio et al., 2013; Nabavi et al., 2015). Sensory evaluations further confirmed the effectiveness of the treatments in maintaining visual appeal and textural integrity. Treated samples exhibited reduced discoloration, tissue softening, and dehydration compared to controls, underscoring their potential for extending the marketability of fresh-cut products. The obtained results in this research settled that the combination of cysteine and EOs proved to be a promising strategy for enhancing the quality and functional properties of fresh-cut 'Blanca de Tudela' artichokes. It has been revealed that the use of cysteine in combination with 150 µL of EOs effectively reduced browning, preserved phenolic content and antioxidant activity, and maintained structural and sensory quality during storage. These findings contribute to the development of natural, sustainable solutions for extending the shelf-life of fresh-cut vegetables, addressing both consumer preferences and industry needs.

However, while the results demonstrate the effectiveness of cysteine and EOs in improving the shelf-life and quality of fresh-cut artichokes, some limitations were noted. The treatments were unable to completely halt browning and quality loss, particularly in the later stages of storage. This indicates that enzymatic browning is a multifactorial process influenced by other intrinsic and extrinsic factors that can affect PPO activity and phenolic oxidation. In this context, the second and third investigations (Publications 2, and 3) aimed to delve deeper into this aspect, specifically sought to identify and understand the factors that influence phenolic content, shedding light on the underlying mechanisms that impact browning and overall quality in artichokes. These studies were dedicated to exploring the pivotal role of phenolic content as a determinant of susceptibility to browning.

The second research (**Publication 2**) (Giménez-Berenguer et al., 2025) evaluated the suitability for fresh-cut processing of three different artichokes cultivars ('Lorca', 'Tupac' and 'Green Queen'), attending to their flower head orders (main, secondary, and tertiary). Specifically, it investigated how these two factors influence key quality parameters, including total phenolic content, browning evolution, and sensory acceptability attributes of fresh-cut artichokes heart slices during cold post-processing storage. This work aimed to provide insights into how intrinsic plant characteristics and post-harvest behaviour impact the commercial potential of minimally processed artichokes. The results revealed that total phenolic content varied significantly among cultivars and flower head orders. Tertiary heads consistently showed the highest phenolic concentrations within all cultivars, with the 'Green Queen' cultivar exhibiting overall higher phenolic levels than 'Lorca' and 'Tupac.' However, this increased phenolic content was closely linked to a greater susceptibility to enzymatic browning (Amodio et al., 2020a; Cefola et al., 2012; Görgüç et al., 2024; Pompili et al., 2023; Ricceri and Barbagallo, 2016; Rizzo et al., 2021; Tortosa-Díaz et al., 2025), particularly evident in the tertiary heads of all cultivars. Browning progression during storage followed a hierarchical trend, with tertiary heads browning most rapidly, while main heads demonstrated slower browning, especially in 'Lorca' and 'Tupac' cultivars. Sensory evaluations supported these findings, showing that main heads from 'Lorca' and 'Tupac' retained better visual quality and overall acceptability throughout storage. In contrast, 'Green Queen', despite its higher phenolic content, scored lower due to its accelerated browning and diminished visual appeal, which limited its shelf-life and consumer preference (Amodio et al., 2020a; Constabel and Barbehenn, 2008; Giménez et al., 2023; Pandino et al., 2017a).

The strong relationship between browning and sensory acceptability was further confirmed through correlation analysis. A significant negative correlation was observed across all cultivars and flower head orders, with correlation coefficients exceeding -0.9 throughout the storage period. This inverse relationship indicated that as browning index increased, sensory acceptance scores declined, reinforcing the crucial impact of visual appearance on consumer perception. The strongest correlation was observed in the main heads of 'Lorca' and 'Tupac', where browning developed more slowly, allowing for better preservation of visual quality

and overall acceptability. Conversely, in 'Green Queen', where phenolic content was highest, the correlation remained strong but slightly lower, particularly in tertiary heads, which browned more rapidly. These findings highlight the significant influence of cultivar and flower head order on post-harvest quality parameters. The study confirmed the interplay between phenolic content and browning susceptibility in determining the suitability of artichokes for fresh-cut products.

