



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



"El saber de mis hijos
hará mi grandeza"

UNIVERSIDAD DE SONORA
DEPARTAMENTO DE INVESTIGACIÓN Y POSGRADO EN ALIMENTOS
Programa de Posgrado en Ciencias y Tecnología de los Alimentos

**DESARROLLO DE ESTRATEGIAS DE RIEGO
DEFICITARIO CONTROLADO Y SELECCIÓN DEL
PORTAINJERTO PARA MEJORAR LA CALIDAD Y
FUNCIONALIDAD DE LOS PISTACHOS (*Pistacia vera*)**

TESIS DOCTORAL

LUIS NOGUERA ARTIAGA

2020



UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE
ESCUELA POLITÉCNICA SUPERIOR DE ORIHUELA

**PROGRAMA DE DOCTORADO EN RECURSOS Y TECNOLOGÍAS
AGRARIAS, AGROAMBIENTALES Y ALIMENTARIAS**

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Esta Tesis Doctoral, titulada "desarrollo de estrategias de riego deficitario controlado y selección del portainjerto para mejorar la calidad y funcionalidad de los pistachos (*Pistacia vera*)", se presenta bajo la modalidad de **tesis por compendio de las siguientes publicaciones:**

Noguera-Artiaga, L., Lipan, L., Vázquez-Araújo, L., Barber, X., Pérez-López, D., y Carbonell-Barrachina, Á. A. (2016). Opinion of Spanish consumers on hydrosustainable pistachios. *Journal of Food Science*, 81(10), S2559-S2565. doi: 10.1111/1750-3841.13501

Noguera-Artiaga, L., Pérez-López, D., Burgos-Hernández, A., Wojdyło, A., y Carbonell-Barrachina, Á. A. (2018). Phenolic and triterpenoid composition and inhibition of α -amylase of pistachio kernels (*Pistacia vera* L.) as affected by rootstock and irrigation treatment. *Food Chemistry*, 261, 240-245. doi: 10.1016/j.foodchem.2018.04.033

Noguera-Artiaga, L., Salvador, M. D., Fregapane, G., Collado-González, J., Wojdyło, A., López-Lluch, D., y Carbonell-Barrachina, Á. A. (2019). Functional and sensory properties of pistachio nuts as affected by cultivar. *Journal of the Science of Food and Agriculture*, 99(15), 6696-6705. doi: 10.1002/jsfa.9951

Noguera-Artiaga, L., García-Romo, J. S., Rosas-Burgos, E. C., Cinco-Moroyoqui, F. J., Vidal-Quintanar, R. L., Carbonell-Barrachina, Á. A., y Burgos-Hernández, A. (2019). Antioxidant, antimutagenic and cytoprotective properties of hydroSOS pistachio nuts. *Molecules*, 24(23), 4362. doi: 10.3390/molecules24234362

Noguera-Artiaga, L., Sánchez-Bravo, P., Hernández, F., Burgos-Hernández, A., Pérez-López, D., y Carbonell-Barrachina, Á. A. (2020). Influence of regulated deficit irrigation and rootstock on the functional, nutritional and sensory quality of pistachio nuts. *Scientia Horticulturae*, 261, 108994. doi: 10.1016/j.scienta.2019.108994

Noguera-Artiaga, L., Sánchez-Bravo, P., Pérez-López, D., Szumny, A., Calín-Sánchez, Á., Burgos-Hernández, A., y Carbonell-Barrachina, Á. A. (2020). Volatile, sensory and functional properties of hydroSOS pistachios. *Foods*, 9, 158. doi: 10.3390/foods9020158

En Orihuela, a 29 de enero de 2020.

Esta memoria ha sido presentada por D. Luis Noguera Artiaga, Graduado en Ciencia y Tecnología de los Alimentos y Máster en Técnicas Avanzadas para la Investigación y Producción en Fruticultura, para la obtención del título de doctor.

D. Luis Noguera Artiaga

Esta Tesis Doctoral ha sido dirigida por el Dr. Ángel Antonio Carbonell Barrachina, Catedrático de Universidad del Departamento Tecnología Agroalimentaria de la Universidad Miguel Hernández de Elche, y codirigida por el Dr. Armando Burgos Hernández, Profesor-Investigador de Tiempo Completo del Departamento de Investigación y Posgrado en Alimentos de la Universidad de Sonora (México), los cuales autorizan su presentación bajo la modalidad de tesis por compendio de publicaciones.

Dr. Ángel A. Carbonell Barrachina

Dr. Armando Burgos Hernández



Dr. D. Ángel Antonio Carbonell Barrachina, Catedrático de Universidad y Coordinador 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 “**Desarrollo de estrategias de riego deficitario controlado y selección del portainjerto para mejorar la calidad y funcionalidad de los pistachos (*Pistacia vera*)**” de la que es autor el Graduado en Ciencia y Tecnología de los Alimentos **D. Luis Noguera Artiaga** ha sido realizada bajo la dirección del **Dr. Ángel Antonio Carbonell Barrachina** (UMH) y la codirección del **Dr. Armando Burgos Hernández** (Universidad de Sonora, México), actuando como tutora de la misma la Dra. Francisca Hernández García (UMH). Considero que la tesis es conforme en cuanto a forma y contenido a los requerimientos del Programa de Doctorado ReTos-AAA por tanto, es apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a diez de febrero de dos mil veinte.

Prof. Dr. Ángel A. Carbonell Barrachina

ÍNDICE DE CALIDAD DE LAS PUBLICACIONES

Opinion of Spanish consumers on hydrosustainable pistachios

Autores

Luis Noguera-Artiaga, Leontina Lipan, Laura Vázquez-Araújo, Xabier Barber, David Pérez-López y Ángel A. Carbonell-Barrachina

Revista

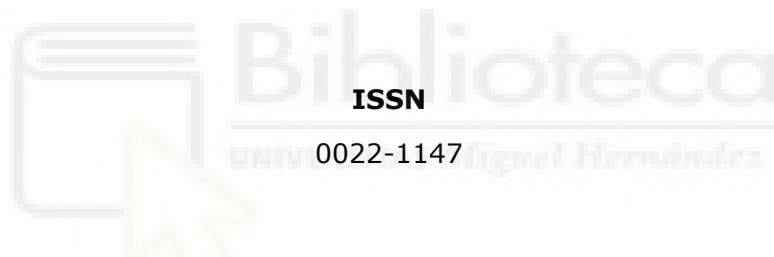
Journal of Food Science

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Ámbito de la publicación (categoría JCR)

Food Science and Technology

Cuartil

Q2

Rango

52/130

Factor de impacto

1,815 (Año 2016)

Factor de impacto (5 años)

2,192

Phenolic and triterpenoid composition and inhibition of α -amylase of pistachio kernels (*Pistacia vera* L.) as affected by rootstock and irrigation treatment

Autores

Luis Noguera-Artiaga, David Pérez-López, Armando Burgos-Hernández, Aneta Wojdyło y Ángel A. Carbonell-Barrachina

Revista

Food Chemistry

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Dr. Paul Finglas

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Food Science and Technology

Cuartil

Q1

Rango

7/135

Factor de impacto

5,399 (Año 2018)

Factor de impacto (5 años)

5,488

Functional and sensory properties of pistachio nuts as affected by cultivar

Autores

Luis Noguera-Artiaga, María Desamparados Salvador, Giuseppe Fregapane, Jacinta Collado-González, David López-Lluch y Ángel A. Carbonell-Barrachina

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Dr. Mark Shepherd y Prof. Andrew Waterhouse

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Agriculture, Multidisciplinary

Cuartil

Q1

Rango

9/57

Factor de impacto

2,422 (Año 2018)

Factor de impacto (5 años)

2,733

Antioxidant, antimutagenic and cytoprotective properties of hydroSOS pistachio nuts

Autores

Luis Noguera-Artiaga, Joel S. García-Romo, Ema C. Rosas-Burgos, Francisco J. Cinco-Moroyoqui, Reyna L. Vidal-Quintanar, Ángel A. Carbonell-Barrachina y Armando Burgos-Hernández

Revista

Molecules

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Editores

Dr. Derek J. McPhee

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Ámbito de la publicación (categoría JCR)

Chemistry, Multidisciplinary

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Q2

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67/172

Factor de impacto

3,060 (Año 2018)

Factor de impacto (5 años)

3,380

Influence of regulated deficit irrigation and rootstock on the functional, nutritional and sensory quality of pistachio nuts

Autores

Luis Noguera-Artiaga, Paola Sánchez-Bravo, Francisca Hernández, Armando Burgos-Hernández, David Pérez-López y Ángel A. Carbonell-Barrachina

Revista

Scientia Horticulturae

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10.1016/j.scienta.2019.108994

Editores

G. Colla, W.W. Guo, S. Kondo, P. Martinez-Gómez, B. Pennisi y D. Schwarz



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Ámbito de la publicación (categoría JCR)

Horticulture

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Q1

Rango

5/36

Factor de impacto

1,961 (Año 2018)

Factor de impacto (5 años)

2,315

Volatile, sensory and functional properties of hydroSOS pistachios

Autores

Luis Noguera-Artiaga, Paola Sánchez-Bravo, David Pérez-López, Antoni Szumny, Ángel Calín-Sánchez, Armando Burgos-Hernández y Ángel A. Carbonell-Barrachina

Revista

Foods

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Food Science & Technology

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Al *ola k ase*, porque soy lo que soy, en buena parte, gracias a vosotros. Porque el mundo está lleno de cabrones y tener a unos cerca siempre ayuda.

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A mis padres y a mi hermano Eduardo

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Yugos os quieren poner
gentes de la hierba mala,
yugos que habéis de dejar
rotos sobre sus espaldas.



Biblioteca
UNIVERSITAS Miguel Hernández

Si me muero, que me muera
con la cabeza muy alta.

Muerto y veinte veces muerto,
la boca contra la grama,
tendré apretados los dientes
y decidida la barba.

Cantando espero a la muerte,
que hay ruiseñores que cantan
encima de los fusiles
y en medio de las batallas.

Miguel Hernández

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ESTRUCTURA DE LA TESIS DOCTORAL

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

- **Resumen:** descripción de los resultados y conclusiones más relevantes (en castellano e inglés).
- **Introducción:** descripción de las características generales del cultivo del pistacho y de sus propiedades agronómicas y funcionales.
- **Objetivos:** objetivo global y objetivos parciales de la investigación.
- **Resumen de la metodología:** breve descripción de la metodología empleada para la consecución de los objetivos.
- **Publicaciones científicas:** Transcripción literal de las publicaciones científicas por las que se compone esta tesis, estructurada en los siguientes cinco capítulos:

I. Atributos de calidad de diferentes cultivares de pistacho

Este capítulo consiste en un artículo científico titulado "*Functional and sensory properties of pistachio nuts as affected by cultivar*", publicado en la revista *Journal of the Science of Food and Agriculture*. En él se refleja la información obtenida tras estudiar las propiedades físico-químicas, funcionales y sensoriales de ocho cultivares de pistacho.

II. Concepto de hidrosostenibilidad

Este capítulo está compuesto por un artículo científico titulado "*Opinion of Spanish consumers on hydrosustainable pistachios*", publicado en la revista *Journal of Food Science*. El artículo contiene información sobre el desarrollo del concepto hidroSOStenible o hidroSOS obtenida mediante el empleo de grupos focales, con el fin de obtener información que permita a los productores obtener un producto con una calidad diferenciada.

III. Calidad física, química y sensorial de pistachos hidroSOS

Este capítulo está formado por dos artículos científicos. El primero de ellos se titula "*Influence of regulated deficit irrigation and rootstock on the functional, nutritional and sensory quality of pistachio nuts*" y está publicado en la revista *Scientia Horticulturae*, mientras que el segundo, está titulado "*Influence of rootstock and*

regulated deficit irrigation on pistachio quality" y está publicado en la revista *Foods*. En ambos se estudia el efecto que el empleo de diferentes tratamientos de riego deficitario controlado y portainjertos tienen sobre la calidad físico-química, funcional y sensorial de los pistachos. Esta última, se estudió mediante el empleo de análisis sensorial descriptivo con panel entrenado y un estudio afectivo desarrollado en España, México y Polonia.

IV. Estudio de la calidad funcional de pistachos hidroSOS

Este capítulo consiste en un artículo científico titulado "*Phenolic and triterpenoid composition and inhibition of α -amylase of pistachio kernels (*Pistacia vera* L.) as affected by rootstock and irrigation treatment*", publicado en la revista *Food Chemistry*. En él se estudió la influencia que el riego deficitario controlado y el empleo de diferentes portainjertos tuvo sobre el contenido polifenólico, contenido de triterpenoides, capacidad antioxidante y capacidad de inhibición de alfa-amilasa de los pistachos obtenidos.

V. Propiedades antimutagénicas y citoprotectivas

Este capítulo, contiene información sobre la incidencia que el riego deficitario controlado y el empleo de diferentes portainjertos tienen sobre las propiedades antimutagénicas y citoprotectivas de los frutos de pistacho. Está formado por una publicación titulada "*Antioxidant, antimutagenic and cytoprotective properties of hydroSOS pistachio nuts*" y está publicado en la revista *Molecules*.

- **Resumen de los resultados, discusión y conclusiones:** en el que se hace un breve resumen de los resultados obtenidos en cada uno de los capítulos.
- **Conclusiones generales:** conclusiones de la Tesis Doctoral.
- **Investigaciones futuras:** breve descripción de futuras investigaciones que pueden desarrollarse a partir de los resultados obtenidos.
- **Referencias bibliográficas:** en las que se indican las referencias empleadas en las secciones complementarias a las publicaciones.

RESUMEN

ABSTRACT

RESUMEN

La necesidad de optimizar el uso del agua en los agrosistemas es un hecho asumido por agricultores y organismos reguladores. Esta situación irreversible nos obliga a convivir con la escasez de recursos hídricos y a desarrollar herramientas capaces de asegurar la competitividad de la fruticultura. Sin embargo, la mayoría de las estrategias de riego deficitario no están basadas en niveles de déficit hídrico concretos en las plantas, ni está claro que conlleven una mejora de las características funcionales y sensoriales de los frutos. En este sentido, la bibliografía existente es escasa y contradictoria, debido fundamentalmente a que: (i) en ciertas publicaciones falta precisión a la hora de definir el estado hídrico del cultivo estudiado (duración del estrés, velocidad de imposición de este, etc.) y (ii) se presupone la existencia de una relación lineal entre déficit hídrico y acumulación de compuestos bioactivos.

La hipótesis principal de esta Tesis Doctoral radica en que, no hay relación lineal entre el nivel de compuestos bioactivos de los frutos y el nivel de estrés hídrico (controlado). Mientras que bajo un estrés hídrico elevado se produce una importante regulación estomática y el CO₂ se destina al mantenimiento del metabolismo primario, bajo un déficit moderado/suave se redistribuye el CO₂ hacia la formación de metabolitos secundarios, en detrimento del crecimiento. Esto da lugar a frutos con mayor contenido en compuestos bioactivos cuando el déficit hídrico ha sido controlado. Además, las situaciones de estrés hídrico provocan una acumulación de sustancias antioxidantes, como respuesta fisiológica a la eliminación de radicales libres formados.

Por ello, pensamos que mediante el manejo preciso de niveles de déficit hídrico "suave" es factible conseguir mínimas mermas en la producción y generar a su vez productos de máxima calidad. Estos productos, por ser el resultado de una optimización del uso del agua y tener una calidad elevada, tendrán una identidad marcada que los diferenciará del resto de su categoría, facilitando su inclusión en mercados internacionales más exigentes.

Bajo estas premisas, se desarrolló el concepto "hidroSOStenible" y se aplicó al cultivo del pistacho, demostrando que los consumidores valoran positivamente los productos saludables, los que respetan el medio ambiente y los que tienen buenas propiedades funcionales, estando dispuestos a pagar hasta un 10 % más por este tipo de productos.

Una vez desarrollado el concepto y puesto en práctica se realizó un estudio de la calidad de pistachos procedentes de ocho cultivares, para realizar una caracterización completa de los frutos obtenidos. Una vez elegido aquél que, bajo los

criterios estudiados, consideramos como el de mejor calidad, cultivar Kerman, se realizó un estudio combinado de diferentes tratamientos de riego deficitario controlado y portainjertos para estudiar su influencia sobre la calidad física, química, funcional y sensorial de los pistachos obtenidos.

Se demostró que un tratamiento de riego deficitario controlado leve aplicado durante la fase fenológica II del cultivo del pistacho es capaz de provocar una mejora en la calidad física, funcional, química y sensorial de los frutos obtenidos, manteniendo inalteradas las propiedades antimutagénicas y citotóxicas que los compuestos presentes en este fruto tienen frente a células cancerígenas

El empleo del portainjerto *P. terebinthus* durante el cultivo del pistacho condujo a frutos de mayor calidad (morfológica, textura, ácidos grasos, capacidad antioxidante y contenido de polifenoles) que los obtenidos mediante los portainjertos *P. atlantica* y *P. integerrima*.

Este hecho supone un incuestionable avance para la fruticultura, ya que se pueden obtener productos de mayor calidad y con un claro valor añadido al percibirse como productos sostenibles y respetuosos con el medioambiente.



ABSTRACT

The need to accelerate the use of water in agrosystems is a fact assumed by farmers and regulatory councils. This irreversible situation forces us to live with the scarcity of water resources and develop reliable tools to guarantee the competitiveness of fruticulture. However, most of the deficit irrigation strategies are not based on concrete water deficit levels in the plants, nor it is clear whether it implies an improvement of the functional and sensory characteristics of the fruits. In this sense, the existing literature is scarce and contradictory, mainly due to the fact that: (i) in certain publications there is a lack of precision in defining the hydric state of the crop studied (duration of stress, speed of imposition of the same, etc.) and (ii) the existence of a linear relationship between the water deficit and the accumulation of bioactive compounds is assumed.

The main hypothesis of this Thesis is that there is no linear relationship between the level of bioactive compounds of the fruits and level of water stress (controlled); while under high water stress there is an important stomatal regulation and CO₂ is used to maintain the primary metabolism, under a moderate deficit, CO₂ is redistributed to the formation of secondary metabolites to the detriment of growth. This situation results in products with a higher content of bioactive compounds in those fruits in which the water deficit has been controlled. In addition, situations of water stress cause an accumulation of antioxidant substances, as a physiological response to the elimination of free radicals formed.

Therefore, it is believed that through the precise management of water deficit levels it is feasible to achieve minimal production losses and in turn generate top quality products. These products, as a result of an optimization of water use and having a high quality, will have a marked identity that will differentiate them from the rest of their category, facilitating their inclusion in more demanding international markets.

Under these premises, the concept of "hydroSOSustainable products" was developed and applied to pistachio farming and commercialization, demonstrating that consumers positively value healthy products, those that respect the environment and those that have good functional properties, even willing to pay up to 10 % more for this type of products.

Once the concept was developed and put into practice, a study of the quality of pistachios from eight cultivars was carried out, to perform a complete characterization of the obtained nuts. Once the one that, under the studied criteria, was considered as that having the best quality (cultivar Kerman), was chosen, a

combined study of different treatments of controlled deficit irrigation and rootstocks was carried out to study their influence on the physical, chemical, functional and sensory quality of the pistachios obtained.

It was shown that a soft regulated deficit irrigation treatment, applied during the phenological phase II of pistachio cultivation, is capable of causing an improvement in the physical, functional, chemical and sensory quality of the affected fruits, unaltered the antimutagenic and cytotoxic properties that their compounds have against cancer cells.

The use of the *P. terebinthus* rootstock during pistachio cultivation led to fruits of higher quality (morphological, texture, fatty acids, antioxidant capacity and polyphenol content) than those produced using *P. atlantica* and *P. integerrima* rootstocks.

This fact represents an unquestionable advance for fruticulture, because it allows farmers to obtain products of higher quality and with a clear added value when perceived as sustainable and environmentally friendly products.



INTRODUCCIÓN

1

Biblioteca
UNIVERSIDAD Miguel Hernández



1. INTRODUCCIÓN

El pistacho (*Pistacia vera* L.) es un árbol con gran tolerancia a la sequía, lo que lo convierte en una alternativa muy interesante para su cultivo en la zona con clima mediterráneo. En España se ha triplicado el área cultivada de este cultivo, debido principalmente al rendimiento económico del mismo. Es un fruto seco que goza de gran aceptación en prácticamente todo el mundo, llegando a tener precios muy elevados, por ejemplo, en la zona de centro y Suramérica (en las que llega a ser el fruto seco más costoso). Esta aceptación está basada principalmente en sus propiedades sensoriales, entre las que destaca una elevada crujibilidad y un sabor característico que lo diferencian del resto. Esto lo convierte también en un ingrediente muy utilizado en preparaciones culinarias, como por ejemplo en dulces, tartas, y/o helados. Es un fruto que destaca por sus propiedades funcionales, como pueden ser su perfil de ácidos grasos, composición mineral, composición polifenólica o por tener una gran capacidad antioxidante.

Esta Tesis Doctoral se enmarca dentro de la línea de investigación que, desde hace años, viene desarrollando el Grupo de Investigación Calidad y Seguridad Alimentaria (Universidad Miguel Hernández de Elche) sobre el concepto de hidrosostenibilidad.

El desarrollo de la fruticultura pasa por saber adaptarse a los nuevos escenarios que, en un futuro muy cercano, van a surgir fruto del cambio climático, el avance de la desertificación y el incremento poblacional. Todos ellos, van a provocar una reducción en la cantidad de agua dulce disponible, haciendo indispensables estudios de caracterización de cultivos con el fin de adaptar las necesidades de estos a la disponibilidad de este bien preciado recurso.

Este escenario fuerza a los investigadores a emplear estrategias que reduzcan el agua destinada a los cultivos y, concretamente, a estudiar los momentos en los cuales esta no repercute de manera significativa en la calidad de los frutos obtenidos. Una de estas estrategias, riego deficitario controlado (RDC), ha sido la que se ha puesto en práctica durante el desarrollo de esta Tesis Doctoral. Atendiendo a la bibliografía existente, se han llevado a cabo multitud de caracterizaciones de este cultivo mediante el estudio de la respuesta al estrés hídrico aplicado mediante RDC, aunque estas se han centrado en la respuesta fisiológica del cultivo y en la producción obtenida, dejando de lado la calidad de los frutos. Esta Tesis Doctoral surge para suplir el vacío de conocimiento sobre la influencia que el RDC tiene sobre la calidad física, química, funcional y sensorial de los frutos de pistacho.

La hipótesis principal de esta Tesis Doctoral radica en que, no hay relación lineal entre el nivel de compuestos bioactivos de los frutos y el nivel de estrés hídrico (controlado); mientras que bajo un estrés hídrico elevado se produce una importante regulación estomática y el CO₂ se destina al mantenimiento del metabolismo primario; bajo un déficit moderado/suave se redistribuye el CO₂ hacia la formación de metabolitos secundarios en detrimento del crecimiento. Esto da lugar a frutos con mayor contenido en compuestos bioactivos en aquellos cultivos en los que el déficit hídrico ha sido controlado. Además, las situaciones de estrés hídrico provocan una acumulación de sustancias antioxidantes, como respuesta fisiológica a la eliminación de radicales libres formados. Sin embargo, para una misma especie, el incremento de la actividad antioxidante o acumulación de sustancias del metabolismo secundario no siempre parece seguir unas pautas fáciles de describir o relacionar con el estado hídrico; en la mayor parte de los casos la falta de correlación se debe a que el estado hídrico no es completamente comparable por una inadecuada o insuficiente descripción.

Por ello, pensamos que mediante el manejo preciso de niveles de déficit hídrico "suave" es factible conseguir mínimas mermas en la producción y generar a su vez productos de máxima calidad. Estos productos, por ser el resultado de una optimización del uso del agua y tener una calidad elevada, tendrán una identidad marcada que los diferenciará del resto de su categoría, facilitando su inclusión en mercados internacionales más exigentes.

La elección del portainjerto en el cultivo del pistacho es una de las decisiones más importantes para el desarrollo de la plantación. Tradicionalmente se ha considerado el pistachero como un árbol muy resistente a la sequía y la salinidad, y la caracterización de los portainjertos se ha llevado a cabo desde el punto de vista de resistencia a enfermedades o adaptación a diferentes tipos de suelo, sin tener en cuenta la calidad de los frutos obtenidos. Durante el desarrollo de esta Tesis Doctoral se estudió la influencia que el empleo de diferentes portainjertos tiene sobre la calidad de los frutos de pistacho, con el fin ofrecer a los agricultores la información necesaria para poder tomar una elección basada en la calidad de los frutos obtenidos y/o en la demanda de los consumidores.

1.1. CARACTERÍSTICAS GENERALES DEL CULTIVO

Desde finales del paleolítico los pistachos han formado parte de la alimentación humana. Existen evidencias arqueológicas del periodo del neolítico en las que queda

de manifiesto que el pistacho, junto las aceitunas, almendras, uvas e higos eran frutos muy comunes en la dieta del ser humano (Couceiro, 2013).

El pistacho se introdujo en la Europa actual a través de Italia, bajo el mandato del emperador Tiberio (14-37 d.C.). De ahí, se difundió por el norte de África hasta Hispania. Sin embargo, fueron los árabes los que extendieron su cultivo y asentaron el mismo en la región mediterránea. Tras la reconquista, se comenzaron a eliminar los árboles machos por improductivos. Esto se tradujo en un cese de producción por parte de los que sí lo eran (hembras) lo que provocó la desaparición del cultivo en la Península Ibérica. No fue hasta finales de los años 80 cuando gracias al *Institut de Recerca en Tecnologia d'Aliments* (IRTA) se reintrodujo de nuevo y comenzaron cultivos en la zona de Lleida (Couceiro, 2013).

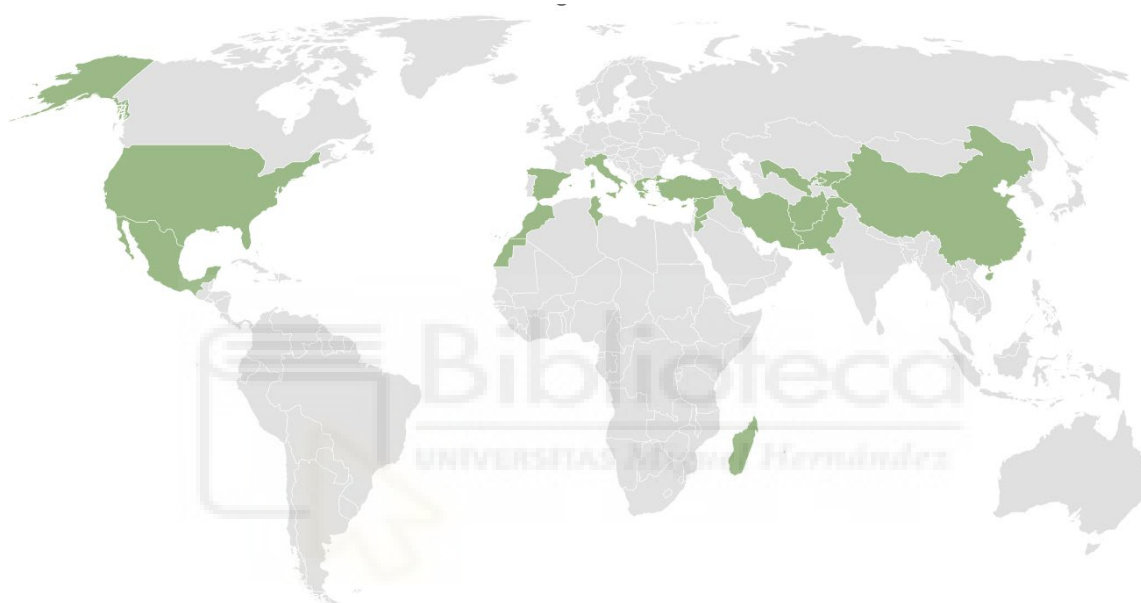
Pistacia vera L. pertenece a la familia de *Anacardiaceae*, orden *Sapindales*, clase *Magnoliopsida* y división *Magnoliophyta*. Es una planta desértica con gran tolerancia al suelo salino que produce un fruto conocido como pistacho o alfóncigo. Crece hasta unos 10 m de altura, tiene hojas pinnadas con 3 o 5 folíolos de 10-20 cm de largo que se desprenden en la estación fría, y presenta flores masculinas y femeninas en árboles diferentes. Su desarrollo es muy lento, lo que lo convierte en una especie muy longeva, es un cultivo bastante resistente a temperaturas extremas y requiere de entre siete a diez años para lograr una producción considerable (alcanzando su pico en unos 20 años). El fruto está cubierto por una piel carnosa y fina, de color verde; en su interior, bajo una cáscara dura y blanquecina, se encuentra la semilla de un color verde pálido, cubierta por una piel fina de color violáceo (en función del cultivar este color es más o menos intenso). Cuando el fruto madura, cambia la piel a color rojizo y su cáscara rompe debido a la presión ejercida en su interior por el fruto provocando un característico sonido audible (fase conocida como eclosión o dehiscencia) (Couceiro, 2013).

Para que el árbol tenga una brotación adecuada es preciso la acumulación, durante el reposo invernal, de un determinado número de horas frío (HF). Estas, en función del cultivar, varían entre las 800 y 1200 HF para los cultivares tempranos y tardíos, respectivamente (Gijón, Gimenez, Pérez-López, *et al.*, 2011). Por otra parte, el pistachero es muy sensible a las heladas durante el periodo de floración y cuajado del fruto, llegando a causar estas grandes pérdidas de producción (Guerrero, Gijón, Moriana y Couceiro, 2006). De igual forma ocurre con la humedad ambiental durante los meses de crecimiento del fruto; durante junio, julio y agosto, humedades superiores al 40 % son limitantes para mantener un correcto estado sanitario del fruto (Arpaci, Atli, Tekin y Burak, 2006).

Aunque se adapta a cualquier tipo de suelo, el pistachero funciona mejor en suelos profundos, pedregosos, moderadamente salinos y calcáreos; con textura franca o franco-arenosa, bien drenados y permeables; cumpliendo la máxima de “a mayor profundidad de suelo, mayor producción” (Couceiro, Guerrero y Gijón, 2010; Ferguson, 2005).

El cultivo del pistacho está distribuido, principalmente, en la zona mediterránea, Asia, Oriente medio y América del norte (Imagen 1).

Imagen 1. Países en los que se cultiva el pistacho de forma extendida.



1.2. PRODUCCIÓN E IMPORTANCIA ECONÓMICA

De todas las especies del género *Pistacia*, el pistachero (*Pistacia vera*) es la que un mayor interés comercial tiene por su producción de frutos comestibles (pistachos); del resto de especies se emplean sus semillas (principalmente) para la producción de jabones y aceites.

A nivel mundial, actualmente hay cultivadas 770.000 hectáreas de pistacheros que producen un total de 1.115.066 toneladas de pistachos (FAOSTAT, 2019). En los últimos 20 años el área cultivada se ha duplicado por lo que las producciones de pistachos se han triplicado (Imagen 2). Esto es debido, en gran parte, al gran interés que ha suscitado este cultivo en Estados Unidos y China, que han incrementado

enormemente sus producciones, pasando a ser el segundo y tercer país (respectivamente) en cuando a producción a nivel mundial. Un mercado dominado claramente por Irán, cuyas producciones representan más de un 50 % con respecto a la producción total (Imagen 3). España, a pesar de ser el séptimo productor mundial, representa apenas un 0,7 %.

Imagen 2. Área cultivada (ha) y producción (t) de pistachos en todo el mundo, en los últimos 20 años.

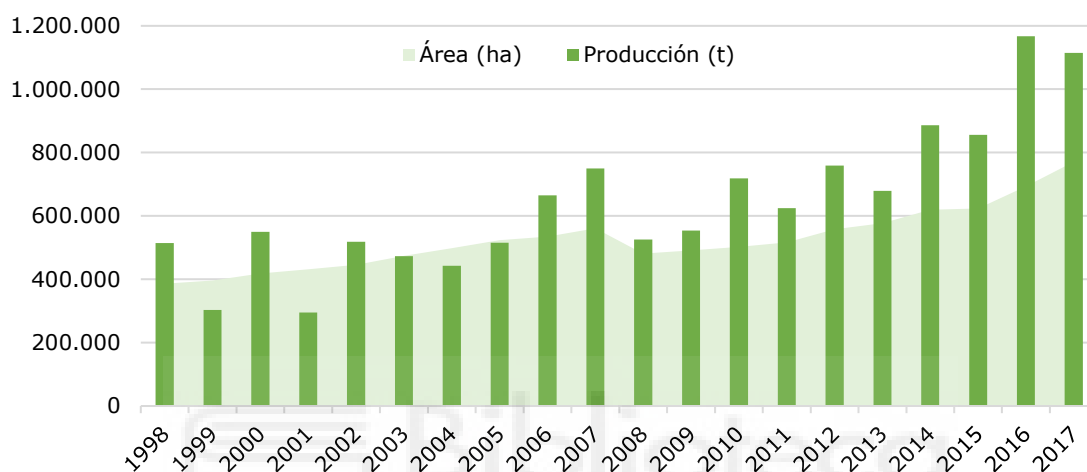
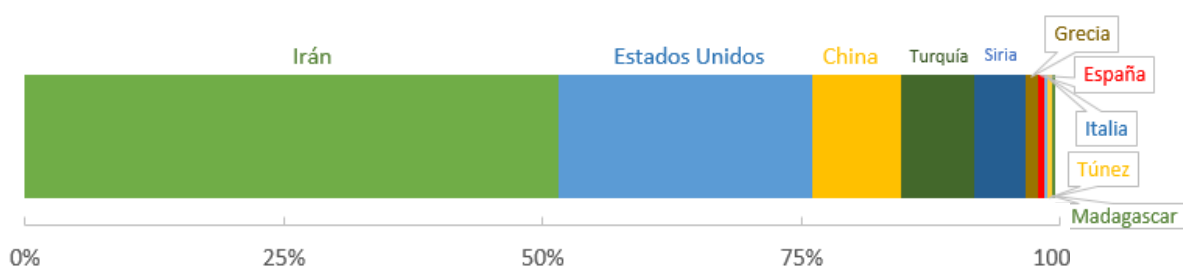
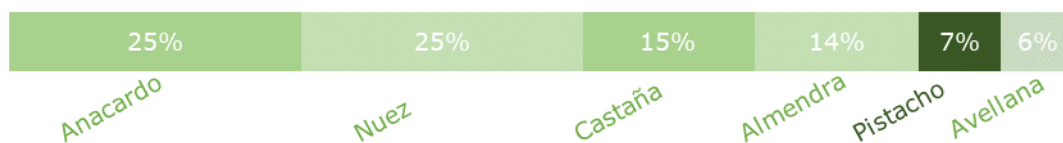


Imagen 3. Principales países productores de pistachos en el mundo (% de producción total)



A pesar de tener unas producciones muy elevadas, el pistacho es el quinto fruto seco producido a nivel mundial (Imagen 4). El anacardo y la nuez (25 % cada uno de ellos) son los frutos secos que más se producen en el mundo, seguidos de la castaña (15 %), la almendra (14 %), el pistacho (7 %) y la avellana (6 %) (FAOSTAT, 2019).

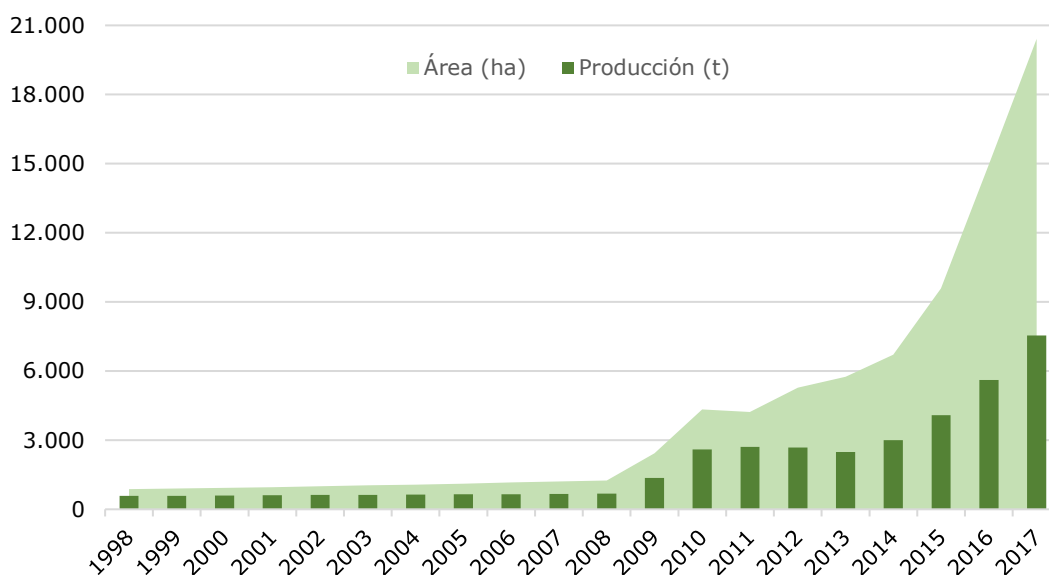
Imagen 4. Distribución de la producción de frutos secos (%) en el mundo.



En España, en los últimos 10 años, las plantaciones de pistacheros se han multiplicado por 10 (Imagen 4), lo que pone de manifiesto que este es un cultivo en auge (FAOSTAT, 2019). Actualmente hay cultivadas unas 20.000 hectáreas, siendo Castilla la Mancha la comunidad que mayor número aporta. Como ya se ha comentado, el pistachero requiere de unos 10 años para tener producciones considerables y no es hasta los 20 años cuando alcanza su pico productivo, por lo que, a pesar de este incremento tan elevado de plantaciones, la producción apenas se ha duplicado en estos años. Será en los próximos 5 años cuando estas plantaciones jóvenes incrementen de manera notable la producción de este fruto.

Con respecto al resto de frutos secos, el pistacho ocupa la quinta posición, de entre todos los frutos secos producidos en España, con un 2 %. La producción nacional está claramente dominada por la almendra, que representa un 82 % de la producción total; seguida de castañas, nueces y avellanas con un 5, 5 y 3 %, respectivamente (FAOSTAT, 2019).

Imagen 5. Área cultivada (ha) y producción (t) de pistachos en España.



1.3. PORTAINJERTO

El injerto es una técnica milenaria (existen evidencias de su uso por el pueblo chino en el año 1000 a. C.). Presumiblemente, el hombre tomó esta idea tras observar cómo surgían, de manera natural, uniones entre ramas de los árboles que quedaban expuestas al frotarse las cortezas entre sí (por la acción del viento o de alguna inclemencia externa) (Valentini, 2003). El portainjerto (patrón o pie) aporta el sistema radical y al menos una porción de tallo (lignificado o no) a la copa o parte superior de un nuevo individuo (injerto). De esta unión se obtiene una planta proveniente de dos individuos diferentes, portainjerto e injerto, que mantienen intacta su información génica (Imagen 6). Esta planta tiene características diferentes a las que presentaría cada uno de los individuos si se desarrollaran como plantas individuales. El principal uso de esta técnica es permitir la multiplicación de una variedad, además de conferir a la planta una adaptación que le permita, entre otras cosas, ser resistente a enfermedades, a diferentes tipos de suelo, a mejorar su longevidad, su producción, y/o la calidad de la misma (Campana y Ochoa, 2008).

El pistachero es una especie que no tiene gran capacidad de enraizamiento, por lo que es necesario el uso de portainjertos para su propagación vegetativa (Moriana *et al.*, 2018). Los estudios sobre portainjertos en pistachos no son muy numerosos y, en general, se centran en comparar la respuesta productiva (rendimiento del cultivo) y/o la resistencia a enfermedades. La selección del portainjerto es una de las decisiones más importantes para el desarrollo de este tipo de cultivo; *P. atlantica*, *P. integerrima* e híbridos entre *P. atlantica* x *P. integerrima* son los principales portainjertos empleados en los Estados Unidos; *P. francs* se usa en Turquía; mientras que *P. mutica*, *P. khinjuk* y *P. francs* son los más utilizados en Irán (Acar, Kafkas, Kapchina-Toteva y Ercisli, 2017; Gijón, Gimenez, Pérez-López, *et al.*, 2011).

Imagen 6. Diferentes tipos de injertos: (de izquierda a derecha) púa, inglés, corona y astilla (Portalfruticola, 2017).

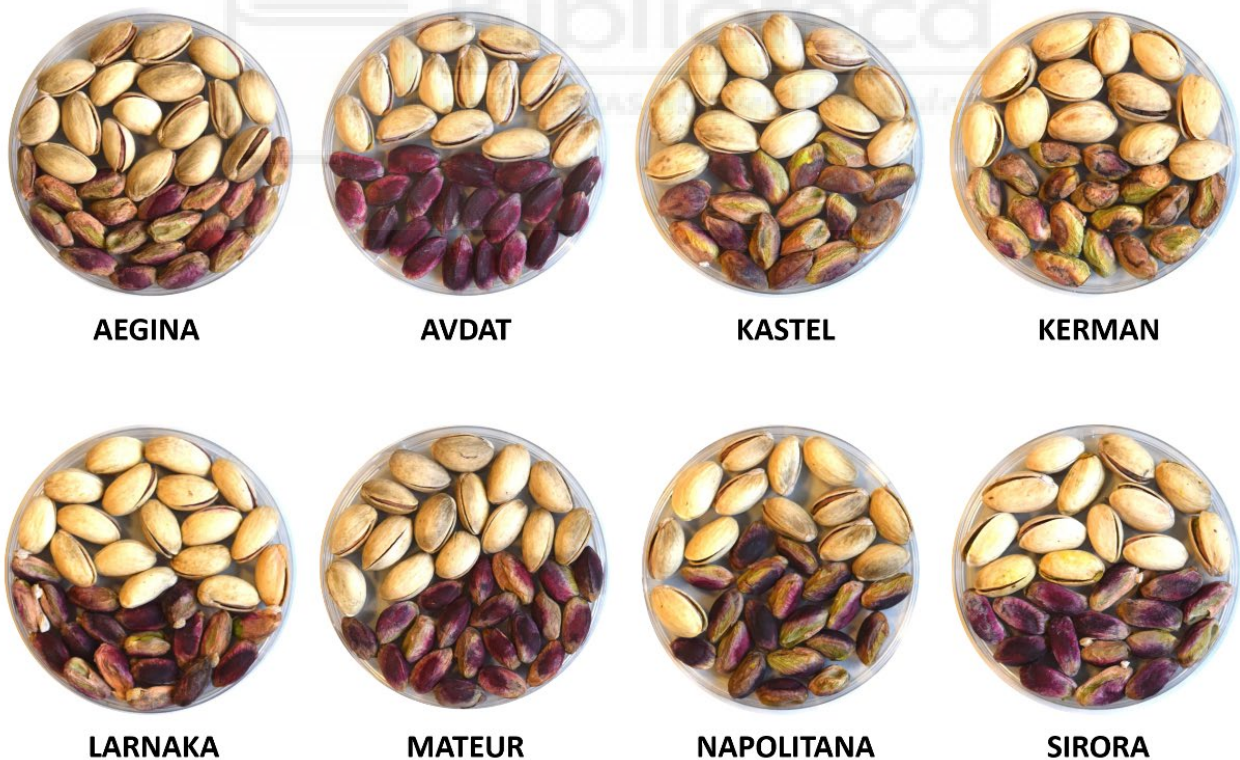


1.4. CULTIVARES

Los diferentes cultivares de pistacho (*Imagen 7*) que se pueden encontrar a lo largo del mundo pueden clasificarse en dos grupos bien diferenciados: aquellos cuyo origen se sitúa en Asia central y occidental, caracterizados por producir frutos de tonalidad amarillo-verdosa, grandes y redondeados; y, aquellos originarios y cultivados en la zona mediterránea, caracterizados por frutos de tonalidad verde intenso, de tamaño pequeño y alargados (Ferguson, 2005).