Phenolic compounds, while critical for the antioxidant and nutritional value of artichokes, act as substrates for enzymatic browning reactions catalysed by PPO (Pompili et al., 2023; Tortosa-Díaz et al., 2025). This dual role presents a challenge for high-phenolic cultivars like 'Green Queen', and artichokes such as tertiary heads, which are more prone to rapid visual quality degradation during storage (Cefola et al., 2012; Licciardello et al., 2017; Lombardo et al., 2015b; Pandino et al., 2017a; Todaro et al., 2010). These findings align with previous research, which reported a positive correlation coefficient of 0.80 between TPC and browning, highlighting the direct influence of phenolic compounds on discoloration processes (Starowicz et al., 2021). Conversely, main heads from 'Lorca' and 'Tupac' showed slower browning rates and better sensory attributes, suggesting that their lower phenolic content make them more suitable for fresh-cut processing. These cultivars and flower head orders provide a practical solution for minimizing enzymatic browning without relying heavily on chemical anti-browning treatments (Bonasia et al., 2010). Additionally, the sensory evaluation demonstrated the commercial advantage of selecting cultivars and head orders with balanced phenolic content, which offers extended shelf-life while maintaining consumer appeal.

The research underscores the importance of tailoring post-harvest strategies to the specific characteristics of the cultivar and flower head order. While high-phenolic cultivars like 'Green Queen' may necessitate greater additional anti-browning interventions, 'Lorca' and 'Tupac' offer a better balance of visual quality and nutritional properties, offering a more favourable option for fresh-cut markets due to their natural lower susceptibility to browning.

The third study (**Publication 3**) (Giménez-Berenguer et al., 2022), sought to build upon the findings of the third by addressing the complex interplay of factors that contribute to enzymatic browning in fresh-cut artichokes. Recognizing that

browning is influenced not only by external treatments but also by intrinsic characteristics of the artichokes themselves, such as cultivar and flower head order (Giménez-Berenguer et al., 2025), the 'Lorca' cultivar was selected to further explore its adaptability for the development of fresh-cut products. Within this framework, an additional focus was placed on examining how the internal developmental stage influences total phenolic content and PPO enzyme activity, providing deeper insights into the factors that govern enzymatic browning and quality retention. The study conducted on the 'Lorca' cultivar revealed that key components such as the flower head order as well as the internal development stage were identified as critical determinants of phenolic levels and post-harvest quality, with substantial variability attributable to these factors. Tertiary and secondary artichoke heads, characterized by their higher phenolic content and increased PPO activity, were found to be more susceptible to browning, limiting their suitability for fresh-cut processing. Conversely, main heads, exhibiting lower phenolic content and PPO activity, demonstrated reduced browning susceptibility, making them better candidates for ready-to-eat applications. This relationship between phenolic concentration and browning aligns with previous studies emphasizing the critical role of PPO in enzymatic browning reactions and its correlation with phenolic substrate availability (Brecht et al., 2003; Starowicz et al., 2021). The internal development stage also played a pivotal role, with advanced stages showing the lowest phenolic content and PPO activity, while younger stages exhibited higher phenolic levels and browning susceptibility. This observation underscores the importance of harvesting artichokes at optimal developmental stages for specific processing and consumption purposes. While higher phenolic levels contribute to the nutritional and antioxidant benefits of artichokes, they also pose challenges for fresh-cut processing due to enhanced browning. Digital image analysis further corroborated these findings by quantifying browning intensity through changes in hue angle over time. Tertiary heads displayed the most significant colour shifts, indicating a pronounced browning process. This method proved effective in objectively assessing browning susceptibility and its direct correlation with phenolic content and PPO activity. The results suggest that the 'Lorca' cultivar's main heads hold greater potential for fresh-cut applications, given their lower browning rates and improved visual stability. This is crucial for consumer acceptance, as appearance significantly influences

purchasing decisions (Pandino et al., 2017a; Ricceri and Barbagallo, 2016; Rizzo et al., 2021, 2019).

The reduced browning in main heads also implies fewer post-harvest interventions, potentially lowering processing costs and increasing marketability (Brecht et al., 2003). However, tertiary and secondary heads, with their higher phenolic content, could be better suited for fresh consumption, prioritizing nutritional benefits over visual quality. The study highlights the potential of using phenolic content and PPO activity as indicators for classifying artichokes based on their processing aptitude. These insights could inform industry practices, enabling the selection of cultivars and harvesting strategies that balance nutritional quality with post-harvest stability. However, significant knowledge gaps remain in fully understanding the pre- and post-harvest behaviour of artichokes, as the analysis of variance components for phenolic content in Publication 3 revealed that more than 50% of the variation remains unexplained.