Los principales cultivares de Irán son *Ahmad Aghaee*, *Akbari*, *Badabi Zarand*, *Kalleghouchi* y *Ohadi*; en Estados Unidos, el mayoritario es *Kerman*; en Turquía, *Kermez* y *Siirt*; en Siria, *Ashoury*; en Italia, el cultivar *Napoletana*; en Grecia, *Aegina*; en Túnez, *Mateur*; en Chipre, *Larnaka*; y en España los principales cultivares son *Kerman* y *Larnaka* (Couceiro, 2013). Es frecuente el uso de varios cultivares femeninos en cada una de las plantaciones con el fin de diversificar el riesgo de la misma (Gijón, 2013).

Imagen 7. Diferentes cultivares de pistacho





1.5. RELACIONES HÍDRICAS

La respuesta del pistachero al estrés hídrico está bien caracterizada, desde un punto de vista agronómico (Gijón *et al.*, 2010; Gijón, Gimenez, Perez-López, *et al.*, 2011; Memmi, Couceiro, Gijón y Pérez-López, 2016; Memmi, Gijón, Couceiro y Pérez-López, 2016; Moriana *et al.*, 2018). Está considerado un cultivo resistente a la sequía, por lo que las condiciones en las que se establece el cultivo suelen ser normalmente deficitarias en agua. Esta situación se apoya en el coeficiente de cultivo (Kc) del pistachero (relación entre la evapotranspiración real de cada cultivo y la evapotranspiración de referencia en esas condiciones y clima), siendo uno de los más altos dentro de los árboles de hoja caduca, superando el valor de 1 durante la mayoría de la estación de crecimiento.

Gracias a su gran resistencia al estrés hídrico se ha estudiado con gran detalle la respuesta del cultivo al riego deficitario controlado (RDI, por sus siglas en inglés). Esta técnica es una estrategia de riego mediante el cual el árbol está sujeto a déficit hídrico en etapas fenológicas específicas, consideradas menos sensibles y sin afectar significativamente el rendimiento o los beneficios económicos (Behboudian y Mills, 1997; Carbonell-Barrachina, Memmi, Noguera-Artiaga, del Carmen Gijón-López, *et al.*, 2015; Gijón, Gimenez, Perez-López, *et al.*, 2011).

El pistachero tiene tres fases fenológicas bien diferenciadas (D.A Goldhamer, 1995) que se establecen en función de los cambios de tamaño y peso del fruto durante su desarrollo (**Imagen 8**):

-  **FASE I:** Tiene lugar una vez ya ha cuajado el fruto, al final de la floración. En esta fase se produce el crecimiento del fruto hasta alcanzar su tamaño definitivo, pero sin que se produzca crecimiento del grano en su interior. Puesto que durante este periodo se produce el crecimiento del fruto, como hemos comentado, es una fase muy sensible al estrés hídrico, ya que cualquier detrimento en el aporte hídrico se verá reflejado en un menor tamaño del fruto, por lo que se producirá un rajado prematuro o una disminución de tamaño del grano durante el desarrollo de este.
-  **FASE II:** es el periodo que acontece inmediatamente después de la fase I y en él se produce el engrosamiento y endurecimiento de la cáscara dura (endocarpio), sin que el grano comience su crecimiento. Los cambios que ocurren durante esta fase no tienen ninguna incidencia significativa en el crecimiento del grano por lo que está considerada como la fase menos sensible

al estrés hídrico (Gijón, 2013; Goldhamer, Viveros y Salinas, 2006; Memmi, Gijón, *et al.*, 2016).

FASE III: este es el periodo en el cual el grano comienza y finaliza su desarrollo, es decir, es la fase en la que se forma el fruto final y objeto del cultivo. Durante esta, el fruto incrementa de tamaño y se produce la dehiscencia. No hace falta decir que, de las tres, esta es la fase más sensible al estrés hídrico, por lo que el árbol debe estar recuperado del estrés sometido durante la fase anterior para evitar un detrimento de su crecimiento (Gijón, 2013; Gijón, Gimenez, Pérez-López, *et al.*, 2011; Memmi, Couceiro, *et al.*, 2016).

Imagen 8. Frutos de pistacho en las tres fases fenológicas del cultivo (Gijón, 2013).



1.6. COMPUESTOS FUNCIONALES

El fruto se caracteriza nutricionalmente por tener un gran aporte energético, un perfil de ácidos grasos saludable, ser rico en potasio y un alto contenido vitamínico (Tabla 1).

Los pistachos han sido recientemente clasificados entre los primeros 50 productos alimenticios con mayor poder antioxidante. Son una rica fuente de compuestos fenólicos, principalmente polifenoles, entre los que destacan la epicatequina, quercetina, kaempferol, cianidín-3-*O*-galactósido y cianindín-3-*O*-glucósido (Dreher, 2012). Se ha demostrado en el pistacho que los polifenoles son bioaccesibles durante la digestión humana simulada, liberando más del 90 % de su contenido total en el compartimento gástrico (Mandalari *et al.*, 2013). Es decir, los pistachos son frutos que tienen una gran cantidad de compuestos bioactivos y el cuerpo humano es capaz de asimilarlos y usarlos.

El cáncer es una de las principales causas de muerte en el mundo. Está causado por la incidencia de factores externos como los factores ambientales, carcinógenos, virus, productos químicos y/o radiación, entre otros, y por supuesto, también tiene gran incidencia el historial genético (Namvar *et al.*, 2012).

Entre todos los factores anteriormente citados, la exposición a mutágenos es uno de los que mayor incidencia presenta, causando la mutación de genes que están directamente involucrados en la regulación del ciclo celular. A día de hoy, los cánceres que causan un mayor número de muertes en el mundo son el de pulmón, colon, estómago, hígado y mama (World Health Organization, 2018).

Se ha demostrado que muchos productos de origen natural tienen el potencial de desencadenar la apoptosis en un gran número de tipos de células cancerosas humanas. Hoy en día, sustancias obtenidas de estos productos se están utilizando clínicamente como terapia de apoyo en el tratamiento de muchos cánceres (Acquaviva *et al.*, 2016). Esto demuestra que es necesario buscar nuevos compuestos en plantas no estudiadas hasta ahora con el fin de obtener nuevas sustancias que puedan tener efecto inductor de apoptosis (Cragg y Newman, 2005; Khazir, Mir, Pilcher y Riley, 2014).

Tabla 1. Composición nutricional de 100 g de pistachos (BEDCA, 2019).

	Valor	Unidad
Proximales		
Energía	594	kcal
Grasas	49,2	g
Proteínas	17,6	g
Agua (humedad)	8,6	g
Hidratos de Carbono		
Fibra dietética total	6,5	g
Carbohidratos	15,7	g
Grasas		
Ácidos grasos monoinsaturados	26,35	g
Ácidos grasos poliinsaturados	15,63	g
Ácidos grasos saturados	6,21	g
Vitaminas		
Vitamina A	25	ug
Vitamina E	5,2	mg
Folato	58	ug
Niacina	1,45	mg
Riboflavina	0,2	mg
Tiamina	0,69	mg
Vitamina B-6	1,27	mg

2

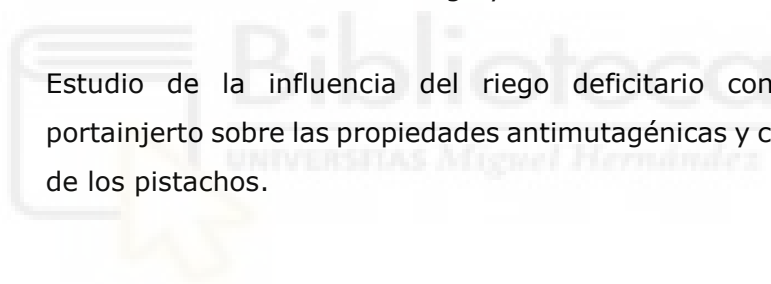
OBJETIVOS



El objetivo global de la Tesis Doctoral fue establecer estrategias de riego deficitario controlado y emplear distintos portainjertos para mejorar la eficiencia del uso del agua en el cultivo del pistachero y la calidad de los pistachos.

Para la consecución de este fin se establecieron los siguientes objetivos parciales:

- I. Estudio de los atributos de calidad de diferentes cultivares de pistacho.
- II. Evaluación del concepto de hidrosostenibilidad.
- III. Estudio de las propiedades físicas, químicas y sensoriales de pistachos obtenidos mediante diferentes tratamientos de riego y mediante distintos portainjertos.
- IV. Estudio de la calidad funcional de los pistachos obtenidos mediante diferentes tratamientos de riego y mediante distintos portainjertos.
- V. Estudio de la influencia del riego deficitario controlado y el portainjerto sobre las propiedades antimutagénicas y citoprotectivas de los pistachos.



3 | MATERIALES Y MÉTODOS



Material, condiciones experimentales y tratamientos

Los pistachos empleados en cada uno de los estudios descritos a lo largo de esta Tesis Doctoral fueron cultivados en la finca experimental "La Entresierra", localizada en Ciudad Real, España, (latitud 3°56' O, longitud 39° N y una altitud sobre el nivel del mar de 640 m). Esta zona se caracteriza por tener un clima mediterráneo con una lluvia anual promedio de 397 mm. Las parcelas experimentales tienen un suelo franco-arcilloso (Petrocalcic Palexeralfs), con un horizonte petrocálcico discontinuo localizado a 0,5 m de profundidad, un pH de 8.1, una conductividad baja (0.2 dS m^{-1}), 1,05 % de materia orgánica, 0,12 % de nitrógeno, $17 \times 10^{-4} \text{ mol kg}^{-1}$ de potasio y una gran capacidad de intercambio catiónico ($0.186 \text{ mol kg}^{-1}$).

En el primero de los estudios englobados dentro de esta Tesis Doctoral, se analizaron pistachos procedentes de 8 cultivares: Aegina, Avdat, Kastel, Kerman, Larnaka, Mateur, Napolitana y Sirora. Se obtuvieron los frutos procedentes de 4 árboles (de cada uno de los cultivares bajo estudio), plantados con un marco de plantación de $7 \times 6 \text{ m}$ ($238 \text{ árboles ha}^{-1}$).

Para el resto de los estudios solo se empleó un cultivar: Kerman. Se utilizó un diseño factorial completamente al azar, en el que hubo 18 parcelas en el campo, con 2 réplicas y 2 factores: (i) tratamiento de riego y (ii) portainjerto. Cada parcela experimental consistió en 2 árboles que se utilizaron para las mediciones, rodeados por 10 árboles en las mismas condiciones (portainjertos y tratamientos de riego).

Los portainjertos empleados fueron *P. Atlantica*, *P. Integerrima* y *P. Terebinthus*.

Los tratamientos de riego evaluados fueron los siguientes:

- T0 (control): los árboles se regaron hasta asegurar condiciones no limitantes de este recurso en el suelo, es decir, 100 % ETo (coeficiente de evapotranspiración del cultivo) de la semana anterior, calculado de acuerdo con el coeficiente diario de evapotranspiración (ecuación de Penman-Monteith), un factor de corrección al cultivo basado en la época del año y teniendo en cuenta el tamaño del dosel (Allen, Pereira, Raes y Smith, 1998; Fereres y Goldhamer, 1990; D. A. Goldhamer, 1995).
- T1: durante el transcurso de la fase fenológica II del cultivo, se suprimió el riego al cultivo hasta mantener un potencial hídrico del tallo inferior a $-1,5 \text{ MPa}$.
- T2: durante el transcurso de la fase fenológica II del cultivo, se suprimió el riego hasta mantener el potencial hídrico del tallo en unos -2 MPa .

Durante las fases fenológicas I y III, el riego fue el mismo para todas las parcelas experimentales. Las relaciones hídricas del cultivo se caracterizaron de acuerdo a las estudiadas previamente por Memmi, Gijón, Couceiro y Pérez-López (2016).

Caracterizaciones morfológicas y físicas

Una vez obtenidos los frutos, se llevaron a cabo las siguientes determinaciones: peso del fruto completo, cáscara y nuez con una balanza Mettler AG204 y una precisión de 0,1 mg; largo, ancho y alto de la parte comestible con un calibre electrónico (Mitutoyo, 500-97-20); color CIEL $*a*b*$ mediante colorímetro Minolta CR-300 (se rellenaron placas Petri con 25 pistachos triturados y se midió el color en varios puntos de esta); y, el número de pistachos comerciales y no comerciales (porcentaje de los que estaban abiertos, cerrados y defectuosos).

Ácidos grasos

La determinación de los ácidos grasos presentes en los frutos de pistacho se llevó a cabo mediante la metilación de estos, de acuerdo con el método descrito por Carbonell-Barrachina, Memmi, Noguera-Artiaga, Gijon-Lopez, *et al.* (2015). El análisis se realizó en un cromatógrafo de gases con espectrómetro de masas (Shimadzu GC-17 QP-5050A) equipado con una columna de naturaleza polar (Suprawax 280) empleando helio como gas portador a un flujo de 1,1 mL min⁻¹.

Compuestos volátiles

La extracción de los compuestos volátiles se realizó mediante el método de determinación en fase sólida del espacio de cabeza (HS-SPME, por sus siglas en inglés). Un gramo de muestra finamente molida se puso en un vial, se añadió un agitador magnético, se cerró con papel de aluminio, se introdujo una fibra Supelco DVB/CAR/PDMS (divinilbenzeno/carboxeno/polidimetilsiloxano) y se mantuvo en agitación durante 25 min a 45 °C. Una vez finalizado el tiempo de extracción de los compuestos volátiles, la fibra se inyectó en un cromatógrafo de gases con espectrómetro de masas Saturn 2000 Varian Chrompack, equipado con una columna HP-5. Se empleó helio como gas portador a un flujo de 1,0 mL min⁻¹.

Los compuestos se identificaron mediante tres métodos analíticos: índice de retención de Kovats (mediante el empleo de patrones de hidrocarburos alifáticos),

tiempos de retención de compuestos químicos puros y espectros de masas de bibliografía (NIST05 y Adams 2012).

Composición mineral

Para la determinación del contenido mineral se digirieron 0,5 g de pistachos, mediante digestión húmeda en bloque digestor (Selecta Digest 20) empleando ácido nítrico (65 % v/v) a 130 °C. La cuantificación de los compuestos se llevó a cabo en un espectrómetro de absorción-emisión atómica (Unicam Solaar 969). Los minerales Ca, Mg, Cu, Fe, Mn y Zn se determinaron por absorción, mientras que K y Na se determinaron por emisión.

Ácidos orgánicos y azúcares

La identificación y cuantificación de los principales azúcares y ácidos orgánicos presentes en el pistacho se realizó de acuerdo al protocolo puesto a punto por Hernández, *et al.* (2016). Se llevó a cabo una extracción con buffer fosfato, se homogenizó, centrifugó y filtró. Posteriormente, se determinaron los compuestos bajo estudio en un cromatógrafo líquido de alta eficiencia (HPLC, por sus siglas en inglés), empleando como eluyente ácido fosfórico (0,1 %) con un flujo de 0,5 mL min⁻¹, en una columna Supelcogel TM C-610H. La absorbancia se midió a 210 nm con un detector de diodos (DAD, por sus siglas en inglés). Para la identificación y cuantificación de los compuestos, se emplearon rectas de calibrado elaboradas a partir de compuestos químicos puros.

Actividad antioxidante

Como bien es sabido, es necesario analizar la actividad antioxidante mediante el empleo de varios métodos de determinación ya que cada uno de estos tiene un mecanismo distinto de acción. En esta Tesis Doctoral, se emplearon los métodos ABTS⁺ (Re *et al.*, 1999), FRAP (Benzie y Strain, 1996) y DPPH (Brand-Williams, Cuvelier y Berset, 1995).

Para la preparación de los extractos se trituraron los pistachos y a 0,5 g de estos se les añadieron 10 mL de extractante (MeOH/agua, 80:20 v/v) acidificado con HCL (1 %). Se sonicó la muestra en un ultrasonidos durante 10 min y se centrifugó a 10.000 x g durante otros 10 min.

Diez microlitros del sobrenadante se mezclaron con 990 μL de reactivo ABTS⁺ o FRAP. Tras 10 minutos de reacción, se midió la absorbancia a 734 nm para ABTS⁺ y 593 nm en el caso del FRAP. En el método DPPH, se mezclaron 10 μL del sobrenadante con 40 μL de MeOH y se agregaron a 950 μL de solución DPPH^{*}.

Contenido de polifenoles total

El mismo extracto que se preparó para el análisis de la actividad antioxidante se utilizó para determinar el contenido total de polifenoles, siguiendo el método de Folin-Ciocalteu. Para ello, se tomaron 0,1 mL de este extracto, 2 mL de agua, 0,2 mL de reactivo de Folin y se dejó reaccionar durante 3 minutos a temperatura ambiente. Pasado ese tiempo, se añadió 1 mL de carbonato sódico y se dejó incubar durante 1 hora. La absorbancia se determinó a 765 nm usando un espectrofotómetro UV-visible Helios Gamma UVG-1002E. Se realizó una curva patrón de ácido gálico para la cuantificación de las muestras.

Identificación y cuantificación individual de polifenoles

Para el caso de la identificación individual de los polifenoles presentes en las muestras de pistachos se pesaron 0,6 g de muestra (finamente molida) y se les añadieron 5 mL de metanol (30 % v/v) acidificado con ácido ascórbico (1 %), y 3 mL de hexano. La mezcla se trituró con un homogeneizador, se introdujo en un ultrasonidos durante 15 min y se dejó reposar 24 h en oscuridad y temperatura ambiente. Pasado ese tiempo, se centrifugó y se filtró. Este filtrado se analizó en un cromatógrafo líquido de alto (ultra) rendimiento (UPLC, por sus siglas en inglés) acoplado a un espectrómetro de masas QToF (LC-PDA-MS-Qtof), con una columna Waters BEH C18 y un flujo de 0,45 mL min⁻¹ (Wojdyło, Nowicka, Carbonell-Barrachina, y Hernández, 2016).

Los compuestos polifenólicos se monitorizaron en las siguientes longitudes de onda: 520 nm (antocianinas), 360 nm (flavonoles y flavanonas), 320 nm (ácidos fenólicos) y 280 nm (flavan-3-oles). La cuantificación se realizó inyectando concentraciones conocidas de compuestos químicos puros.

Procianidinas poliméricas

La determinación de procianidinas poliméricas se llevó a cabo mediante la floroglucinólisis de las muestras de pistacho, de acuerdo al método previamente

descrito por Wojdyło, Carbonell-Barrachina, Legua, and Hernández (2016). Las muestras (0,05 g) se trataron con una disolución de HCl / MeOH / floroglucinol / ácido ascórbico [0,8 mL de la solución metanólica de floroglucinol (75 g L⁻¹) y ácido ascórbico (15 g L⁻¹)]. Tras 30 minutos de reacción, se añadió una disolución acuosa de acetato de sodio (1,2 mL) para detener la reacción del floroglucinol (4 °C). Los aductos de fluoroglucinol obtenidos se analizaron en un UPLC equipado con una columna Waters BEH RP Shield C18, empleando longitudes de onda de excitación/emisión de 278/330 nm. Las procianidinas B1 y B2 se usaron como compuestos de referencia y se calculó la tasa de conversión de (-)-epicatequina y (+)-catequina. El grado medio de polimerización se obtuvo calculando la relación molar de las unidades de extensión y flavan-3-ol terminales a unidades terminales (Wojdyło, Oszmiański, y Bielicki, 2013).