To address this, the fourth investigation (**Publication 4**) (Giménez et al., 2022) not only evaluated the influence of flower head order, but also considered the impact of harvest date. Previous studies have highlighted that phenolic content can be influenced by multiple factors, including harvest timing, agronomic practices and post-harvest management (Ceccarelli et al., 2010; Lattanzio et al., 2009; Lombardo et al., 2018, 2012, 2010; Palma et al., 2023; Pandino et al., 2013a, 2013b, 2012; Petropoulos et al., 2018b; Spanu et al., 2018). Therefore, we aimed to determine whether harvest date influences the quality and bioactive profile of one of the most traditionally cultivated artichoke varieties in Spain, 'Blanca de Tudela' cultivar. To achieve this, two simultaneous studies were conducted: on the one hand, artichokes were analysed individually across different harvest dates, both in winter and spring, following the two cops cycle characteristic of this cultivar; on the other hand, a post-harvest storage experiment was performed at two key harvest points, representative of winter and spring production, to evaluate the behaviour and quality evolution of the artichokes during cold storage. Results demonstrated that bioactive compounds were not uniformly distributed across the different flower head orders. Tertiary heads consistently exhibited higher antioxidant activity and higher levels of phenolic compounds, including hydroxycinnamic acids and luteolin derivatives, followed by

secondary and main heads. Despite seasonal variations in environmental conditions, there were no significant differences in total phenolic and antioxidant content between spring and winter harvests, suggesting that flower head order plays a more dominant role in determining phenolic concentration than the seasonal timing of harvest. These findings align with our prior research suggesting that phenolic accumulation is influenced by metabolic activity and flower head development (Publication 2 and Publication 3) (Giménez-Berenguer et al., 2025, 2022). However, the lack of seasonal variations contrasts with studies (Licciardello et al., 2017; Pandino et al., 2017b), where differences on phenolic content have been reported among artichoke cultivars and harvest times. Those researchers informed that the early harvested artichoke had lower phenolic content than the latest ones. These differences may be because the floral order was not considered as a factor of study in those investigations. The primary heads, the ones with the lower phenolic content, are the first to grow on the plant (Carrión Pávez, 2013) and, therefore, the first ones to be harvested.

Postharvest storage experiment revealed distinct respiration rates across flower head orders and harvest seasons. Artichokes harvested in spring showed significantly higher respiration rates compared to winter harvests, with tertiary heads maintaining lower RR than secondary and main heads throughout storage. These findings underscore the crucial role of tertiary heads' metabolic activity in prolonging shelf-life, as it is well established that higher respiration rates accelerate the deterioration of fresh products (Kader, 2002). WL and firmness degradation, critical quality parameters (Sabi et al., 2013), also varied by flower head order. Tertiary and secondary heads experienced the least weight loss and better firmness retention, maintaining their marketability for longer periods. This suggest that smaller heads with tighter bracts are less prone to water loss under refrigerated conditions. In contrast, main heads exhibited the highest weight and firmness losses rates, which could compromise their visual and sensory quality for fresh market applications. The enhanced preservation of these attributes where also linked to the higher antioxidant activity (Noreen et al., 2017; Turkiewicz et al., 2019) and phenolic content in tertiary and secondary heads, which likely mitigate oxidative stress and maintain better structural integrity, further supporting their suitability for prolonged storage (Aryal et al., 2019; Côté et al., 2010). In general terms, phenolic

content, composed of hydroxycinnamic acids and luteolin derivates, increased across the cold storage period. This raise may be due to the activation of phenylpropanoid biosynthesis as an abiotic stress response to cold conditions (Handa et al., 2019; Saltveit, 2009; Smirnov, 2015). The extent of this increase was greater in tertiary and secondary heads, reinforcing their enhanced storage adaptability. The findings of this study provide valuable insights into the phenolic dynamics of 'Blanca de Tudela' artichokes and their response to flower head order, harvest timing, and post-harvest storage. The characterization of these factors significantly improves the understanding of the quality and bioactive properties of entire artichokes, leading to practical implications such as allowing the best ones to be selected when longer storage periods are needed for export market.