Triterpenoides

Para determinar los triterpenoides presentes en las muestras de pistacho se siguió el protocolo previamente descrito por Wojdyło, Nowicka, *et al.* (2016). La identificación y cuantificación de los ácidos oleanólico, betulínico y ursólico se realizó utilizando un sistema UPLC ACQUITY Ultra Performance LC con administrador de disolventes binario (Waters Corp.), una columna UPLC BEH C18 (1,7 µm, 2,1 × 150 mm, Waters Corp.), un espectrómetro de masas QToF (Waters Corp.) equipado con una fuente ESI (ionización por electrospray) que funciona en modo negativo, y metanol-acetonitrilo (15:85, v / v) como disolvente de elución. Los compuestos se monitorizaron a 210 nm; las m/z para los ácidos betulínico, ursólico y oleanólico fueron 455,3452, 455,3365, 455,3496, respectivamente, y sus tiempos de retención fueron 6,80, 7,50 y 7,85 min, respectivamente.

α-Amilasa y α-glucosidasa

El efecto inhibitorio de la α-amilasa se analizó mediante la cuantificación de los grupos reductores liberados del almidón por la reducción del ácido 3,5-dinitrosalicílico (DNS), mientras que en el caso de la inhibición de la actividad de α-glucosidasa, la determinación se realizó midiendo la cantidad de glucosa hidrolizada a partir de p-nitrofenil-α-D-glucopiranosido. Luego, se midió la absorbancia a 540 y 405 nm (para α-amilasa y α-glucosidasa, respectivamente) usando un espectrofotómetro de PC UV-2401 (Shimadzu, Kyoto, Japón).

Antimutagenicidad

Para los ensayos de antimutagenicidad se prepararon extractos de igual manera que para la cuantificación de polifenoles. La actividad antimutagénica del extracto de pistacho se evaluó utilizando el ensayo de mutagenicidad estándar, según lo descrito por Maron and Ames (1983), con *Salmonella typhimurium* TA100, en presencia de azida de sodio como mutágeno (control positivo). Los extractos de pistacho se diluyeron en dimetilsulfóxido (DMSO) estéril y se enriquecieron con azida de sodio suficiente como para alcanzar concentraciones de 20, 2 y 0,2 $\mu\text{g mL}^{-1}$. La actividad antimutagénica se publicó como el porcentaje de revertantes (inducidas por azida de sodio) inhibidas debido a la presencia del extracto de pistacho.

Citotoxicidad

Para determinar la actividad citotóxica de los compuestos con actividad biofuncional presentes en el pistacho frente a líneas celulares de cáncer humano, se empleó el ensayo MTT (Roche, kit de proliferación celular I, Roche, Cat. No. 11-465-007-001). En una placa de 96 pocillos, se sembraron 10.000 células en cada pozo y se suspendieron en 100 μL de medio de cultivo. Tras 24 h de incubación a 37 °C y atmósfera controlada (5 % de CO_2), se añadieron 100 μL de medio de cultivo sin suero fetal bovino y los extractos de pistacho resuspendidos en DMSO estéril (0,5 %) hasta obtener una concentración final de 100 $\mu\text{g mL}^{-1}$, y se mantuvieron los cultivos celulares durante otras 24 h, en las mismas condiciones.

Se utilizaron las líneas celulares humanas A-549 (carcinoma de pulmón), HeLa (adenocarcinoma de cuello uterino epitelial), MDA-MB-231 (adenocarcinoma de mama), HCT 116 (carcinoma de colon) y ARPE-19 (epitelio retiniano no canceroso). Las líneas celulares se obtuvieron de la Colección de Cultivos Tipo Americana (ATCC). Previo al ensayo se comprobó que los cultivos celulares no mostraron ninguna evidencia de daño celular. Como control positivo se empleó cisplatino [cis-diamminedicloroplatino (II)] a una concentración de 50 $\mu\text{g mL}^{-1}$ para todas las líneas celulares estudiadas, excepto para MDA-MB-231, en cuyo caso la concentración fue de 100 $\mu\text{g mL}^{-1}$. La absorbancia se midió al día siguiente mediante un lector de placas ELISA.

Citoprotección frente al daño oxidativo (H_2O_2)

El primer paso a la hora de estudiar la capacidad que tienen los compuestos funcionales presentes en el pistacho para proteger a células normales frente al daño

oxidativo fue realizar un ensayo de dosis respuesta con diferentes concentraciones de H₂O₂ (20, 10, 1, 0,5, 0,25, 0,125 y 0,062 mM) para elegir aquella que conseguía inducir un daño celular de alrededor de un 85 %.

Las células normales, ARPE-19, se sembraron en placas de 96 pocillos (10.000 células en cada pocillo), se suspendieron en 100 µl de DMEM y se incubaron durante 24 h, a 37 °C y una atmósfera controlada (5 % de CO₂). Tras la adición del agente oxidativo (H₂O₂), se reemplazó el medio celular (por un volumen igual de medio libre de suero DMEM) y se siguió el protocolo MTT original. De esta forma, se evaluó el daño celular que este agente era capaz de provocar.

Una vez obtenida la concentración a estudiar se sembraron nuevas células, en las mismas condiciones descritas anteriormente, y se les añadieron los extractos obtenidos de pistachos a una concentración de 100 µg mL⁻¹, durante 4 y 24 h. Tras el tiempo de incubación, el medio se recuperó de los pozos y se incubaron con 10 mM de H₂O₂ durante 30 minutos (en iguales condiciones). Una vez acabado el tiempo de reacción se siguió el protocolo previamente descrito para analizar el daño celular (MTT).



Grupos focales y análisis de datos conjunto

Se llevaron a cabo cuatro grupos focales con una duración de 60 minutos (cada uno de ellos), con al menos 10 consumidores por grupo, que generalmente consumían pistachos o almendras al menos 1 o 2 veces por semana, para establecer los atributos clave que los consumidores esperan en pistachos cultivados bajo riego deficitario controlado.

En estas sesiones se trataron temas relacionados con los hábitos del consumidor, la salud, las prácticas agrícolas, y las propiedades sensoriales de los frutos secos. Para garantizar que todos los consumidores tenían el mismo conocimiento sobre los temas a tratar, al comienzo de cada sesión grupal, se proporcionaron nociones sobre los frutos secos y el uso del agua en la agricultura.

Toda la información recopilada en estas sesiones la organizó un grupo de expertos (profesores altamente experimentados pertenecientes a áreas de trabajo relacionadas con la agricultura y la tecnología de los alimentos).

Con los conceptos surgidos, se llevó a cabo un análisis conjunto, agrupando los términos en 3 categorías (agricultura, salud y atributos sensoriales) y creando un total de 45 combinaciones con los términos de estas, para que un total de 90 consumidores las agruparan de acuerdo con su preferencia de compra.

Análisis sensorial descriptivo

Doce panelistas entrenados (7 mujeres y 5 hombres), con más de 500 h de experiencia en estudios sensoriales con frutos secos, con edades comprendidas entre 24 y 61 años, participaron en los estudios de análisis sensorial descriptivo de las muestras de pistacho.

Para el análisis, se sirvieron 20 pistachos a cada uno de los panelistas y de cada uno de los tratamientos o cultivares, en platos de plástico desechables con tapa, en una sala de degustación a temperatura controlada (22 °C), con iluminación de luz fluorescente y natural. Para la evaluación de las muestras se creó un léxico con 13 atributos relativos a la apariencia de las muestras, 6 atributos relativos al sabor y 6 atributos de textura. Para la cuantificación de la intensidad de los atributos estudiados, el panel utilizó una escala numérica de 0 a 10, con incrementos de 0,5 unidades, donde 10 representó una intensidad extremadamente fuerte y 0 ninguna intensidad.

Análisis sensorial afectivo

Para conocer la opinión que los consumidores tienen sobre los pistachos obtenidos mediante riego deficitario controlado, se realizaron estudios de consumidores en España, México y Polonia. En el estudio se analizaron pistachos de cultivar Kerman injertado sobre *P. atlantica* y sometidos a los tres tratamientos de riego estudiados a lo largo de esta Tesis Doctoral.

Al menos 60 consumidores fueron reclutados en cada país, a los que se les preguntó sobre la frecuencia de consumo de frutos secos y la disposición a probar los pistachos. Los consumidores declararon que tenían entre 18 y 70 años, no tenían restricciones de dieta o alergias, comían frutos secos al menos una vez por semana y estaban dispuestos a probar los pistachos. En el caso específico de Polonia, para confirmar que no hubo interpretaciones erróneas importantes durante el proceso de traducción, los cuestionarios se tradujeron del español al polaco, y luego nuevamente al español.

Se sirvieron diez pistachos a cada panelista, en tarrinas de plástico desechable (y sin olor), codificadas con números de tres dígitos y a temperatura ambiente. Entre las muestras, para limpiar el paladar de los panelistas se utilizaron galletas saladas sin sal y agua potable.

Los consumidores respondieron usando una escala hedónica de 9 puntos, donde 9 = me gusta muchísimo y 1 = me disgusta muchísimo. Luego, se pidió a los

consumidores que indicaran su orden de preferencia para las muestras y señalaran los motivos de su elección entre todos los atributos en estudio (tamaño, pelado, color, pistacho-ID, tostado, dulce, ácido, postgusto, aceitosidad, dureza, crujibilidad, friabilidad y adhesividad). Por último, se hicieron preguntas relativas al grado de satisfacción total y la intención de compra.

Análisis estadístico

Los datos obtenidos en esta Tesis Doctoral son los valores medios de (al menos) 3 repeticiones. Para la obtención de estos se realizó un análisis de varianza de dos vías (ANOVA) y un test de rangos múltiples de Tukey para hacer una comparación de las medias obtenidas. En los casos en los que se proporcionan datos expresados como porcentaje, estos fueron transformados por la función arcoseno antes del análisis estadístico. Las diferencias se consideraron estadísticamente significativas en $p < 0,05$. Todos los análisis estadísticos se llevaron a cabo empleando el software XLSTAT (versión 2014.1).



4

PUBLICACIONES



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AS AFFECTED BY CULTIVAR**

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FUNCTIONAL AND SENSORY PROPERTIES OF PISTACHIO NUTS AS AFFECTED BY CULTIVAR

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ABSTRACT

BACKGROUND: Modern agriculture allows farmers to choose among different cultivars of the same fruit to fulfill their agronomic needs and consumers' demands; however, there are only few studies describing and comparing key functional and sensory properties of different pistachio cultivars. The main objective of this study was to compare eight pistachio cultivars by analyzing key functional properties (phenolic compounds, polymeric procyanidins, antioxidant activity, inhibition of α -amylase and β -glucosidase), aromatic compounds (GCMS), and sensory properties with a trained panel.

RESULTS: By combination of LC-PDA-MS-QToF and electrospray ionization were determined 2 phenolic acids, 9 flavonols, 1 anthocyanin and 3 flavan-3-ols in pistachio cultivars, with a total concentration ranged from 500 to 6065 mg 100 g⁻¹ dry weight (dw); total polymeric procyanidins concentrations oscillated between 348 and 5919 mg 100 g⁻¹ dw, being (-)-epicatechin the major monomer contributor; Pinene was the most abundant volatile compound (\sim 200 mg kg⁻¹ dw); and, in case of sensory analysis of samples, 23 sensory attributes were found significantly different.

CONCLUSION: The cultivar "Larnaka" stood out as having the best functional profile (high polyphenolic content, high antioxidant activity, and high values of α -amylase and β -glucosidase inhibition), while the cultivars "Kastel" and "Kerman" showed the most attractive sensory properties, mainly the most intense flavor.

Keywords: *Pistacia vera* L. cultivars; GC-MS; antioxidant activity; phenolic compounds; LC-PDA-MS-QToF.

1. INTRODUCTION

The pistachio (*Pistacia vera* L.) belongs to the *Anacardiaceae* family. This nut is originated in western Asia and Asia Minor, where it can still be found growing wild. Nowadays, it is commercially cultivated in countries such as Iran, Turkey, United States, Syria, Greece, Italy, and Spain. The Islamic Republic of Iran is the first world pistachio producer with a production of 241,759 tons, whereas Spain is the tenth producer with 2,500 tons (Food and Agriculture Organization of the United Nations, 2015).

The pistachio tree has been cultivated for a long time in semi-arid areas and it is considered as a desert-tolerant plant mainly for its ability to survive under extreme water stress conditions.(Memmi, Gijón, Couceiro, & Pérez-López, 2016) The importance of pistachio is due to its fruit, which is formed by the shell and the edible kernel that has a thin skin and light green flesh with a distinctive and attractive flavor (Dreher, 2012; Saitta, La Torre, Potortì, Di Bella, & Dugo, 2014).

Pistachio has been used as a folk remedy since prehistoric times due to its nutritional value and long storage life. Recently, it has been demonstrated that consumption of pistachio significantly decreases the oxidative stress and improves both total cholesterol and LDL levels. Indeed, the United States Food and Drug Administration approved the first qualified health claim (July 2003) specific to seeds and the risk of heart disease, quoting that "*scientific evidence suggests but does not prove that eating 1.5 ounces (42.5 g) per day of most nuts, as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease*". In addition, its antioxidant capacity and anti-inflammatory potential have been also reported, and was basically linked to its high total phenolic content.(Alasalvar & Bolling, 2015; Mandalari et al., 2013) Therefore, nowadays, pistachio kernels are consumed roasted and salted as snacks, and they are also incorporated into a wide array of food products such ice cream, salads, fermented meats and bakery products (Dreher, 2012; Saitta et al., 2014).

Even though the chemical composition of the pistachio seeds is complex and is still not completely known, some studies have reported that among its micro-constituents the following are the most relevant, unsaturated fatty acids [both MUFAs (monounsaturated fatty acids) and PUFAs (polyunsaturated fatty acids)], proteins, dietary fiber, vitamin K, and phytochemicals, such as phytosterols, lutein (xanthophyll carotenoid), γ -tocopherol, and some polyphenols. In addition, they are considered an excellent source of K, P, Mg, and Ca.(Fabani et al., 2013) All these components can act synergistically helping to promote cardiovascular health, glycemic and weight control, and promoting protective effect against colorectal and breast cancer (Dreher, 2012; Saitta et al., 2014). Some studies have stated that the

chemical composition of pistachio oil depends on environmental factors such as climate, geographical origin and soil type, among others, and some intrinsic factors such as variety (Chahed et al., 2008).

The aim of this work was to study the functional, aromatic, and sensory characteristics of eight pistachio cultivars to fully characterize them and to gather as much information as possible to help farmers and consumers to take proper decisions when growing and buying pistachios and pistachio-based products, in particular regarding their healthy and organoleptic properties.

2. MATERIAL AND METHODS

2.1. Chemicals

Standards including 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl) striazine (TPTZ), potassium persulfate, acetic acid, phloroglucinol, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). (+)-Catechin, and (–)-epicatechin, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, procyanidin B1 and B2, eriodictyol-3-*O*-glucoside, quercetin and keampferol-3-*O*-glucoside were obtained from Extrasynthese (Lyon Nord, France). Ascorbic acid and acetonitrile for ultra-pressure liquid chromatography (UPLC, gradient grade) were obtained from Merck (Darmstadt, Germany).

2.2. Plant material and experimental design

The experiment was carried out on an experimental farm "*El Chaparrillo*", Ciudad Real, Spain (39° 00' N, 3° 56' W, altitude 640 m) in crop season 2014-15. The plant material consisted of 4 pistachio trees of eight cultivars (cvs.) Kerman, Avdat, Larnaka, Mateur, Napolitana, Aegina, Kastel and Sirora (Figure 1). Tree spacing followed a 7 × 6 m pattern (238 trees ha⁻¹). The climate of the area had an average annual rainfall of 397 mm, mostly distributed outside a four-month summer drought period, and soil is a shallow clay-loam (*Petrocalcic Palixeralfs*) of 0.5 m depth, and a discontinuous *petrocalcic* horizon of around 0.5 m. All the pistachio trees were subjected to the same farming/ agronomical practices (irrigation, fertilization, pruning, and harvesting).

2.3. Polyphenols extraction

Polyphenols from grounded samples were extracted following the procedure previously described by Noguera-Artiaga, Pérez-López, Burgos-Hernández, Wojdyło, and Carbonell-Barrachina (2018). After extraction, samples were filtrated through a

0.20 µm membrane filter (Millex Smplicity® Filters Membrane) immediately before analysis.

2.4. Determination of polyphenols by LC-PDA-MS-QToF

Chromatographic analyses were carried out on a UPLC BEH C18 column (1.7 µm, 2.1 × 100 mm, Waters Corp.; Milford, USA) at 30 °C. The mobile phase used consisted of two solvents: water-formic acid (2.5 % formic acid, v/v) as solvent A, and acetonitrile pure as solvent B. The injection volume was 10 µL and elution was performed at a flow rate of 0.45 mL min⁻¹. The linear gradient started maintaining from 0 to 1 minute the 99 % A, reaching the 0 % A in the minute 12 and from 12.5 to 13.5 min the initial composition (99 % A) was used for re-equilibrating the column. Identification of the compounds was made using an Acquity UPLC system (Waters, Milford, MA), and a Micromass Q-ToF spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative mode. The eluent was passed to the electrospray source with the following conditions: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 130 °C, desolvation temperature of 350 °C and desolvation gas (nitrogen) flow rate 300 L h⁻¹. The full scan mass covered the range from 100 to 1500 *m/z*. For identification of polyphenols, spectral data from all peaks were accumulated at 280 nm (flavan-3-ols), 320 nm (phenolic acids), 360 nm (flavonols and flavanones), and 520 nm (anthocyanins). All Q/TOF-MS chromatograms are presented as the base peak intensity (BPI) chromatograms and scaled to 12.400 counts per second, cps (=100 %). Injection of standard solutions of known concentrations was carried out for quantification of polyphenols, according to Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016). Results were expressed as mg *per* 100 g dry weight (dw) and all data were obtained in triplicate. The leucine enkephalin was used as internal reference compound and the lock mass correction was ±1.000 for the mass window.

2.5. Polymeric procyanidins

Pistachio samples (0.05 g) were treated with a solution of HCl/MeOH/phloroglucinol/ascorbic acid [0.8 mL of the methanolic solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L)]. After 30 min, an aqueous sodium acetate solution (1.2 mL) was added to stop the reaction of phloroglucinol at 4 °C (Wojdyło et al., 2016). Phloroglucinol adducts were analyzed following the methodology previously described by Noguera-Artiaga et al. (2018). All determinations were performed in triplicate and expressed as mg *per* 100 g dw.

2.6. Analysis of antioxidant activity

The solvent prepared for analysis of antioxidant activity was prepared following the method previously described by Wojdyło, Jáuregui, Carbonell-Barrachina, Oszmiański, and Golis (2013). The free radical scavenging capacities were determined using the ABTS⁺ method described by Re et al. (1999) and FRAP (ferric reducing antioxidant power) method described by Benzie and Strain (1996). Determinations of ABTS⁺ and FRAP methods were performed using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). All antioxidant capacity analyses were done in triplicate, and results were expressed as mmol Trolox *per* 100 g dw.

2.7. α -Amylase and α -glucosidase inhibition

The α -amylase and α -glucosidase inhibition were assayed according to the procedure described by Nowicka, Wojdyło, and Samoticha (2016). The α -amylase inhibitory effect was assayed by the reducing groups released from starch by the reduction of 3,5-dinitrosalicylic acid (DNS), while in the case of the inhibition of α -glucosidase activity, the determination was carried out by measuring the amount of glucose hydrolyzed from *p*-nitrophenyl- α -D-glucopyranoside. Then, the absorbance was measured at 540 and 405 nm (for α -amylase and α -glucosidase, respectively) using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). The analysis was run in triplicate and results were expressed as IC₅₀ (mg of dried nut mL⁻¹).

2.8. Extraction of volatile aroma compounds

The extraction of the volatile compounds of the pistachios fruits was carried out using the headspace solid-phase micro-extraction method (HS-SPME). Five grams finely ground pistachios sample was mixed with 1 g of NaCl, stirred with a magnetic bar (500 rpm) and hermetically placed in 50 mL vials with polypropylene caps and PTFE/silicone septa. Vials were equilibrated for 10 min at 40 °C (to simulate mouth temperature during the chewing process) and, after this equilibration time, a 50/30 μ m DVB/CAR/PDMS fiber (length of 2 cm) was exposed to the sample headspace for 50 min at 40 °C. Volatile extractions were run in triplicate.

2.9. Chromatographic analysis of volatile compounds by GC-MS

The isolation and identification of the volatile compounds were performed on a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GCMS QP-5050A. The GC-MS system was equipped with a Restek Rxi-1301 Sil MS column (30 m \times 0.25 mm, 1 μ m film thickness; Restek Corporation, Bellefonte, PA, U.S.A.). Helium was used as carrier gas at a flow rate of 0.6 mL min⁻¹ in a splitless mode. The oven temperature

started at 80 °C and was increased by 3 °C min⁻¹ up to 210 °C; after 1 min of stabilization the temperature was increased by 25 °C min⁻¹ up to 300 °C, and it were hold for 5 min. Detector and injector temperatures were 300 and 230 °C, respectively. Desorption time of the samples was 3 min (230 °C).

Most of the volatile compounds were identified using different analytical methods: (1) retention indices [Kovat's index, where retention time (RT) of each peak is normalized to the RT of adjacently eluting n-alkanes (alkane standard C8-C20)], (2) GCMS retention indices (authentic chemicals), and (3) mass spectra (NIST05 spectral library collection). Identification was considered tentative when it was based on only mass spectral data. Results were expressed as a relative concentration (mg kg⁻¹) by each one of the volatile compounds.

2.10. Sensory analysis

Twelve highly trained panelists (7 female and 5 male), with more than 500 h of testing experience with nuts, aged between 24 and 61 years, and associated with the AgroFood Department of the Universidad Miguel Hernández de Elche (Orihuela, Alicante, Spain), participated in this study. For the analysis, 20 fruits for each panelist were served in coded disposable covered plastic plates, in a tasting room at room temperature (22±2 °C) with an illumination of fluorescent light. Six 1 h-sessions were held for the evaluation of 8 samples by triplicate (4 samples in each session).

Thirteen appearance, 6 flavor and 6 texture descriptive attributes were used for the evaluation of the samples. For the quantification of the intensity of samples attributes, the panel used a numerical scale from 0 to 10, with increments of 0.5 units, where 10 represents extremely strong and 0 none intensity.

2.11. Statistical analysis

The data was subjected to two-way analysis of variance (ANOVA) and later to Tukey's multiple-range test to compare the means. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were done using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD).

3. RESULTS AND DISCUSSION

3.1. Characterization of phenolic compounds.

It is important to emphasize the fact that polyphenols present antioxidant and anti-inflammatory properties (Duthie, Gardner, & Kyle, 2003; C Gentile et al., 2012). Polyphenols represent the main dietary antioxidant; it has been demonstrated that these compounds have higher antioxidant capacity *in vitro* compared with other

bioactive compounds such as vitamins and carotenoids (Mandalari et al., 2013; Richelle, Tavazzi, & Offord, 2001) .

The combination of LC-PDA-MS-QToF analysis and electrospray ionization (ESI) mass spectrometry used revealed the presence of a wide range of polyphenols in the analyzed pistachio nuts samples: 2 phenolic acids, 9 flavonols, 1 anthocyanin and 3 flavan-3-ols (as dimer of procyanidins and (-)-epicatechin and (-)-epicatechin-*O*-hexoside) (Table 1). These phenolic compounds were identified according to their retention times, molecular mass, fragmentation patterns, characteristic spectra, and by using some literature references (Fabani et al., 2013; Grace et al., 2016; Gültekin-Özgüven, Davarcı, Paslı, Demir, & Özçelik, 2015; Regueiro et al., 2014).

Previously, other authors (Saitta et al., 2014; Tomaino et al., 2010) identified in pistachio samples hydroxybenzoic acids (as gallic and protocatechuic acids) and hydroxycinnamic acid (as chlorogenic acid), flavan-3-ols (as [+] -catechin and [-]-epicatechin), flavonols (as glycosides of quercetin), flavones (as luteolin), isoflavones (as daidzein), and flavanones (as aglycones of eriodictyol and their glycosides), being gallic acid, (+)-catechin, and isoquercetin the major compounds identified.

The content of phenolic compounds in different pistachio samples ranged from 500 mg 100 g⁻¹ dw of samples of the "Sirora" cultivar to 6065 mg 100 g⁻¹ dw of "Larnaka" cultivar samples (Table 2). The concentration of phenolic compounds found in this work was similar to those reported in pistachio cultivated in other countries, such as Turkey (461 mg GAE 100 g⁻¹ dw), United States of America (~400 mg GAE 100 g⁻¹ dw), Italy (178.57 mg GAE 100 g⁻¹ dw), Austria (492 mg of GAE 100 g⁻¹ dw), and Greece (1442 mg of GAE 100 g⁻¹ dw) (Ballistreri, Arena, & Fallico, 2009; C Gentile et al., 2012; Kornsteiner, Wagner, & Elmadfa, 2006; Wu et al., 2004; Yang, Liu, & Halim, 2009). Besides, Tsantili, Konstantinidis, Christopoulos, and Roussos (2011) indicated that differences between studies on the same cultivar could be ascribed to field and/or measuring conditions and postharvest handling before sampling.

Pistachios are the only nuts that contain anthocyanins, which are the pigments of the skin responsible for the colors of many vegetables and fruits.(Tomaino et al., 2010) The most abundant compound of this family (cyanidin-3-*O*-galactoside) in the studied cultivars ranged between 12.3 and 130.6 mg 100 g⁻¹ dw, with samples of the cultivars "Kastel" and "Avdat" having the lowest and highest anthocyanin content, respectively.

All pistachio cultivars studied here had higher anthocyanin content than those previously analyzed by Ballistreri et al. (2009) and Seeram et al. (2006), who studied the effect of ripeness and drying or roasting process on anthocyanin content in Californian and Italian (var. *Bianca*) pistachios. The "Aegina", "Mateur" and "Sirora" samples had similar cyanidin-3-*O*-galactoside contents than that found by Bellomo

and Fallico (2007) in pistachio samples of different geographic origin and at different ripening stage. Cultivars "Larnaka" and "Napolitana" had similar content of anthocyanin than observed for cyanidin-3-*O*-glucoside by Tomaino et al. (2010) in var. *Bronte*, and for cyanidin-3-*O*-galactoside by Bellomo and Fallico (2007) in var. *Agrigento*. All these findings indicated that anthocyanin content may be influenced by several factors including cultivar, geographical origin, ripening stage and industrial processing.

3.2. Characterization of polymeric procyanidins

By comparing their retention times and UV and mass spectral properties with those of pure standards, 3 flavan-3-ols and 4 polymeric procyanidins were identified in pistachio seeds [(-)-epicatechin, (-)-epicatechin-*O*-hexoside, and dimer of procyanidins], and were evaluated as polymeric procyanidins after phloroglucynolysis reaction. The total polymeric procyanidins concentrations oscillated between 348 and 5919 mg 100 g⁻¹ dw, with (-)-epicatechin units being the major monomer contributor to these compounds (Table 2); this wide range indicated the huge variability in the content of proanthocyanidins in pistachio nuts. The highest polymeric procyanidins concentration was found in samples of the "Larnaka" cultivar (5919 mg 100 g⁻¹ dw), whereas the lowest ones (from 348 to 506 mg 100 g⁻¹ dw) were found in the cultivars "Aegina", "Avdat" and "Sirora", respectively. Fabani et al. (2013) studied the (+)-catechin and epicatechin contents in pistachios cultivated in Argentina (*Pistacia vera*, variety "Kerman") showing that these compounds presented a content of 16 and 3 µg g⁻¹ dw, respectively. Also, C. Gentile et al. (2007) reported that pistachios (*Pistacia vera* L., variety "Bronte") cultivated at the region of Sicilia (Italy) had a content of 2680 µg g⁻¹ of proanthocyanidins including not only monomers. These data indicate that "Larnaka" (5919 µg g⁻¹ dw), "Kastel" (4600 µg g⁻¹ dw), and "Napolitana" (3825 µg g⁻¹ dw) cultivars were richer in procyanidins than those reported in the Sicilian varieties. In addition, Gu et al. (2003) reported that pistachios, in general, had a procyanidin content of 109.9 µg g⁻¹ indicating that had the second highest content of these chemical group in the nuts family after pecans. However, data from the current study indicated that pistachios grown in Spain would occupy the first place in terms of the content of flavan-3-ols monomers.

3.3. Antioxidant activity

Plants, in general, are rich in secondary metabolites, which play an important role in scavenging free radicals. This fact not only allows these plants to be considered as a source of multifunctional properties, but they can be seen as possible sources for new drugs aimed at various types of diseases (Chadha, Engle, Hughes,

Ledesma, & Weinberger, 2011; Dastmalchi, Dorman, Vuorela, & Hiltunen, 2007). Due to the complex nature of those phytochemicals, it is necessary to use more than one method to evaluate antioxidant capacity of plant materials. For this reason, in this study the antioxidant activity of the different pistachio cultivars was evaluated by different chemical assays: ABTS⁺ and the ferric-reducing antioxidant power (FRAP) assays. Generally, good correlation is found between both methods, as they use the same single electron transfer mechanism. ABTS⁺ method is based on the ability of antioxidant to reduce the ABTS⁺ radical and is one of the most applied method for its high sensitivity, practicality, speed and stability. In addition, this method allows to confirm the antiradical capacity of the hydrophilic and lipophilic antioxidants, because it can be used in both organic and aqueous solvent systems in comparison with other tests with antioxidants (Rakholiya, Kaneria, & Chanda, 2017). The free radical scavenging capacities of ABTS⁺ showed that "Kerman" (6.21 mmol Trolox 100 g⁻¹ dw) and "Larnaka" (5.88 mmol Trolox 100 g⁻¹ dw) and "Napolitana" cultivars presented the highest antioxidant capacity values (Table 3); whereas the lowest were found for "Mateur" and "Sirora" (0.40 and 0.46 mmol Trolox 100 g⁻¹ dw respectively). The antioxidant activity of the hydrophilic fraction of pistachios was also measured by the FRAP method. This method is based on the ability of an antioxidant to reduce Fe⁺³ in the presence of 2,4,6-Tripyridyl-s-triazine (TPTZ), forming a Fe⁺²-TPTZ complex (Kaneria, 2017). Results obtained by FRAP method were comparable to those from ABTS⁺, although FRAP method exhibited that not only "Larnaka" (3.61 mmol Trolox 100 g⁻¹ dw) and "Kerman" (3.19 mmol Trolox 100 g⁻¹ dw) presented the highest antioxidant capacity, but also samples of the cultivars "Napolitana" (3.61 mmol Trolox 100 g⁻¹ dw) and "Avdat" (3.19 mmol Trolox 100 g⁻¹ dw) showed high values of this key functional parameter (Table 3). Once again "Sirora" showed the lowest FRAP antioxidant activity.

These results showed that the total content of phenolic compounds was not directly proportional to antioxidant activity, except in the case of the cultivar "Sirora", which had the lowest content in both parameters. In this sense, ABTS⁺ and FRAP assays data showed that type of phenolics (composition) rather than the amounts is responsible for high antioxidant activity. This can be contrasted by the amount of gallic acid found in the samples. Cultivars "Larnaka" and "Kerman" had high contents of this compound and were in turn the ones with the best antioxidant behavior in the two methods studied. This finding agreed with other authors who reported that differences in antioxidant activities of plant extracts could be due to different structures of plant extracts from phenolic acids and flavonoids compounds as well as their derivatives (Rababah, Hettiarachchy, & Horax, 2004; Rajaei, Barzegar, Mobarez, Sahari, & Esfahani, 2010).

3.4. α -Amylase and α -glucosidase activities

α -Amylase and α -glucosidase are two of the enzymes (pancreatic and intestinal, respectively) responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides, which are the compounds suitable for absorption. The inhibition of these enzymes may be a good strategy to regulate the metabolic alterations related to type 2 diabetes and hyperglycaemia (Nowicka et al., 2016). Therefore, in this study, the inhibition of these enzymes was measured in pistachios and results are presented in Table 3 as IC_{50} (mg of dried nut mL^{-1}).

All the samples studied showed very low inhibition values for both enzymes, which indicated the great inhibitory power of pistachio nuts. Although the differences among samples for both enzymes studied were very small (less than 5 units), significant statistical differences were found. In the case of α -amylase inhibition, "Kerman" and "Mateur" samples showed the lowest IC_{50} value of all the studied cultivars (5.49 and 5.56 mg of dried nut mL^{-1} , respectively), followed by "Larnaka" and "Aegina" (6.56 and 6.71 mg of dried nut mL^{-1} , respectively). The rest of the samples presented higher values, with "Napolitana" and "Sirora" samples having the highest values, with an average value of 10.5 mg of dried nut mL^{-1} . Similar results were observed in case of the α -glucosidase enzyme, in which, again, the samples that presented a lower IC_{50} value were "Kerman", "Mateur", "Aegina" and "Larnaka" samples (values lower than 0.05 mg of dried nut mL^{-1}).

To the best of our knowledge, the α -amylase and α -glucosidase inhibitory activity of pistachios nuts, comparing the cultivar effect, has not been previously evaluated and reported elsewhere. Therefore, the data presented in this study can be considered as the first report in the literature stating this point.

3.5. Characterization of volatile compounds

It is important to highlight that fresh pistachio samples were analyzed in this study, and that most of the compounds characteristic of pistachio flavor are developed during roasting of the nuts. Thirteen volatile compounds were isolated in fresh pistachio fruits by HS-SPME GC-MS (Table 4): 2 alcohols (1-methoxy-2-propanol and 1-hexanol), 2 aldehydes (hexanal and nonanal), 6 terpenes (α -thujene, α -pinene, β -pinene, *trans*- β -ocimene, limonene and terpinolene), 1 pyrrole (1-methylpyrrole) and 2 additional compounds (acetic acid and benzyl acetate). α -Pinene was the most abundant compound (109-333 $mg\ kg^{-1}$ of dried nut) in all samples, followed by 1-methylpyrrole (13-48 $mg\ kg^{-1}$ of dried nut). The content of these two main compounds represented ~94% of the total volatile molecules; thus, it can be concluded that they are key compounds in the characteristic odor of this nut. The sensory descriptors of these compounds are related to woody and

herbaceous notes (Table 4). Aegina and Napolitana samples had the highest concentration of total volatile compounds (375 and 370 mg kg⁻¹ of dried nut, respectively) due to, mainly, they were the two samples that had the highest concentration of α -pinene (333 and 321 mg kg⁻¹ of dried nut, respectively). The small differences in the contents of the minor compounds could be behind the small differences in the odor, aroma and flavor of these pistachio samples. For example, the small but statistically significant differences observed in hexanal, β -pinene, and limonene can be used to explain the more complex sensory profile of some of the samples.

These results agreed with those obtained by other researchers such as Hojjati, Calín-Sánchez, Razavi, and Carbonell-Barrachina (2013) and Penci et al. (2013), who also obtained that α -pinene was one of the predominant compounds in the aromatic composition of pistachio and its essential oil.

3.6. Sensory analysis

Out of the 25 sensory attributes studied (Table 5), significant differences were found in 23 of them (Table 6). Only in the attributes bitter and astringent, there were no significant differences, probably due to the fact that the intensity of these attributes was very low in this specific nut.

Considering the visual attributes studied in the pistachio samples (Table 6), the "Kerman" and "Mateur" samples were characterized by the whiteness of their shell and the uniformity in the fruit size, which make them very attractive for consumers. On the other side, samples of the cultivars "Kastel" and "Sirora" were characterized by the dark color of their shell. As for the color of the nut skin, "Avdat" and "Larnaka" samples had the most intense purple color, while "Kerman" and "Kastel" were the samples that presented the lowest intensity of this color and instead had an intense green color (Table 6).

Regarding flavor, "Avdat" stood out mainly because of its sweetness, with an intensity of 6.0 *versus* an average intensity of 3.2 for the rest of the samples (Table 6). This high sweetness found in this sample, predominated over the rest of the flavor attributes studied (nut flavor, pistachio ID, and aftertaste) and perhaps masked their sensory perception and intensity, leading to lower values as compared to the other cultivars. "Kerman" sample showed the highest intensity of key attributes of nut flavor (6.8), pistachio-ID (7.9), and aftertaste (6.6), (Table 6), attributes that are very important when assessing the sensory quality of this nut, because they are buying- and satisfaction-drivers, according to our own experience with nut consumers. In a similar study carried out by Tsantili et al. (2010), the "Kerman" cultivar was preferred by consumers among other varieties, because it had the best

sensory profile. These results are directly related to those obtained in the analysis of the volatile composition of the samples. In this, "Kerman" sample had a slightly but statistically significant higher concentration of limonene, which is a key compound in the aroma of this fruit because it gives aromatic citrus notes.

Regarding texture, all studied samples presented a characteristic dry nut texture; that is to say, the samples were hard, crispy, had a certain intensity of cohesiveness and adhesiveness, at the same time that they lacked almost completely juiciness or oiliness in the mouth. "Larnaka" and "Aegina" samples were the most representative of this type of texture; that is, they had the highest intensity of hardness and crispiness (these are the texture attributes most appreciated by consumers (Carbonell-Barrachina et al., 2015)).

4. CONCLUSIONS

This was the first study establishing the relationship between pistachio cultivar with its functional properties – mainly related to phenolics - and sensory attributes. After comparing the results obtained, it has been demonstrated that pistachios of the cultivar "Larnaka" had the highest contents of polyphenols and polymeric procyanidins, while samples of the cultivars "Aegina", "Avdat" and "Sirora" had the lowest ones. Besides, samples of the cultivars "Kerman" and "Larnaka" had the highest values of antioxidant activity as well as high inhibitory effect of α -amylase and α -glucosidase; on the other hand, "Sirora" had the opposite behavior. Regarding flavor, "Kerman" was the cultivar with the highest flavor intensity, which might be linked, among other factors, with slightly higher limonene concentration. "Kerman" and "Mateur" samples stood out for their skin whiteness, while "Avdat" and "Larnaka" had the most intense skin color (purple). Finally, "Aegina" and "Larnaka" had the highest intensities of key texture attributes, including hardness and crispiness. Consequently, it is not easy to make a final recommendation on which cultivar is the best one, and this recommendation must be done according to specific objectives; for instance, if pistachio nuts with high functionality or healthy properties are required, "Larnaka" and "Kerman" are the best options. Whereas, "Kerman" is most suitable if flavor characteristics are demanded.

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REFERENCES

- Alasalvar, C., & Bolling, B. W. (2015). Review of nut phytochemicals, fat-soluble bioactives, antioxidant components and health effects. *British Journal of Nutrition*, 113(S2), S68-S78. doi:10.1017/S0007114514003729
- Ballistreri, G., Arena, E., & Fallico, B. (2009). Influence of Ripeness and Drying Process on the Polyphenols and Tocopherols of *Pistacia vera* L. *Molecules*, 14(11), 4358-4369.
- Bellomo, M., & Fallico, B. (2007). Anthocyanins, chlorophylls and xanthophylls in pistachio nuts (*Pistacia vera*) of different geographic origin. *Journal of Food Composition and Analysis*, 20(3-4), 352-359.
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1), 70-76.
- Carbonell-Barrachina, Á. A., Memmi, H., Noguera-Artiaga, L., Gijón-López, M. d. C., Ciapa, R., & Pérez-López, D. (2015). Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *Journal of the Science of Food and Agriculture*, 95(14), 2866-2873.
- Chadha, M., Engle, L., Hughes, J. d. A., Ledesma, D., & Weinberger, K. (2011). 10 AVRDC–The World Vegetable Center’s Approach to Alleviate Malnutrition. Combating micronutrient deficiencies: food-based approaches, 183-197.
- Chahed, T., Bellila, A., Dhifi, W., Hamrouni, I., M’hamdi, B., Kchouk, M., & Marzouk, B. (2008). Pistachio (*Pistacia vera*) seed oil composition: geographic situation and variety effects. *Grasas y aceites*, 59(1), 51-56.
- Dastmalchi, K., Dorman, H. D., Vuorela, H., & Hiltunen, R. (2007). Plants as potential sources for drug development against Alzheimer’s disease. *Int J Biomed Pharm Sci*, 1(2), 83-104.
- Dreher, M. L. (2012). Pistachio nuts: composition and potential health benefits. *Nutrition Reviews*, 70(4), 234-240. doi:10.1111/j.1753-4887.2011.00467.x
- Duthie, G. G., Gardner, P. T., & Kyle, J. A. (2003). Plant polyphenols: are they the new magic bullet? *Proceedings of the Nutrition Society*, 62(3), 599-603.
- Fabani, M. P., Luna, L., Baroni, M. V., Monferran, M. V., Ighani, M., Tapia, A., . . . Feresin, G. E. (2013). Pistachio (*Pistacia vera* var Kerman) from Argentinean cultivars. A natural product with potential to improve human health. *Journal of Functional Foods*, 5(3), 1347-1356. doi:https://doi.org/10.1016/j.jff.2013.05.002
- Food and Agriculture Organization of the United Nations. (2015). FAOSTAT statistics database. Retrieved from <http://www.fao.org/faostat/en/#data/QC>