Building upon the findings of the previous study, the subsequent research (**Publication 5**) (Giménez-Berenguer et al., 2023) sought to delve deeper into the functional implications of these bioactive compounds. Recognizing the pivotal role of phenolic content not only in determining post-harvest quality but also in providing resilience against environmental stressors (Kumar et al., 2023), this new investigation shifted focus to explore how the total phenolic content and, consequently, the antioxidant potential, of 'Blanca de Tudela' artichokes influences their capacity to tolerate frost injury when exposed to low temperatures. This approach aims to address critical challenges in regions where climatic variability and frost events significantly impact crop viability and marketability. The research revealed a marked relationship between flower head order and susceptibility to frost injury. Tertiary heads, which are the smallest, showed the highest resistance to frost, with most artichokes in this category exhibiting minor or no injury. Conversely, main heads, characterized by their larger size, were the most vulnerable, with the majority classified as having severe frost injury. This susceptibility could be linked to their lower TPC and TAA levels, as these compounds play a protective role against oxidative stress induced by frost conditions (Aryal et al., 2019; Babbar et al., 2015; Mougiou et al., 2020). This aligns with studies in other crops where antioxidant capacity correlates with frost tolerance, such as avocados (Weil et al., 2019) and olives (Hashempour et al., 2014). However, this is the first time that this relationship has been reported in artichokes. In this sense, this study offered a pioneering comprehensive evaluation of phenolic compounds and their impact on frost injury

in artichokes. Seven hydroxycinnamic acids, with 5-O-caffeoylquinic acid as the predominant compound, and three luteolin derivatives were identified. Across all compounds, concentrations were significantly higher in non-frosted artichokes compared to frosted ones. For instance, non-frosted samples exhibited nearly double the hydroxycinnamic acid content of frosted samples. The TAA mirrored the trends observed for TPC, with non-frosted artichokes demonstrating significantly higher antioxidant activity than frosted artichokes. The higher concentrations observed in tertiary heads likely explain their greater resistance to frost injury. This supports the hypothesis that a robust antioxidant system, mitigates frost-induced oxidative damage. The relationship between TAA, TPC, and frost tolerance has been extensively documented in other crops (Hashempour et al., 2014; Neugart et al., 2012; Oh et al., 2009; Weil et al., 2019) and is reaffirmed in this study for the first time in artichokes. This research provides a novel understanding of the biochemical and physiological factors underpinning frost tolerance in artichokes. By linking phenolic content and antioxidant activity to frost resistant, it offers valuable insights for developing strategies to mitigate frost-related losses in artichoke production. These findings not only advance scientific knowledge but also have practical implications for improving the sustainability and profitability of artichoke cultivation.

Having thoroughly examined the challenges and potential solutions related to artichokes, the focus of the research shifted to another key crop within the *Cynara cardunculus* species: cardoon. This vegetable offers untapped potential, particularly in their seeds, which hold promise for diverse applications in food (Fernández et al., 2006; Khaldi et al., 2021; Petropoulos et al., 2018a), feed (Barbosa et al., 2020; Cabiddu et al., 2019; Cajarville et al., 2000; Gominho et al., 2018; Zumbo et al., 2022), and bioindustry (Barbanera et al., 2021; Barracosa et al., 2019; Bartocci et al., 2016; De Domenico et al., 2016; Mirpoor et al., 2021; Silva et al., 2022; Zayed et al., 2020). However, the lack of information around this vegetable emphasizes the need for further investigation into genetic and environmental interactions. In this sense, the subsequent investigation (**Publication 6**) delved into the characterization of cardoon seeds, analysing thirteen genotypes (including commercial varieties, genotypes developed at the University of Catania, and wild species), across three consecutive growing seasons. This study sought to evaluate the nutritional stability

and mineral profiles of these seeds, providing valuable insights into their consistency and suitability for various innovative uses.

This study confirms that both genotype and growing season significantly influence the ash content and mineral composition of cardoon seeds with notable interaction effects between these factors. Aligning with previous research, the concentration and bioavailability of chemical compounds are influenced by a complex interplay of environmental conditions (including temperature, precipitation, and soil characteristics associated with different growing seasons), along with genetic background and developmental stage (Borah et al., 2009; Kopsell et al., 2004; Mandim et al., 2022a; Pandino et al., 2011a; Petropoulos et al., 2019; Raigón et al., 2008). These results highlight the need to account for both genetic and environmental variables when optimizing cardoon seed production to enhance its nutritional profile. Commercial CC exhibited higher ash content than wild genotypes, contrary to the results reported by Petropoulos et al. (2019). Catania university-developed genotypes showed the highest concentrations of most macro- and micro-minerals, particularly N, K, Zn, Fe, Mn, and Cu, and the same trend in the order of quantity of mineral content was also observed in other studies (Petropoulos et al., 2019, 2018c). Wild cardoon genotypes accumulated greater Ca levels but had generally lower mineral concentrations, in contrast with previous studies where wild genotypes exhibited higher ash content than commercial ones (Petropoulos et al., 2019).

Growing season also played a crucial role, with substantial fluctuations in ash and mineral content across the three seasons. Notably, ash and K levels were lowest in the second season, likely due to higher rainfall, which may have diluted soil mineral concentrations and affected nutrient uptake by plants (Xu et al., 2021). Quite the opposite for the levels of Ca, Fe and Zn in S2, since the rains could have favoured the accumulation of these elements in the seeds (Choukri et al., 2020; Sorooshzadeh et al., 1999). These variations across seasons suggest an influence of environmental factors on nutrient accumulation, consistent with observations in related species such as globe artichoke (Elia and Conversa, 2007; Lombardo et al., 2017; Pandino et al., 2011a).

The interaction between genotype and growing season also contributed to mineral variability, although to a lesser extent than individual factors. This was particularly evident in Na, N, and Mn content, which varied across seasons and genotypes. In this study, Fe and Mn levels were considerably lower than those reported by Petropoulos et al. (2019, 2018c) but more aligned with the findings of Nemska et al. (2019), highlighting the variability in mineral composition across different studies.

Overall, university-developed genotypes demonstrated enhanced mineral content, making them promising candidates for agricultural and industrial applications. Their higher and more stable concentrations of essential minerals suggest their potential as a valuable genetic resource for future breeding programs aimed at optimizing the macro- and micro-mineral content of cultivated cardoon. These findings emphasize the importance of selecting specific genotypes and optimizing environmental conditions to improve the nutritional and commercial value of cardoon seeds.

CONCLUSIONS

CONCLUSIONES

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CONCLUSIONS

This doctoral thesis comprehensively provided critical insights into the physiological, biochemical, and agronomic factors influencing the commercial and functional quality of artichoke and cardoon cultivation, two under-researched crops despite their high economic and agricultural importance. Through six interconnected studies, significant advancements were made toward understanding the genetic, nutritional, and functional properties of these crops, significantly enhance their competitiveness in the agricultural and industrial sectors. The findings contribute to optimizing production strategies, improving post-harvest management, and expanding their market potential.

I. Innovations in Fresh-Cut Artichoke Preservation.

The application of L-cysteine combined with essential oils (eugenol, thymol, and carvacrol) at a concentration of 150 µL/L effectively reduced enzymatic browning, maintained bioactive properties, and preserved the quality of fresh-cut 'Blanca de Tudela' artichokes during refrigerated storage.

II. Cultivar Comparison for Fresh-Cut Processing.

It was highlighted the significant role of flower head order in determining phenolic content, and quality attributes of 'Lorca', 'Tupac' and 'Green Queen' artichokes. Main heads consistently exhibit lower phenolic content followed by secondary and tertiary heads. Among the evaluated cultivars, 'Lorca' and 'Tupac', especially main artichokes, exhibited superior aptitude for fresh-cut processing due to their lower browning susceptibility compared to 'Green Queen' and tertiary and secondary heads.

III. Role of Developmental Stage in Fresh-Cut Processing.

'Lorca' cultivar was selected for its aptitude to development of fresh-cut products. Results on flower hear order were reaffirmed. Main heads, with lower phenolic content, showed reduced browning susceptibility, confirming their suitability for minimally processed formats. Additionally, artichokes at

advanced developmental stages, despite having lower phenolic content than those at initial stages, also performed well for fresh-cut applications due to their reduced enzymatic browning.

IV. Influence of Flower Head Order and Harvest Time on Artichoke Quality.

It was underscored the crucial influence of flower head order in phenolic content, antioxidant activity, and quality attributes of 'Blanca de Tudela' artichokes. It was confirmed also for this cultivar that tertiary heads consistently exhibit higher phenolic content followed by secondary and main heads. While no seasonal differences were found in phenolic content, tertiary heads maintained better the quality parameters during refrigerated storage period.