- Gentile, C., Allegra, M., Angileri, F., Pintaudi, A., Livrea, M., & Tesoriere, L. (2012). Polymeric proanthocyanidins from Sicilian pistachio (*Pistacia vera* L.) nut extract inhibit lipopolysaccharide-induced inflammatory response in RAW 264.7 cells. *European journal of nutrition*, 51(3), 353-363.
- Gentile, C., Tesoriere, L., Butera, D., Fazzari, M., Monastero, M., Allegra, M., & Livrea, M. A. (2007). Antioxidant activity of Sicilian pistachio (*Pistacia vera* L. var. Bronte) nut extract and its bioactive components. *Journal of agricultural and food chemistry*, 55(3), 643-648.
- Grace, M. H., Esposito, D., Timmers, M. A., Xiong, J., Yousef, G., Komarnytsky, S., & Lila, M. A. (2016). Chemical composition, antioxidant and anti-inflammatory properties of pistachio hull extracts. *Food chemistry*, 210, 85-95.
- Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., Holden, J., Haytowitz, D., & Prior, R. L. (2003). Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *Journal of agricultural and food chemistry*, 51(25), 7513-7521.
- Gültekin-Özgülven, M., Davarcı, F., Paslı, A. A., Demir, N., & Özçelik, B. (2015). Determination of phenolic compounds by ultra high liquid chromatography-tandem mass spectrometry: Applications in nuts. *LWT-Food Science and Technology*, 64(1), 42-49.
- Hojjati, M., Calín-Sánchez, Á., Razavi, S. H., & Carbonell-Barrachina, Á. A. (2013). Effect of roasting on colour and volatile composition of pistachios (*Pistacia vera* L.). *International Journal of Food Science & Technology*, 48(2), 437-443.
- Kaneria, M., K. Rakholiya, J. Sonagara and S. Chanda. (2017). Comparative assessment of antioxidant activity and phytochemical analysis of facultative halophyte *Salvadora oleoides* Decne. and *Salvadora persica* L. *Am. J. Biochem. Mol. Biol.*, 7, 102-110.
- Kornsteiner, M., Wagner, K.-H., & Elmadfa, I. (2006). Tocopherols and total phenolics in 10 different nut types. *Food chemistry*, 98(2), 381-387.
- Mandalari, G., Bisignano, C., Filocamo, A., Chessa, S., Sarò, M., Torre, G., . . . Dugo, P. (2013). Bioaccessibility of pistachio polyphenols, xanthophylls, and tocopherols during simulated human digestion. *Nutrition*, 29(1), 338-344.
- Memmi, H., Gijón, M., Couceiro, J., & Pérez-López, D. (2016). Water stress thresholds for regulated deficit irrigation in pistachio trees: Rootstock influence and effects on yield quality. *Agricultural Water Management*, 164, 58-72.
- Noguera-Artiaga, L., Pérez-López, D., Burgos-Hernández, A., Wojdyło, A., & Carbonell-Barrachina, Á. A. (2018). Phenolic and triterpenoid composition and inhibition of α -amylase of pistachio kernels (*Pistacia vera* L.) as affected by

rootstock and irrigation treatment. *Food Chemistry*, 261, 240-245.
doi:<https://doi.org/10.1016/j.foodchem.2018.04.033>

- Nowicka, P., Wojdyło, A., & Samoticha, J. (2016). Evaluation of phytochemicals, antioxidant capacity, and antidiabetic activity of novel smoothies from selected *Prunus* fruits. *Journal of Functional Foods*, 25, 397-407.
doi:10.1016/j.jff.2016.06.024
- Penci, M. C., Martinez, M. L., Fabani, M. P., Feresin, G. E., Tapia, A., Ighani, M., . . . Wunderlin, D. A. (2013). Matching changes in sensory evaluation with physical and chemical parameters. *Food and Bioprocess Technology*, 6(12), 3305-3316.
- Rababah, T. M., Hettiarachchy, N. S., & Horax, R. (2004). Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. *Journal of agricultural and food chemistry*, 52(16), 5183-5186.
- Rajaei, A., Barzegar, M., Mobarez, A. M., Sahari, M. A., & Esfahani, Z. H. (2010). Antioxidant, anti-microbial and antimutagenicity activities of pistachio (*Pistachia vera*) green hull extract. *Food and Chemical Toxicology*, 48(1), 107-112.
- Rakholiya, K. D., Kaneria, M. J., & Chanda, S. V. (2017). Isolation, validation and characterization of major bioactive constituents by various techniques from mango ripe seed. In M. Meghwal, Goyal, M.R., Kaneria, M. (Ed.), *Food Technology: Applied research and production* (pp. 273-314): AppleAcademic Press, New Jersey, USA.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26(9-10), 1231-1237.
- Regueiro, J., Sánchez-González, C., Vallverdú-Queralt, A., Simal-Gándara, J., Lamuela-Raventós, R., & Izquierdo-Pulido, M. (2014). Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap–Orbitrap mass spectrometry. *Food chemistry*, 152, 340-348.
- Richelle, M., Tavazzi, I., & Offord, E. (2001). Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *Journal of agricultural and food chemistry*, 49(7), 3438-3442.
- Saitta, M., La Torre, G. L., Potortì, A. G., Di Bella, G., & Dugo, G. (2014). Polyphenols of pistachio (*pistacia vera* L.) oil samples and geographical differentiation by principal component analysis. *Journal of the American Oil Chemists' Society*, 91(9), 1595-1603.

- Seeram, N. P., Zhang, Y., Henning, S. M., Lee, R., Niu, Y., Lin, G., & Heber, D. (2006). Pistachio skin phenolics are destroyed by bleaching resulting in reduced antioxidative capacities. *Journal of agricultural and food chemistry*, 54(19), 7036-7040.
- Tomaino, A., Martorana, M., Arcoraci, T., Monteleone, D., Giovinazzo, C., & Saija, A. (2010). Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie*, 92(9), 1115-1122.
- Tsantili, E., Konstantinidis, K., Christopoulos, M., & Roussos, P. (2011). Total phenolics and flavonoids and total antioxidant capacity in pistachio (*Pistachia vera* L.) nuts in relation to cultivars and storage conditions. *Scientia Horticulturae*, 129(4), 694-701.
- Tsantili, E., Takidelli, C., Christopoulos, M., Lambrinea, E., Rouskas, D., & Roussos, P. (2010). Physical, compositional and sensory differences in nuts among pistachio (*Pistachia vera* L.) varieties. *Scientia Horticulturae*, 125(4), 562-568.
- Wojdyło, A., Jáuregui, P. N. N., Carbonell-Barrachina, A. n. A., Oszmiański, J., & Golis, T. (2013). Variability of phytochemical properties and content of bioactive compounds in *Lonicera caerulea* L. var. *kamtschatica* berries. *Journal of agricultural and food chemistry*, 61(49), 12072-12084.
- Wojdyło, A., Nowicka, P., Carbonell-Barrachina, Á. A., & Hernández, F. (2016). Phenolic compounds, antioxidant and antidiabetic activity of different cultivars of *Ficus carica* L. fruits. *Journal of Functional Foods*, 25, 421-432.
- Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of agricultural and food chemistry*, 52(12), 4026-4037.
- Yang, J., Liu, R. H., & Halim, L. (2009). Antioxidant and antiproliferative activities of common edible nut seeds. *LWT-Food Science and Technology*, 42(1), 1-8.

Figure1. External appearance of samples of different cultivars of pistachios

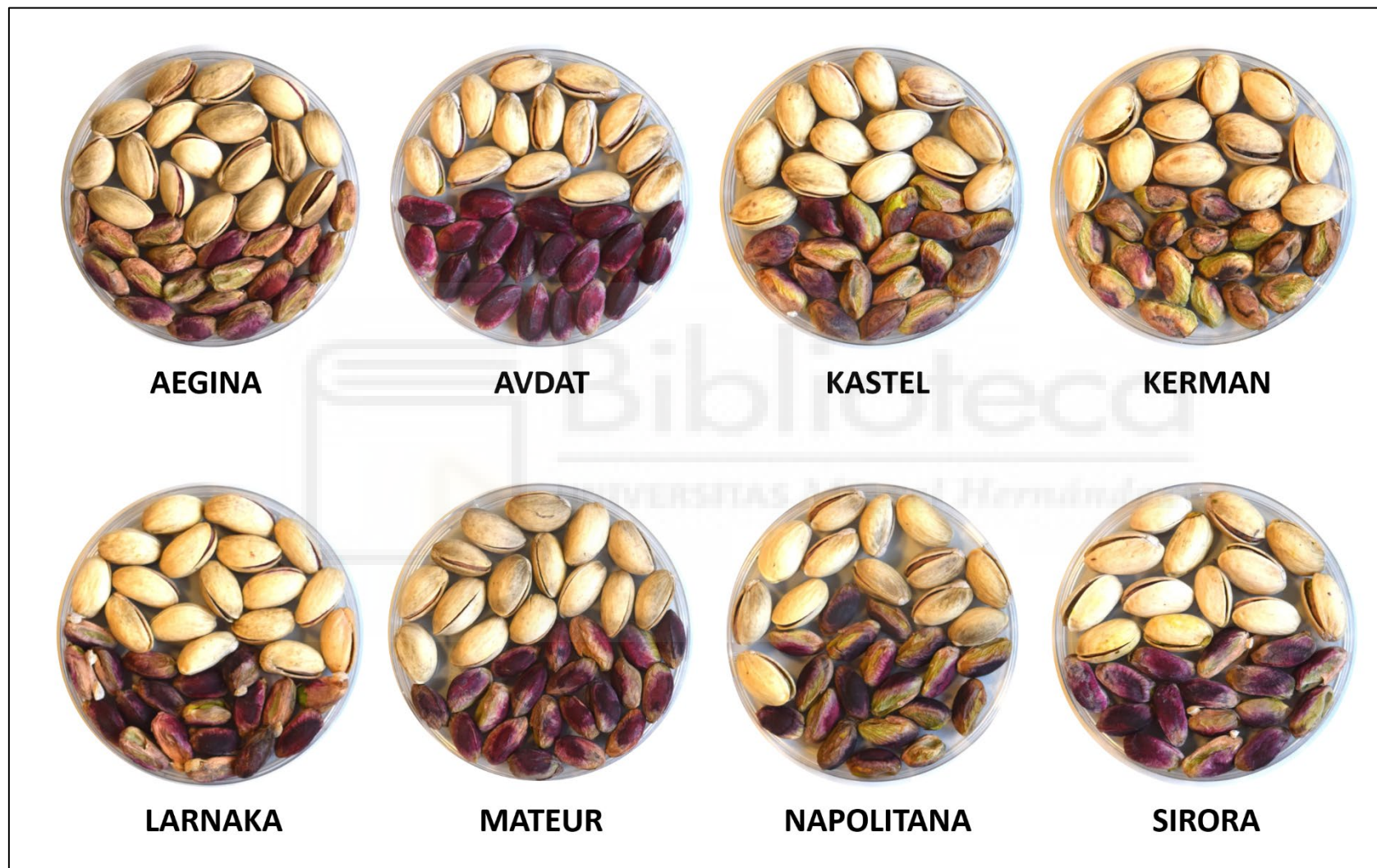


Table 1. Identification of phenolic compounds found in pistachios nuts using LC-MS-QTof/PDA.

Compounds	Retention time (min)	λ_{\max} (nm)	MS [M-H] ⁻ (m/z)	MS/MS (m/z)
Gallic acid	1.76	269	168.97	
Protocatechuic acid	2.74	258, 293	152.97	
Cyanidin-3- <i>O</i> -galactoside	3.68	287, 517	449.07	287.11
Procyanidin dimer	3.92	274	577.06	289.02
Kaempferol-3- <i>O</i> -hexoside	4.14	272, 350	447.02	284.98
(-)-Epicatechin	4.28	270	289.01	245.04
(-)-Epicatechin- <i>O</i> -hexoside	4.31	283	451.06	289.02/323.07
Myricetin- <i>O</i> -xyloside	5.34	254,305,366	449.03	317.05/281.09
Myricetin- <i>O</i> -galloyl-deoxyhexoside	5.86	266, 356	615.18	463.12/317.05
Myricetin- <i>O</i> -hexoside	5.87	269, 356	479.13	317.05
Quercetin-3- <i>O</i> -rutinoside	6.61	350	609.06	301.02
Quercetin-3- <i>O</i> -galactoside	6.78	346	463.02	301,02
Eriodictyol-3- <i>O</i> -glucoside	6.88	283	449.03	287.12
Kaempferol-3- <i>O</i> -hexoside	6.97	352	447.02	285.05
Quercetin-triglucoside	10.04	354	625.20	301.01



Table 2. Quantification of phenolic compounds (mg 100 g⁻¹ dw) in different pistachio cultivars.

Compounds	ANOVA [†]	Aegina	Avdat	Kastel	Kerman	Larnaka	Mateur	Napolitana	Sirora
		(mg 100 g ⁻¹ dw)							
Gallic acid	**	3.48 ab [‡]	3.90 ab	2.65 b	2.96 ab	6.64 a	4.07 ab	2.91 ab	2.37 b
Protocatechuic acid	***	3.03 bc	5.12 ab	2.36 bc	3.45 bc	6.86 a	2.12 c	2.35 bc	1.76 c
Keampferol-3- <i>O</i> -hexoside	***	3.47 a	0.64 c	1.10 bc	1.17 bc	2.29 ab	1.14 bc	0.72 c	1.05 bc
Myricetin- <i>O</i> -xyloside	***	6.08 c	27.12 a	2.24 c	3.32 c	22.26 ab	5.78 c	9.40 bc	5.47 c
Myricetin- <i>O</i> -galloyl-deoxyhexoside	***	1.91 c	8.13 a	1.13 c	1.96 c	6.56 ab	2.42 c	2.51 c	3.05 bc
Myricetin- <i>O</i> -hexoside	**	6.67 b	9.99 a	2.72 c	2.13 c	11.46 a	1.86 c	4.45 bc	1.98 c
Quercetin-3- <i>O</i> -rutinoside	NS	1.04	9.52	4.80	4.29	9.02	5.56	8.77	3.67
Quercetin-3- <i>O</i> -galactoside	**	1.79 abc	2.95 ab	1.04 c	1.45 bc	3.46 a	2.37 abc	1.88 abc	1.95 abc
Eriodictyol-3- <i>O</i> -glucoside	*	0.72 b	7.70 a	0.34 b	0.40 b	2.96 ab	0.85 ab	1.03 ab	0.91 ab
Kaempferol-3- <i>O</i> -hexoside	NS	5.68	11.37	3.16	4.46	8.35	4.93	9.03	6.94
Quercetin-triglucoside	NS	6.26	5.46	3.91	5.69	10.63	5.32	4.42	5.73
Cyanidin-3- <i>O</i> -galactoside	*	27.01 b	130.59 a	12.29 c	16.35 bc	55.92 b	27.36 b	46.95 b	27.41 b
Polymeric Procyanidins [‡]	***	505.72 e	348.13 e	4600.97 b	1348.94 d	5919.35 a	1585.23 d	3824.68 c	437.80 e
Total	***	572.85 e	570.65 e	4638.70 b	1396.57 d	6065.77 a	1649.01 d	3919.24 c	500.11 e

[†]NS: not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same raw, were not significantly different ($p < 0.05$), Tukey's least significant difference test. [‡]Polymeric procyanidins included sum of Procyanidin dimer, (-)-Epicatechin, (-)-epicatechin-*O*-hexoside and other polymeric flavan-3-ols.

Table 3. Antioxidant activity and determination of α -amylase and α -glucosidase inhibition IC_{50} (mg dried nut mL^{-1}) of pistachio cultivars.

Pistachio cultivar	ABTS⁺ (mM Trolox 100 g⁻¹ dm)	FRAP	α-Amylase [IC50 (mg mL⁻¹)]	α-Glucosidase
ANOVA test[†]	***	***	***	***
Aegina	4.397 d [‡]	2.506 b	6.71 d	<0.05 d
Avdat	4.562 cd	3.191 ab	7.41 c	2.49 c
Kastel	1.521 e	1.090 cd	8.41 b	5.94 a
Kerman	6.210 a	3.191 ab	5.39 e	<0.05 d
Larnaka	5.881 ab	3.617 a	6.56 d	<0.05 d
Mateur	0.402 f	1.515 c	5.56 e	<0.05 d
Napolitana	5.276 bc	3.306 ab	10.21 a	3.67 b
Sirora	0.456 f	0.257 d	10.99 a	3.69 b

[†] ***: significant at $p < 0.001$. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and same factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test.



Table 4. Concentration of volatile compounds (mg kg⁻¹), retention time, RT (min), Kovat's index (KI), and sensory descriptors of pistachio cultivars.

Compound	Sensory descriptor	RT (min)	KI	ANOVA [†]	Concentration (mg kg ⁻¹)							
					Aegina	Avdat	Kastel	Kerman	Larnaka	Mateur	Napolitana	Sirora
Acetic acid	Vinegar	3.529	682	*	3.39 bc [‡]	2.02 c	2.07 c	1.97 c	4.54 b	1.57 c	6.21 a	6.11 a
1-Methoxy-2-propanol	<i>nf</i>	4.282	720	NS	1.85	2.11	1.06	1.62	1.83	1.37	1.50	0.52
1-Methylpyrrole	Woody, smoky, herbaceous	5.516	782	**	15.25 c	18.03 c	27.41 b	48.67 a	45.06 a	22.65 bc	31.57 b	13.67 c
Hexanal	Fatty, green	6.877	835	**	9.19 a	0.29 c	0.78 c	3.08 b	0.35 c	0.49 c	1.71 c	0.86 c
1-Hexanol	Green, herbaceous, woody	9.231	912	*	1.07 a	1.26 a	0.23 b	1.23 a	0.31 b	0.12 b	0.12 b	0.43 b
α -Thujene	Woody, green, herbal	10.262	938	NS	0.56	0.68	0.08	0.25	0.53	0.38	0.14	0.24
α -Pinene	Woody	10.651	948	***	333 a	185 c	109 d	281 b	255 b	183 c	321 a	201 c
β -Pinene	Woody	12.636	999	**	6.57 b	5.45 b	0.96 e	3.63 d	8.98 a	4.72 c	3.35 d	3.12 d
<i>trans</i> - β -Ocimene	Green, tropical, woody	13.794	1025	NS	0.39	0.87	0.33	0.34	0.85	0.18	0.21	0.45
Limonene	Lemon, orange, citrus	14.740	1046	*	0.98 d	1.03 d	1.65 c	3.79 a	2.42 b	1.64 c	2.80 b	2.45 b
Terpinolene	Plastic	17.381	1103	*	0.29 b	2.08 a	0.31 b	0.07 b	0.25 b	0.30 b	0.30 b	0.90 b
Nonanal	Fruity, citric, rose	19.381	1144	NS	0.68	0.99	0.58	1.20	0.63	0.09	0.17	0.14
Benzyl acetate	Fruity, floral	22.696	1212	*	1.79 a	1.63 a	0.56 c	1.67 a	1.19 b	0.87 b	1.17 b	0.46 c
Total				***	375 a	222 c	146 d	349 b	322 b	218 c	370 a	230 c

Rt = Retention time; *nf* = not found; [†]NS: not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.

[‡] Values (mean of 3 replications) followed by the same letter, within the same raw, were not significantly different ($p < 0.05$), Tukey's least significant difference test.

Table 5. Appearance, flavor and texture attributes, definitions, and references used in the study.

Attributes	Definition	References and intensities
Visual		
Shell color	Visual evaluation of the color intensity of the shell.	Pantone 451C = 8.0
Stains	Percentage of the shell with different color of the general (not own fruit color).	50 % = 10
Thickness	Thickness (equatorial diameter) of the sample.	18 mm = 8.0, 10 mm = 2.0
Length	Length of the sample.	22 mm = 9.0, 15 mm = 2.0
Uniformity	Degree of similarity of size between samples.	80 % = 8.0; 50 % = 1.0
Shape	Three-dimensional fruit size ratio (thickness/length).	1 = 10; 0.5 = 2.5
Texture	That has wrinkles or roughness on its surface and is rough to the touch.	Almond shell = 9.0; Peanut kernel = 1.0
Peel	Ease or difficulty when removing the shell of the fruit.	Oh! Nuts pistachio (roasted) = 9.0; Oh! Nuts pistachio (raw) = 5.0
Skin color	Visual evaluation of the color intensity of the skin.	Pantone 261C = 10; Pantone 460C = 2.0
Kernel color	Visual evaluation of the color intensity of the kernel.	Pantone 4645C = 9.0; Pantone 460C = 2.0
Kernel shape	Three-dimensional kernel size ratio (thickness/length).	1.0 = 10; 0.5 = 2.5
Roughness (amount)	Amount of wrinkles on its surface.	Almond shell = 9.0; Peanut kernel = 1.0
Roughness (type)	Depth of skin wrinkles.	Deep = 10; Shallow = 1
Flavor		
Sweet	The taste stimulated by sugars and other sweet substances.	Sucrose solution 5 g/L= 6.5
Bitter	The taste stimulated by substances such as quinine or caffeine.	Caffeine solution 0.5 g/L= 1.5
Astringent	The puckering or shrinking of the mouth caused by substances such as alum or tannins.	Alum solution 1.5 g/L = 1.5
Nut flavor	The nut-like aromatic that is typical of several different nuts such as almonds and hazelnuts.	Borges <i>Selección Mezcla de Frutos secos</i> = 8.0
Pistachio ID	Aromatics reminiscent of pistachio.	Oh! Nuts pistachio = 8.0
Aftertaste	Time it remains in the mouth the characteristic flavor of pistachio after swallowing or expectorating the sample.	5 s = 1.0; 20 s = 10
Texture		
Hardness	The force required to bite completely through the sample with molar teeth. Evaluate on first bite down with the molars.	Carrots fresh = 7.5; Cheese American Land O'Lakes = 3.0
Crispiness	The intensity of audible noise at first bite with molars.	Cheerios = 5.0
Juiciness	The sensation of moisture released by the pistachios during the first two bites.	Raw carrot (peeled) = 1.5
Oiliness	The amount of oily coating perceived on the mouth after swallowing or expectorating.	Potato chips Lay's = 8.0
Cohesiveness	The amount the sample deforms rather than cuts during first two bites.	Baby food-bananas = 7.0; Granny Smith apple= 2.0
Adhesiveness	The degree to which product sticks on the surface of teeth.	Mushrooms fresh unpeeled = 2.0; MilkyWay bar= 8.5

Table 6. Descriptive analysis of pistachios obtained by different cultivar

Attributes	ANOVA[†]	Aegina	Avdat	Kastel	Kerman	Larnaka	Mateur	Napolitana	Sirora
<i>Visual</i>									
Shell color	***	6.2 b [‡]	6.0 b	3.5 e	6.7 a	5.0 c	7.1 a	6.0 b	4.0 d
Stains	***	4.7 c	6.7 a	5.0 bc	5.0 bc	4.2 c	2.2 d	5.0 bc	5.7 b
Thickness	***	3.0 d	3.2 d	5.4 b	7.0 a	3.4 d	2.7 d	3.0 d	4.2 c
Length	***	5.0 c	5.4 bc	7.0 a	7.0 a	5.2 bc	5.0 c	6.7 a	5.9 b
Uniformity	***	8.7 cd	9.2 bc	8.5 d	9.5 ab	7.7 e	10.0 a	8.2 de	8.7 cd
Shape	***	3.0 de	3.2 de	6.7 b	9.0 a	2.9 de	3.3 d	2.8 e	4.2 c
Texture	***	2.7 bc	2.2 c	2.2 c	3.2 bc	2.5 c	4.0 ab	5.0 a	4.0 ab
Peel	***	7.5 c	9.4 ab	8.5 bc	9.7 a	10.0 a	8.5 bc	7.9 c	9.7 a
Skin color	***	8.2 d	9.2 b	5.2 e	4.5 f	9.9 a	7.8 d	8.2 cd	8.7 bc
Kernel color	***	3.0 d	6.5 a	2.0 e	2.0 e	3.7 bc	4.0 b	6.1 a	3.2 cd
Kernel shape	***	3.2 de	3.7 cd	6.2 b	8.1 a	3.0 e	3.4 cde	3.0 e	4.0 c
Roughness (amount)	***	3.2 bc	1.7 de	4.9 a	2.2 cd	1.0 e	4.0 ab	4.4 ab	1.9 de
Roughness (type)	***	2.5 b	1.5 c	1.9 bc	2.7 ab	1.2 c	2.7 ab	3.5 a	2.2 bc
<i>Flavor</i>									
Sweet	***	2.7 b	6.0 a	3.5 b	2.5 b	3.5 b	3.3 b	4.2 b	2.7 b
Bitter	NS	1.5	1.0	1.3	1.0	1.2	1.0	1.1	1.2
Astringent	NS	1.1	0.5	0.8	0.7	0.7	0.7	0.9	0.9
Nut flavor	*	6.6 ab	5.8 c	6.1 bc	6.8 a	7.0 a	6.0 bc	6.3 b	6.4 b
Pistachio ID	***	5.8 bc	5.5 c	6.3 bc	7.9 a	5.8 bc	5.5 c	6.8 ab	5.5 c
Aftertaste	***	5.5 abc	5.1 c	6.5 ab	6.6 a	5.5 bc	4.9 c	5.9 abc	5.6 abc
<i>Texture</i>									
Hardness	***	6.3 ab	5.7 b	5.4 b	5.1 b	7.5 a	5.2 b	5.3 b	5.5 b
Crispiness	**	4.7 a	3.5 ab	3.8 ab	3.3 ab	4.5 a	3.6 ab	3.0 b	3.0 b
Juiciness	**	3.1 ab	3.5 a	3.3 a	1.9 b	2.5 ab	3.0 ab	3.1 ab	3.7 a
Oiliness	***	6.0 a	5.5 ab	5.4 ab	2.7 c	5.5 ab	3.6 bc	4.2 abc	5.5 ab
Cohesiveness	**	6.5 a	6.3 a	6.4 a	4.9 c	6.1 ab	5.2 bc	5.7 abc	6.0 ab
Adhesiveness	**	2.9 a	3.0 a	2.4 b	3.0 a	3.0 a	2.2 b	2.8 a	2.5 b

[†]NS: not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [‡]Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ($p < 0.05$), Tukey's least significant difference test.

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OPINION OF SPANISH CONSUMERS ON HYDROSUSTAINABLE PISTACHIOS

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ABSTRACT

Fruits and vegetables cultivated under controlled deficit irrigation (CDI) are called hydrosustainable (hydroSOS) products and have its own personality and are environmentally-friendly. Focus groups helped in classifying key farming, sensory, and health concepts associated with CDI-grown pistachios. Besides, focus groups also helped in stating that a logo was needed for these special foods, and that a hydroSOS index is also essential to certify that the products have been controlled by a control board. Conjoint analysis was used to check which attributes could be helpful in promoting CDI-grown pistachios among Spanish consumers in a first step towards the European Union (EU) market. It was clearly proved that the main silo of properties driving the attention of Spanish consumers was that related to health. The most important attributes for pistachios were "product of Spain", "rich in antioxidant", and "crunchy"; this finding was clearly related to the popularity of regional foods, the preoccupation of European consumers for their health, and the joy related to the crunchiness of toasted nuts, respectively. The use of these three concepts, together with the use of the hydroSOS logo, will be essential to promote hydroSOS pistachios among Spanish and EU consumers. Finally, it is important to highlight that in general Spanish consumers were willing to pay an extra amount of 1.0 euros per kg of hydroSOS pistachios. These earnings will be essential to convince Spanish farmers to implement CDI strategies and have a sustainable and environmental-friendly use of the irrigation water.

PRACTICAL APPLICATIONS: The information generated in this study will be essential for farmers as a starting point for promoting their hydroSOS pistachios, and a similar strategy can be implemented for other hydroSOS vegetables and fruits. Nowadays, it is necessary to address consumers' demands to ensure new products' success in the market.

KEYWORDS: conjoint analysis, controlled deficit irrigation, focus group, *Pistachia vera*, water efficiency.

1. INTRODUCTION

The world food production heavily depends on water availability; in fact it depends more on water than on any other type of abiotic stress (Grant 2012). Due to political and environmental reasons, an increase in the availability of water will be quite difficult. Thus, it will be more and more necessary to improve water efficiency; that is, the commercial production *per* unit of applied water (Kijne and others 2003), by implementing new farming technologies (Katerji and others 2008).

A sustainable use of water is the one that does not reduce, on a net basis, the quantity or the quality of this natural resource. Under such conditions and because it is almost impossible to significantly increase water availability, even by using recycled wastewater, the needs must be specially controlled (Feres and González-Dugo 2009). The strategy for the reduction of needs implies: (i) the management of smaller or deficit quantities of irrigation and/or (ii) the use of drought resistant species. Controlled deficit irrigation (CDI) implies the application of water deficit conditions in non-critical periods of the crop to minimally affect crop production and quality (Chalmers and others 1981). Fruits and vegetables cultivated under CDI, "hydrosustainable or hydroSOSustainable" (hydroSOS from now on) products, will have a solid identity. This identity will be based on two facts: (i) water stress will make plants to increase their secondary metabolites, leading to increases in key compounds that can improve the quality and functionality of these products (higher content in bioactive compounds, higher intensity of some sensory attributes, etc.) (Ripoll and others 2014), and (ii) they will be environmentally friendly because the use of a very valuable resource in the world, water, will be optimized in their production.

After the application of CDI in the field, hydroSOS products will be obtained and they must be distinguished from those that have received non-deficit irrigation (Cano-Lamadrid and others 2015; Carbonell-Barrachina and others 2015). In this study pistachio nuts were used as a model of hydroSOS product obtained after CDI, especially because of the high consumer liking for this type of nuts; however, our research team is working with other vegetal products, such as table olives (Cano-Lamadrid and others 2015), and pomegranates (Galindo and others 2014), and similar strategies will be used to differentiate them from their conventional counterparts. Pistachio tree (*Pistacea vera* L.) is considered as one of the most draught and salinity resistant fruit species (Behboudian and others 1986). Around 4,000 ha are dedicated in Spain for cultivation of pistachio (Gijón and others 2009), mainly in the provinces of Ciudad Real and Toledo. This surface makes Spain one of the countries in the EU (basically a pistachio-importing region) with a higher cultivated area, and because of similarities between pistachios and almonds (Spain

is one of the biggest world producers of almonds), pistachio cultivation in Spain has a huge potential.

Water relations and irrigation scheduling have not been studied deeply in pistachio trees. However, the sensitivity of the phenological stages of the crop has been already established; the shell hardening stage is the most resistant one to water stress (Gijón and others 2009), and it is related to changes in the elasticity modulus and the capacity of osmotic adjustment (Gijón and others 2011). On the other hand, the stage of seed growing is the most sensitive to water stress (Goldhamer and Beede 2004). Finally, Memmi and others (2016) has concluded that as much as 43-73% of the irrigation water can be saved by using soft or moderate CDI strategies combined with the use of Ψ_{stem} (midday stem water potential) to manage irrigation scheduling. With the irrigation conditions reported in this study (Memmi and others 2016), no fruit yield reduction was observed.

In a context of change of food habits, the interest of consumers in products with high quality and/or "own personality", including eco-friendly products (hydroSOS pistachios will have their own personality because of their eco-friendly use of irrigation water), has increased in the developed countries. Almlí and others (2011) have recently showed that European consumers believe that the effort made in the production of "special" products, including efforts made to control water stress in plants, is compensated by their specific sensory attributes and their healthy characteristics. The consumers' perception of the quality of foods with "own personality" greatly depends on their personal preferences, cultural and religion influences (Iaccarino and others 2006), demographic and psychological characteristics (Ophuis and van Trijp 1995), product authenticity perceived by the consumer (Kuznesof and others 1997), and quality of expectations. This last factor can be also affected by the geographical origin (Stefani and others 2006), price (Lange and others 1999), nutritional information (Kähkönen and Tuorila, 1998), traditional making technology (Rason and others 2007), and, probably, by cultural conditions, such as how much water has been used for its production (e.g. type of irrigation).

After consideration of the above information, the main aim of this study was to determine the main aspects of pistachios grown under CDI driving consumers purchase intention in order to be able to emphasize these concepts when later promoting the consumption of the later labelled hydroSOS pistachios. Results of the present study will help farmers in successfully promoting any type of hydroSOS vegetal products, including pistachios.

2. MATERIALS AND METHODS

2.1. Focus group

Four 60-min focus groups were conducted, with at least 10 consumers *per* group, who typically consumed pistachios or almonds at least 1 to 2 times per week, to establish the key attributes that consumers expect in pistachios cultivated under controlled deficit irrigation (CDI) strategies. A total of 45 persons were interviewed, covering a wide range of ages (18 to 65), with 63% of the participants being female. Participants were recruited from the SensoFood Solutions (<http://sensofoodsolutions.umh.es>) consumer database, and were screened to ensure they: (i) ate nuts (almonds and/or pistachios), (ii) were not associated with agriculture or food science related industries, and (iii) had lived over 10 years in Spain or the European Union. The focus groups were conducted in the facilities of the Polytechnic Superior School of Orihuela (EPSO), Orihuela, Alicante (Spain).

A trained focus group moderator managed the focus group discussions, which included topics related to (i.) consumer habits (e.g.: if you only had to purchase food items for yourself, compared to purchasing for the whole family, would you have different concerns?), (ii) health (e.g.: what are the special characteristics you look for when choosing a healthy food item? ("light", "high fiber content", etc.), (iii) agricultural practices, including irrigation systems (e.g.: what do you think about information relative to the nuts production system, for example: products labeled as "sustainable"? Should they have information about the use or non-use of pesticides on crops, irrigation system used, etc.? Is this information important to you when choosing a product?), and (iv) sensory properties of different nut products including pistachios (e.g.: what is the main sensory property you think of when thinking of nuts and nut products?). Additional issues raised by the consumers during the group sessions were also discussed; however, the concept of "hydroSOS" products was not discussed during these sessions because it was still under creation. At the very beginning of each group session, definitions of nuts and irrigation water strategies were provided to the consumers; these explanations were provided to ensure that all consumers had initially the same knowledge on nuts and the use of water in agriculture.

The focus groups were recorded and all responses and terms from the participants were first collected, and later organized (**Table 1**) by a group of six highly experienced professors of the Universidad Miguel Hernández de Elche, who belong to different knowledge areas (Vegetal Production, Agriculture, Environment, Food Science and Technology, and Sensory Analysis). The grouping of concepts was later used in the conjoint analysis.

2.2. Conjoint analysis

Conjoint analysis is used frequently in industry and research for measuring consumer trade-offs among behaviors associated with purchasing or adopting products and services (Krieger and others 2003; Mahanna and others 2009; Mahanna and Lee 2010). This statistical tool works by combining simple variables into more complex concepts according to an experimental design (Krieger and others 2003). Jaeger and others (2011) provide additional background on the technique.

In the present study, the concepts obtained from the focus groups, after proper grouping, were used for the conjoint analysis (**Table 1**). The components of the silos [(terms/elements of the 3 mentioned categories: *farming* (3 elements), *sensory* (3 elements), and *health* (5 elements))] were systematically varied to create different combinations with all 3 components belonging to different silos (total of 45 combinations). A total of 45 paper cards were prepared; each card presented one of the aforementioned created combinations. Two examples of a "card concept" are shown in **Figure 1**. The question asked to the consumers was: *How likely would you be interested in purchasing pistachios with the characteristics shown in the card?* Consumers participating in the conjoint analysis experiment were evaluating pistachios in general, and they received no information about hydroSOS products or CDI strategies. A total of 90 consumers were recruited from the SensoFood Solutions database to evaluate the cards.

A completely randomized design was used with a 5% confidence level (α) and fixing the statistical power ($1-\beta$) of the test at 80%; under these conditions a sample size, according to the expected differences among the factors of $n=15$ cards for each individual. Thus, each consumer received an e-mail and rated 15 cards; it was considered that a consumer could rate 15 cards without losing the interest and keeping maximal concentration. A total of 1350 ratings *per product* were obtained (30 ratings *per concepts' combination per product*). The cards were rated on an "interest scale" from 0 to 10, with 0 being "no interest at all" and 10 being "I would definitely buy it", with increments of 0.5 units. The card presentation was randomized causing all consumers to have different combinations of 15 cards to evaluate. A demographic questionnaire was also administrated at the consumers at the end of the survey, and included the following questions: (i) sex (male, female), (ii) range of age (18-24, 25-35, 36-45, 46-55, >56), (iii) are you in charge of buying foods? (yes, no), (iv) are you a frequent consumer of organic foods? (yes, no), (v) how often do you eat nuts (almonds and/pistachios)? (daily, several times a week, once a week, 2-3 times per month, 1 or less per month), and (vi) where do you eat nuts (almonds

and/or pistachios)? (in pubs together with a drink, at home as appetizer, as ingredient in foods, in meetings/parties with friends, others).

Regression with dummy variables was conducted using PROC REG of SAS® (version 9.2; SAS Inst., Cary, N.C., USA) for consumer data to predict the interest score of each concept. Dummy variables regression was used because the elements evaluated were discrete rather than continuous. Additionally, conjoint analysis was also conducted using PROC TRANSREG of SAS® to obtain the part-worth utility of each item and the relative importance of attributes.

The obtained matrix was used to perform linear regression analysis illustrating the drivers for acceptance as positive coefficients in a regression equation and the potentially “dangerous” elements that drive rejection as negative coefficients in a regression equation.

2.3. Sensory evaluation with consumer panel and willingness to pay

Commercial pistachios were purchased from Mercadona supermarkets (Ourense and Alicante, Spain) and were labeled as “hydroSOSustainable” or “conventional” products; this is, the exact same pistachios were presented using two different labels. Pistachios samples were evaluated by 100 consumers, from two different locations: (i) Ourense (Galicia Community, Spain), and (ii) Alicante (Valencia Community, Spain). The main reason to choose these two locations was that the regions of Galicia and Valencia are characterized by having high and low water availability, respectively; thus, the initial hypothesis was that consumers in Valencia should be more aware of the importance of water for the society, especially for agriculture, than those from Galicia.

The whole group of consumers (n=200) consisted of 54 % women and 46% men, aged between 20 and 63 years old. The main requirement for their recruitment was that they consume nuts (almonds and/or pistachios) at least once a week; and an additional recruitment criterion was that they should be responsible for buying foods at their households. Consumers tested the two pistachio samples, coded with 3-digit numbers, in one session; the samples order for each consumer was randomized. Approximately 5-6 pistachios were served, at room temperature together with the appropriate questionnaire, one at a time, and waiting 5 minutes between samples. Unsalted crackers and water were provided to consumers for palate cleansing between samples.

To check the isolated power of the logo hydroSOSustainable, consumers evaluated their products without any additional explanation at the beginning of the

test. Future studies will be performed checking the power of the logo together with the information related to hydroSOS farming.

In each questionnaire, consumers were asked, using 9-point hedonic scales [from 0 to 9, with 5 (the central point of the scale) being “neither like nor dislike it”], about their “global” satisfaction degree, and also their satisfaction degree for saltiness and crunchiness. Finally, a question about their willingness to purchase was also included at the end of the questionnaire. The options for the price of a 125 g bag of pistachios were: 1.25, 1.50, 1.75, 2.00, 2.25, and 2.50 euros. The normal prices of the Mercadona pistachios ranges from 1.75 to 2.00 euros *per* 125 g bag, depending on the season. Thus, there were two prices above and two below the normal market price of this product.

Results from this study are provided as the mean \pm standard error. First, data were subjected to two-way [factor 1 = treatment (conventional or hydroSOS); factor 2 = location (Galicia or Valencia)] analysis of variance (ANOVA), and later data was also subjected to LSD multiple-range test to compare the means. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD).

3. RESULTS AND DISCUSSION

3.1. Focus groups

After the organization of the terms collected during the group sessions, 4 categories or “silos” were found: (i) terms related to *farming* (production of vegetable or fruits), (ii) terms related to *health* (bioactive components of foods and their effects on human health), (iii) terms related to *sensory* attributes of foods, and (iv) terms related to *price*. The willingness to pay for hydroSOS pistachios has been evaluated in this study as a separate experiment; thus, the category on *price* was eliminated from further testing, and 3 silos for the conjoint analysis were included: (i) *farming*, (ii) *health*, and (iii) *sensory*. Authors want to have all the information about the opinion of Spanish consumers on these categories (farming, health, and sensory) to be able to finally get the best possible price for the hydroSOS products; but this last stage will be done with the help of the logo and marketing campaigns based on the results from these 3 categories. Thus, each of these three silos contained only discrete, but not excluding, terms (**Table 1**), rather than quasi-continuous variables with levels. In this way, selecting one concept from one silo did not mean the others were excluded, but that they were less important for the consumer in driving its purchase intention. A similar strategy (discrete terms) was also the choice taken by

Vázquez-Araújo and others (2012), when developing new products based on sorghum. However, other researchers prefer to use a combination of discrete and continuous variables (Kimura and others 2011).

Initially all terms mentioned by the consumers were listed within a particular category or silo; after that, redundant terms were eliminated, and similar or complementary concepts were combined into a single term, representing the general ideas shown by consumers. The final list is shown in **Table 1** and includes 3, 5, and 3 terms within the categories of farming, health, and sensory, respectively.

One of the first things mentioned by the consumers during the group sessions was the need to develop strategies to distinguish CDI products among the ones already in the market; according to some of the consumers “there is a need *to stand out in the crowd*”. In this sense, consumers mentioned that the best way to provide key information regarding the products is to include it in the food package; also a high percentage of people indicated that television, newspapers, and radio (in decreasing order) would be good options to provide information to consumers.

The first strategy developed to distinguish CDI products, in this particular case pistachios, was to design a logo for this category of “new” vegetal products (**Figure 2**), later labelled as hydroSOS. This activity was conducted in parallel to the conjoint analysis experiment, that will be later discussed; this is the main reason why the logo was not used in that analysis. However, in the next studies with other fruits or vegetables, the hydroSOS will be definitely included in the conjoint analysis studies to get a full image of the opinion of the consumers on the logo. The logo was designed in two different languages English and Spanish, because the marketing campaigns will start in Spain, but will be later extended to other countries of the European Union in English, if successful in Spain. As it can be seen, there is a central message (SOS), in the middle of the logo, on the scarcity of water and the need to efficiently use this crucial resource.

Besides, it would be interesting to develop a “hydroSOS index” to certify that pistachios with the hydroSOS logo have been evaluated regarding their hydrosustainability or the way farmers have limited irrigation water. This index should consider factors such as the following ones: (i) knowledge of the crop, including water management at the less sensitive growin stages, (ii) strenght and duration of the water stress, (iii) practical control of the stress, by considering for example how many times the farmer has measured the water potential, etc. Our research team is currently working on establishing this hydroSOS index, but still needs further research before it is finally ready. Besides and according to the

available research on the effects of CDI on pistachio (Memmi and others 2016), it can be stated that high quality (nutritional, functional, and sensory) hydroSOS pistachios can be produced after cultivation with 45% (range from 43-70%) less water than in control trees (commercial irrigation strategies).