V. Frost Injury Tolerance in Artichokes.

It was demonstrated that frost injury susceptibility in 'Blanca de Tudela' artichokes was closely linked to flower head order, phenolic content and antioxidant activity. Tertiary heads showed the highest phenolic content and the highest resistance, highlighting the protective role of these compounds.

VI. Mineral and Nutritional Characterization of Cardoon Seeds.

The inclusion of cardoon seeds in this thesis represents another significant contribution by analysing 13 genotypes over three growing seasons. It was highlighted significant genotypic and seasonal variations in the mineral profile and ash content of cardoon seeds, offering valuable insights into their nutritional consistency and potential applications in food, feed, and bioindustrial sectors.

Overall, the findings of this doctoral thesis contribute to a deeper understanding of the interplay between genetic, agronomic, and environmental factors in determining the commercial and nutritional quality of artichokes and cardoons. With a cross-disciplinary approach combining physiological, biochemical, and agronomic insights, this research not only advances scientific knowledge but

also offers practical implications for breeders, growers, and processors to optimize the production and commercialization of these crops.

Furthermore, these investigations have demonstrated a significant misalignment in artichoke consumption practices. While larger artichokes, or main heads, are predominantly destined for fresh consumption and, tertiary heads are directed toward industrial processing, the findings suggest that this should be reversed. Main heads, with their lower browning susceptibility, would be more suitable for industrial applications, facilitating their processing, while tertiary heads, which are richer in bioactive compounds, should be prioritized for fresh consumption due to their potential health benefits.

These insights lay the groundwork for a transformation in the artichoke industry, fostering a more rational and scientifically driven approach to both consumption and processing strategies.

Figure 22: Artichoke and cardoon heads.

CONCLUSIONES

Esta tesis doctoral ha proporcionado un análisis exhaustivo de los factores fisiológicos, bioquímicos y agronómicos que influyen en la calidad comercial y funcional del cultivo de alcachofa y cardo, dos especies poco estudiadas a pesar de su gran importancia económica y agrícola. A través de seis estudios interrelacionados, se han logrado avances significativos en la comprensión de sus propiedades genéticas, nutricionales y funcionales, lo que permite mejorar su competitividad en los sectores agrícola e industrial. Los hallazgos de esta investigación contribuyen a la optimización de estrategias de producción, la mejora del manejo postcosecha y la ampliación de su potencial de mercado.

I. Innovaciones en la Conservación de Alcachofa Mínimamente Procesada

La aplicación de L-cisteína combinada con aceites esenciales (eugenol, timol y carvacrol) a una concentración de 150 µL/L demostró ser eficaz para reducir el pardeamiento enzimático, mantener las propiedades bioactivas y preservar la calidad de las alcachofas frescas cortadas de la variedad 'Blanca de Tudela' durante el almacenamiento refrigerado.

II. Comparación de Variedades para el Procesado Mínimo de Alcachofa

Se destacó el papel fundamental del orden de floración en la determinación del contenido fenólico y de los atributos de calidad de las alcachofas 'Lorca', 'Tupac' y 'Green Queen'. Las alcachofas principales presentaron un contenido fenólico significativamente menor, seguidas de las alcachofas secundarias y terciarias. Entre las variedades evaluadas, 'Lorca' y 'Tupac', especialmente en las alcachofas principales, mostraron una mayor aptitud para el procesado mínimo debido a su menor susceptibilidad al pardeamiento en comparación con la variedad 'Green Queen' y las alcachofas secundarias y terciarias.

III. Rol del Estado de Desarrollo en la Aptitud para el Procesado Mínimo de Alcachofa

La variedad 'Lorca' fue seleccionada por su aptitud para el desarrollo de productos frescos cortados. Se reafirmaron los resultados sobre el orden de

la floración, donde las alcachofas principales, con menor contenido fenólico, mostraron menor susceptibilidad al pardeamiento, confirmando su idoneidad para elaborar productos mínimamente procesados. Además, las alcachofas en estados avanzados de desarrollo, a pesar de tener menor contenido fenólico que aquellas en estados iniciales, también se comportaron favorablemente como productos frescos cortados debido a su menor pardeamiento enzimático.

IV. Influencia del Orden Floral y la Época de Cosecha en la Calidad de las Alcachofas

Se subrayó la influencia crucial del orden floral en el contenido fenólico, la actividad antioxidante y los atributos de calidad de las alcachofas 'Blanca de Tudela'. También se confirmó que para esta variedad las alcachofas terciarias presentan un mayor contenido fenólico, seguidas de las secundarias y las principales. Aunque no se observaron diferencias estacionales significativas en el contenido fenólico, las alcachofas terciarias conservaron mejor los parámetros de calidad durante el almacenamiento refrigerado.

V. Tolerancia al Daño Por Heladas en Alcachofas

Se demostró que la susceptibilidad al daño por heladas en la variedad 'Blanca de Tudela' está estrechamente relacionada con el orden floral, el contenido fenólico y la actividad antioxidante. Las alcachofas terciarias presentaron el mayor contenido fenólico y la mayor resistencia, lo que resalta el papel protector de estos compuestos.

VI. Caracterización Mineral y Nutricional de Semillas de Cardo

La inclusión del estudio de semillas de cardo en esta tesis representa otra contribución relevante, analizando 13 genotipos durante tres temporadas de cultivo. Se destacaron variaciones significativas en el perfil mineral y el contenido de cenizas, influenciadas tanto por factores genéticos como ambientales, lo que proporciona información valiosa sobre la estabilidad

nutricional de estas semillas y su potencial para aplicaciones en la industria alimentaria, forrajera y bioindustrial.

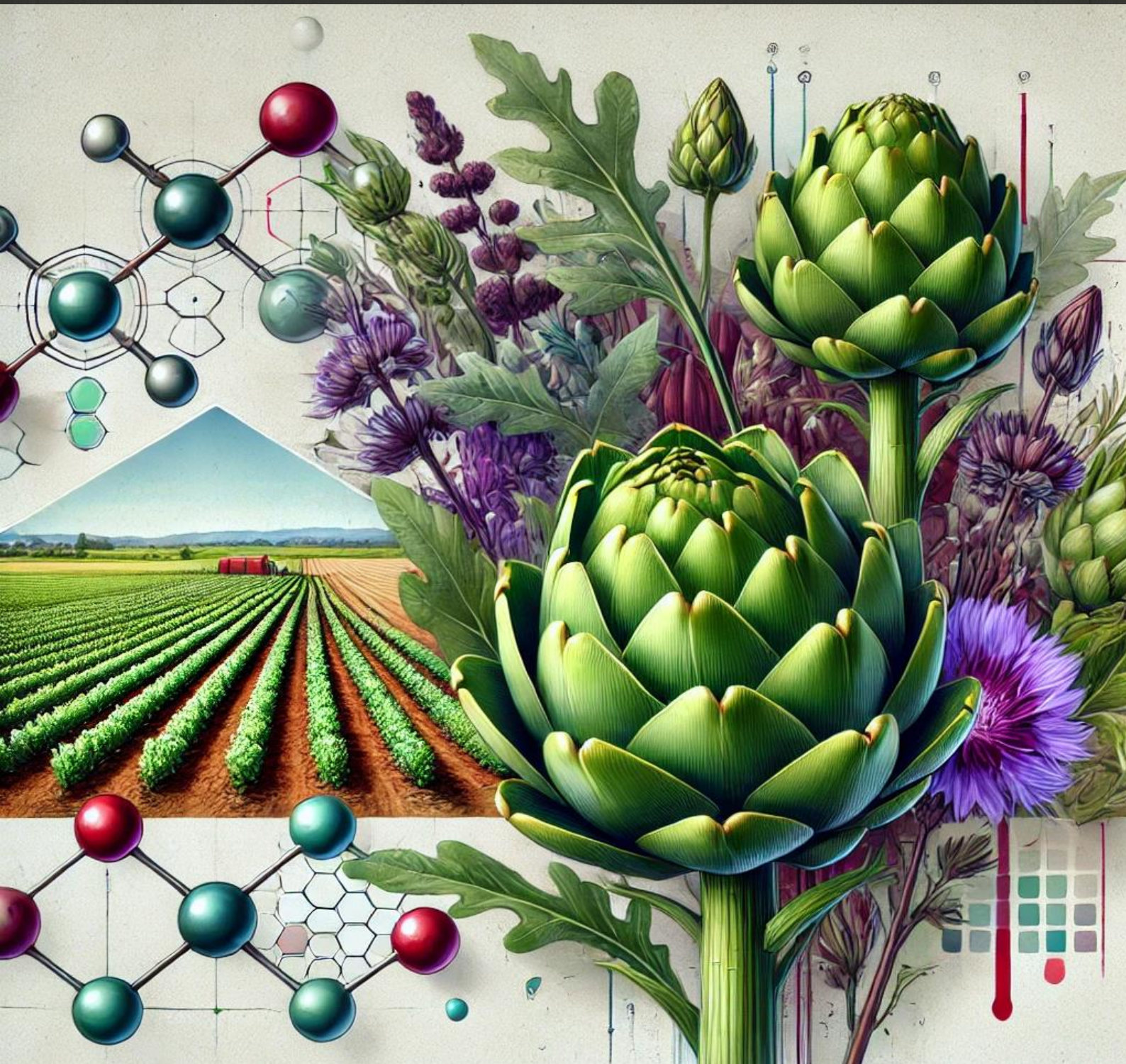
Los hallazgos de esta tesis doctoral contribuyen a una comprensión más profunda de la interacción entre factores genéticos, agronómicos y ambientales en la determinación de la calidad comercial y nutricional de la alcachofa y el cardo. Con un enfoque interdisciplinario que combina componentes fisiológicos, bioquímicos y agronómicos, esta investigación no solo amplía el conocimiento científico, sino que también ofrece aplicaciones prácticas para la mejora de la producción y comercialización de estos cultivos.

Además, esta investigación ha puesto de manifiesto una importante discordancia en las prácticas de consumo de la alcachofa. Mientras que las alcachofas principales, de mayor tamaño, suelen destinarse al consumo en fresco y las terciarias a la industria, los resultados sugieren que esta distribución debería invertirse. Las alcachofas principales, con menor susceptibilidad al pardeamiento, serían más adecuadas para el procesado industrial, mientras que las alcachofas terciarias, más ricas en compuestos bioactivos, deberían priorizarse para el consumo en fresco debido a sus potenciales beneficios para la salud.

Estos hallazgos sientan las bases para una transformación en la industria de la alcachofa, promoviendo un enfoque más racional y basado en la evidencia científica para optimizar tanto su consumo como su procesado.

Figura 23: Alcachofa y cardo.

FUTURE RESEARCH DIRECTIONS



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FUTURE RESEARCH DIRECTIONS

Building upon the findings of this thesis, several promising avenues for future research have been identified to advance the understanding and commercial optimization of artichokes and cardoons. These proposed studies aim to address remaining knowledge gaps and explore new applications:

1. **Development of Consumer-Oriented Processed Products.**

   Once the most suitable cultivars for industrial processing have been identified ('Lorca and Tupac'), future work could focus on the development of new value-added products tailored to current consumer preferences. This may include the application of pre-cooking methods, thermal pretreatments, marination, or other preservation strategies to create products that go beyond the scope of minimally processed (fourth-range) vegetables. The goal would be to generate *ready-to-cook* or *convenience* products with enhanced functionality and market appeal.

2. **Postharvest Strategies for Shelf-Life Extension.**

   Having defined the best suited cultivars for fresh consumption and storage ('Blanca de Tudela' and 'Green Queen'), subsequent research could explore methods to further extend their life. This could involve postharvest treatments such as MAP or CA, which have the potential to delay senescence, preserve quality attributes, and prolong the marketability of fresh products.

3. **Extending Characterization of Artichokes and Cardoons.**

   Future studies should continue with the characterization of artichokes and cardoons. These may include sensory evaluations and aroma profiling of artichokes to understand consumer preferences and characterization of the bioactive compounds present in cardoons to optimize their nutritional, functional, and industrial potential.

4. Sustainability and Waste Valorisation

Future research should also focus on the valorisation of by-products from artichoke and cardoon processing, contributing to a circular economy model. Studies could explore the use of leaves, stems, and other agro-industrial waste in the production of food ingredients, animal feed and biodegradable materials, as well as the production and application of bioactive extracts (source of phenolic compounds) for the pre- and post-harvest elicitation of fruits and vegetables.

These research directions aim to further the understanding and application of artichokes and cardoons, aligning with the evolving demands of consumers, industries, and agricultural practices while supporting sustainability and innovation.

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PhD THESIS

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2025