When considering pistachio *farming* issues, a high percentage of the participants gave not too much importance to concepts such as organic farming, sustainability or eco-friendly strategies; this was especially true for old participants. However, they were willing to pay a slightly higher price for pistachios grown locally or nationally. On the other hand, young participants showed higher interest in the concepts related to the environment and also at the same time for national products or even more for local foods. It is possible that terms such as “sustainable” are not fully understood by the “oldest” consumers; for instance, Farley and Smith (2002) indicated the concept of sustainability is a widespread goal that nearly everyone supports, but almost no one means the same thing when they use the term. In this way, the importance of this concept is underestimated especially by old consumers. However, other researchers (Stolz and Bautista 2015) reported that older consumers showed a significant interest in the environmental impact of their purchases.

The fact that this list has a higher number of terms within the *health* category (5), as compared to 3 in the other two categories, showed the high attention that nowadays consumers pay to the healthy aspects of food (Zhu and others 2016; Hemmerling and others 2015). There is a high number of scientific references supporting the beneficial effects of nuts (Hernandez-Alonso and others 2014; Dreher 2012), including pistachios, and their main components: (i) high energy (Kirbaslar and others 2012), (ii) cholesterol-free (Dreher 2012), (iii) high contents of minerals (Schlörmann and others 2015; Kirbaslar and others 2012), (iv) high content of compounds with antioxidant capacity (Schlörmann and others 2015; Liu and others 2014), and (v) rich polyunsaturated fatty acid profile (Schlörmann and others 2015; Kirbaslar and others 2012).

3.2. Conjoint analysis

Initially and considering only the mean values of the 45 combinations, the best 6 results were obtained for the combinations showed in **Table 2**; all had means above 7.5. The soundness of this ranking is supported by the fact that this same order is shown for both the median and the mean.

Later and after the application of the conjoint analysis, it can be stated that the silo “health” promoting properties of pistachios in general was the most important

category for 43.8% of the consumers participating in this study. From this it can be stated that health promoting properties will be essential to advertise hydroSOS pistachios. This marketing strategy is also supported by previous studies by Carbonell-Barrachina and others (2015) who found that hydroSOS pistachios (stem water potential < -1.3 MPa) showed a fatty acid profile with higher contents of linoleic acid (polyunsaturated and essential fatty acid) than controls, and also showed higher intensities of key sensory attributes, and a greater satisfaction degree among international consumers (Spain, Poland, France, and Slovak Republic). Further research on the effects of RDI on the functionality (full polyphenols profile, antioxidant activity, and α -amylase inhibition activity) of hydroSOS pistachios is ongoing and results will be available in a near future.

The other two factors, "farming" and "sensory" properties, showed similar relative importance, 28.2 and 28.0%, respectively (**Figure 3**). This result is particularly logical because nowadays consumers are very worried about their health and pay big attention to the health information in labeling (Wasowicz and others 2015), marketing and advertisement (Lin 2015). Pohjanheimo and Snadell (2009) reported that age and education were related to food choice motives. Adult consumers (28 to 41 years old) had a university degree and considered food choice motives such as health (e.g. "keeps me healthy"), ethical concern (e.g. "comes from countries I approve politically", and natural content (e.g. "contains no additives") to be highly important. It is important to mention that 67% of the participants in the present study had an age between 25 and 45 years old, and thus are within the age range of adult consumers.

The part-worth utility values for the studied concepts are shown in **Figure 4**. The choice "product of Spain" was the most appreciated farming characteristic. This result from the survey agreed quite well with the comments obtained during the focus groups work, in which a high percentage of participants mentioned that "local foods" were appealing for them. Feldman and Hamm (2015) have concluded that local food is not perceived as expensive, on the contrary to organic food, and this leads to more positive opinions towards this type of products. Besides, consumers stated that they were willing to pay extra-money for products clearly labeled as local because they want to support local farmers and economy. Probably this is the result of previous policies encouraging consumers to buy more locally produced foods; this was the case of the UK government (Chambers and others 2007). Another factor that could be related to the relative importance of consuming local foods is the so called spatial localization (food miles) that has emerged as a dominant issue in environmentally benign alternative food systems (Cleveland and others 2015). On the other hand,

factors such as “eco-friendly” or “sustainable” were less appreciated by Spanish consumers. In the authors’ opinion, concepts such as “eco-friendly” or “sustainable” are still not “fully” understood by the general population and will not be key in driving their food choice. These two terms (eco-friendly and sustainable) were included in the conjoint analysis to evaluate this hypothesis and check whether they were able to drive consumers’ attention and whether the later authors’ hypothesis was right or not. Within the “health” silo, the fact that pistachios are a source of energy (“with the energy of nuts”) did not drive consumers’ willingness to buy pistachios, perhaps because of the worries of consumers regarding fatty products with high levels of energy (**Figure 4B**). On the opposite side, the concepts “rich in antioxidants” and “healthy fatty acids profile” were positively valued by Spanish consumers. These two concepts are supported by recent clinical studies proving the positive effects of nuts on human health, especially cardiovascular disease (Ros 2014, 2015). Thus, these two health claims may be useful when promoting pistachios in Spain and in the European Union.

Finally, the most important factor related to the sensory properties of pistachios was “crunchy”, and also the concept “fun-to-eat” positively affected the opinion of consumers on pistachios. However, the concept of “toasted and salty combination” was not too attractive for the consumers, perhaps because of including the word “salty” which seems not too popular at this time. In this way, even though there is a big controversy regarding the risks associated to salt consumption (Graudal and others 2014; Frisoli and others 2012), it is generally admitted that consumers want to reduce their salt intake and actions are being taken (Zandstra and others 2016). Consequently, it is important that during processing of pistachios it is ensured that they are as crunchy as possible, and the fun associated to its consumption seems also important, and if possible low salt is also highly recommended. However, the social desirability of having toasted and “salty” pistachios in a pub together with a drink is a completely different matter; under that specific situation, the senses will be under control, and the salty side of the pistachios will enhance the pistachios flavor and will help in enjoying them.

3.3. Willingness to pay

HydroSOS products have a competitive disadvantage compared to traditional products because they should cover (i) the reductions in yield by limiting irrigation water, and (ii) the increase of costs due to changes in the irrigation systems, as mentioned by Ingenbleek (2015).

The willingness to pay for pistachios was affected by both the irrigation information (conventional *versus* CDI) and the geographical location (Galicia or Valencia) (**Table 3**). Spanish consumers were willing to pay a significantly ($p<0.01$) higher price for hydroSOS pistachios as compared to conventional samples. In fact, they were willing to pay 1.72 € *per* bag of 0.125 kg, which is equivalent to a final price of 13.76 € kg⁻¹ for hydroSOS nuts, as compared to 12.72 € kg⁻¹ for the conventional ones; this values led to a difference of 1.04 € kg⁻¹. The consumers from Galicia were willing to pay a significantly ($p<0.05$) higher price for the pistachios than those from the Valencia region, exactly 13.60 as compared to 12.88 € kg⁻¹. The initial hypothesis of this study was that Valencia consumers should be willing to pay higher prices because water is scarcer there than in Galicia; however, the real situation was exactly the opposite one. This experimental finding could be due to the fact that all consumers in Spain are aware of the importance of the water, and the drastic reduction in the availability of irrigation water. However, further studies, using audicion methods, will be performed to make a more complete evaluation of the willingness to pay of Spanish and European consumers for hydroSOS products, including pistachios. Besides, differences in the consumers' willigness to pay before and after receiving information about the main characteristics of hydroSOS products will also be assayed in future studies.

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REFERENCES

- Almli VL, Verbeke W, Vanhonacker F, Naes T, Hersleth M. 2011. General image and attribute perceptions of traditional food in six European countries. *Food Qual. Prefer.* 22:129-38.
- Behboudian MH, Walker RR, Törökfalvy E. 1986. Effect of water stress and salinity on photosynthesis of pistachio. *Sci. Hort.* 29:251-61.

- Cano-Lamadrid M, Girón IF, Pleite R, Burló F, Corell M, Moriana A, Carbonell-Barrachina AA. 2015. Quality attributes of table olives as affected by regulated deficit irrigation. *LWT- Food Sci. Technol.* 62:19-26.
- Carbonell-Barrachina AA, Memmi H, Noguera-Artiaga L, Gijón-López MC, Ciapa R, Pérez-López D. 2015. Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *J. Sci. Food Agric.* 95(14):2866-73.
- Chalmers DJ, Mitchell PD, Vanheek L. 1981. Control of peach-tree growth and productivity by regulated water-supply, tree density, and summer pruning. *J. Amer. Soc. Hort. Sci.* 106: 307-12.
- Chambers S, Lobb A, Butler L, Harvey K, Traill WB. 2007. Local, national and imported foods: a qualitative study. *Appetite* 49:208-13.
- Cleveland DA, Carruth A, Mazaroli DN. 2015. Operationalizing local food: goals, actions, and indicators for alternative food systems. *Agric. Hum. Val.* 32(2): 281-97.
- Dreher ML. 2012. Pistachio nuts: composition and potential health benefits. *Nutr. Rev.* 70(4):234-40.
- Farley H, Smith ZA. 2002. Sustainability. If it's everything, is it nothing? Oxford (UK): Routledge, Taylor & Francis Group.
- Feldmann C, Hamm U. 2015. Consumers' perceptions and preferences for local food: a review. *Food Qual. Prefer.* 40:152-64.
- Fereres E, González-Dugo V. 2009. Improving productivity to face water scarcity in irrigated agriculture. In: Sadras V, Calderini D., editors. Crop physiology: applications for genetic improvements and agronomy. Amsterdam: Academic Press. pp 122-43.
- Frisoli TM, Shmieder RE, Grodzicki T, Messerli FH. 2012. Salt and hypertension: is salt dietary reduction worth the effort? *Am. J. Med.* 125(5):433-9.
- Gijón MC, Giménez C, Pérez-López D, Guerrero J, Couceiro JF, Moriana A. 2011. Water relation of Pistachio (*Pistacea vera* L.) as affected by phenological stage and water regimes. *Sci. Hort.* 128:415-22.
- Gijón MC, Guerrero J, Couceiro JF, Moriana A. 2009. Deficit irrigation without reducing yield or nut splitting in pistachio (*Pistacea vera* cv Kerman on *Pistacia terebinthus* L.). *Agric Water Manag.* 96:12-22.
- Goldhamer DA, Beede RH. 2004. Regulated deficit irrigation effects on yield, nut quality and water-use efficiency of mature pistachio trees. *J. Hortic. Sci. Biotech.* 79:538-45.
- Grant OM. 2012. Understanding and exploiting the impact of drought stress in plant physiology. In: Ahmad P, Prasad MNV, Editors. Abiotic stress response in

- plants: Metabolism, productivity and sustainability. New York: Springer. pp 89-104.
- Graudal N, Jürgens G, Baslund B, Alderman MH. 2014. Compared with usual sodium intake, low- and excessive-sodium diets are associated with increased mortality: A meta-analysis. *Am. J. Hypertens.* ajh.hpu028.1
- Hemmerling S, Hamm U, Spiller A. 2015. Consumption behavior regarding organic food from marketing perspective – A literature review. *Org. Agric.* 5(4): 277-313.
- Hernandez-Alonso P, Salas-Salvado J, Baldrich-Mora M, Juanola-Falgarona M, Bullo M. 2014. Beneficial effect of pistachio consumption on glucose metabolism, insulin resistance, inflammation, and related metabolic risk markers: a randomized clinical trial. *Diabetes Care.* 37(11):3098-105.
- Galindo A, Calín-Sánchez A, Collado-Gonzalez J, Ondoño S, Hernández F, Torrecillas A, Carbonell-Barrachina AA. 2014. Phytochemical and quality attributes of pomegranate fruits for juice consumption as affected by ripening stage and deficit irrigation. *J. Sci. Food Agric.* 94:2259-65.
- Iaccarino T, Di Monaco R, Mincione A, Cavella S, Masi P. 2006. Influence of information on origin and technology on the consumer response: The case of *soppressata* salami. *Food Qual. Prefer.* 17:76-84.
- Ingenbleek PTM. 2015. Price strategies for sustainable food products. *Brit. Food J.* 117(2): 915-28.
- Jaeger SR, Harker R, Triggs CM, Gunson A, Campbell RL, Jackman R, Requejo-Jackman C. 2011. Determining consumer purchase intentions: the importance of dry matter, size, and price of kiwifruit. *J. Food Sci.* 76(3):177-84.
- Kähkönen P, Tuorila H. 1998. Effect of reduced-fat information on expected and actual hedonic and sensory ratings of sausage. *Appetite* 30:13-23.
- Katerji N, Mastorilli M, Rana G. 2008. Water use efficiency of crops cultivated in the Mediterranean region: Review and analysis. *Eur. J. Agron.* 28:493–507.
- Kijne JW, Barker R, Molden DJ. 2003. Water productivity in agriculture: limits and opportunities for improvement. Wallingford (UK): CABI Publishing and IWMI (International Water Management Institute).
- Kimera A, Kuwazawa S, Wada Y, Kyutoku Y, Okamoto M, Yamaguchi Y, Masuda T, Dan I. 2011. Conjoint analysis on the purchase intent for traditional fermented soy product (*Natto*) among Japanese housewives. *J. Food Sci.* 76(3):217-24.
- Kirbaslar FG, Turker G, Ozsoy-Gunes Z, Unal M, Dulger B, Ertas E, Kizilkaya B. 2012. Evaluation of fatty acid composition, antioxidant and antimicrobial activity, mineral composition and calorie values of some nuts and seeds from Turkey. *Rec. Nat. Prod.* 6(4):339-49.

- Krieger B, Carpuccio R, Katz R, Moskowitz H. 2003. Next generation healthy soup: an exploration using conjoint analysis. *J. Sens. Stud.* 18:249-68.
- Kuznesof S, Tregear A, Moxey A. 1997. Regional foods: a consumer perspective. *Brit. Food J.* 99:199-206.
- Lange C, Rousseau F, Issanchou S. 1999. Expectation, liking and purchase the effect of order behaviour under economical constraint. *Food Qual. Prefer.* 10:31-9.
- Lin CY. 2015. Promote health or prevent disease? The effects of health-related advertising on eating behavior intention. *Int. J. Environ. Res. Public Health.* 12(4):3517-34.
- Liu YT, Blumberg JB, Chen CYO. 2014. Quantification and bioaccessibility of California pistachio bioactives. *J. Agric. Food Chem.* 62(7):1550-6.
- Mahanna K, Lee SY. 2010. Consumer acceptance of food bars. *J. Sens. Stud.* 25:153-70.
- Mahanna K, Moskowitz H, Lee SY. 2009. Assessing consumer expectations for food bars by conjoint analysis. *J. Sens. Stud.* 24:851-70.
- Memmi H, Gijón MC, Couceiro JF, Pérez-López D. 2016. Water stress thresholds for regulated deficit irrigation in pistachio trees: Rootstock influence and effects on yield quality. *Agric. Water Manage.* 164:58-72.
- Ophuis PAM, van Trijp HCM. 1995. Perceived quality: a market driven and consumer oriented approach. *Food Qual. Prefer.* 6:177-83.
- Pohjanheimo T, Sandell M. 2009. Explaining the liking for yoghurt: the role of sensory quality, food choice motives, health concern and product information. *Int. Dairy J.* 19:459-66.
- Rason J, Martin JF, Dufour E, Lebecque A. 2007. Diversity of the sensory characteristics of traditional dry sausages from the centre of France: relation with regional manufacturing practice. *Food Qual. Prefer.* 18:517-30.
- Ripoll J, Urban L, Staudt M, Lopez-Lauri F, Bidet LPR, Bertin N. 2014. Water shortage and quality of fleshy fruits — making the most of the unavoidable. *J. Exp. Bot.* 65:4097-117.
- Ros E. 2014. Contribution of nuts to the Mediterranean diet. In: *The Mediterranean diet: An evidence-based approach*. London: Academic Press, London. pp. 175-84.
- Ros E. 2015. Nuts and CVD. *Brit. J. Nutr.* 113(S2):S111-20.
- Schlörmann W, Birringer M, Böhm V, Löber K, Jahreis G, Lorkowski S, Müller AK, Schöne F, Gleis M. 2015. Influence of roasting conditions on health-related compounds in different nuts. *Food Chem.* 180:77-85.

- Stefani G, Romano D, Cavicchi A. 2006. Consumer expectations, liking and willingness to pay for specialty foods: do sensory characteristics tell the whole story? *Food Qual. Prefer.* 17:53-62.
- Stolz J, Bautista R. 2015. Corporate sustainability: perception and response by older consumers. *Int. J. Consum. Stud.* 39:343-51.
- Vázquez-Araújo L, Chambers IV E, Cherdchu P. 2012. Consumer input for developing human food products made with sorghum grain. *J. Food Sci.* 77(10):S384-9.
- Wasowicz G, Stysko-Kunkowska M, Grunert KG. 2015. The meaning of colours in nutrition labeling in the context of expert and consumer criteria of evaluating food product healthfulness. *J. Health Psychol.* 20(6):907-20.
- Zandstra EH, Lion R, Newson RS. 2016. Salt reduction: Moving from consumer awareness to action. *Food Qual. Prefer.* 48:376-81.
- Zhu C, Lopez RA, Liu X. 2016. Information cost and consumer choices of healthy foods. *Am. J. Agric. Econ.* 98(1):41-53.



Table 1. Silos and concepts chosen for the conjoint analysis of hydro-sustainable pistachios.

Farming	Sensory	Health
Product of Spain	Crunchy texture	With the energy of nuts
Sustainable	Toasted and salty combination	Cholesterol-free
Eco-friendly	Fun-to-eat	Rich in minerals
		Rich in antioxidants
		Healthy fatty acids profile



Table 2. Six concept combinations getting the highest values in the conjoint analysis.

Farming	Sensory	Health	Mean	SD	Min	Median	Max
PRODUCT OF SPAIN	CRUNCHY TEXTURE	RICH IN	7.9	1.6	5.0	8.0	10.0
		ANTIOXIDANTS					
Sustainable	CRUNCHY TEXTURE	With the energy of nuts	7.7	1.2	6.0	8.0	10.0
PRODUCT OF SPAIN	CRUNCHY TEXTURE	Healthy acids profile	7.7	1.4	4.0	7.8	10.0
PRODUCT OF SPAIN	Toasted and salt combination	Healthy acids profile	7.7	1.4	4.0	7.5	10.0
Sustainable	Toasted and salt combination	RICH IN	7.6	1.3	5.5	7.5	10.0
		ANTIOXIDANTS					
Eco-friendly	CRUNCHY TEXTURE	RICH IN	7.5	1.6	3.5	7.5	10.0
		ANTIOXIDANTS					

Table 3. Overall liking and satisfaction degree on saltiness and crunchiness and willingness to pay for pistachio samples as affected by irrigation treatment and geographical location of the study.

Factor	Overall	Saltiness	Crunchiness	Willingness to Pay
ANOVA Test[†]				
Treatment	NS	NS	NS	**
Location	**	*	NS	*
Treat. × Locat.	NS	NS	NS	NS
LSD Multiple Range Test[‡]				
TREATMENT				
Conventional	6.6	6.0	6.4	1.59 b
HydroSOS	6.9	6.0	6.5	1.72 a
LOCATION				
Galicia	6.5 b	5.7 b	6.6	1.70 a
Valencia	7.0 a	6.3 a	6.4	1.61 b

[†] NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [‡] Values followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), according to LSD multiple range test.

Figure 1. Example of 2 of the cards showed to the respondents of the conjoint analysis survey.

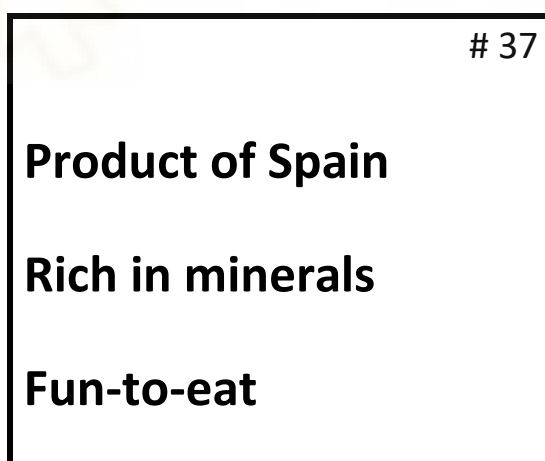
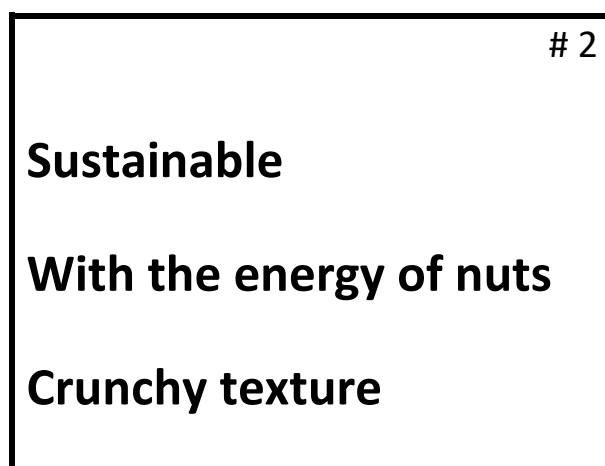


Figure 2. First logo designed for the differentiation of hydro-sustainable products in Spanish and English-speaking countries.

Hydro **S.O.S** *tenibles*

Hydro **S.O.S** *tainable*



Figure 3. Average importance of each of the silos or categories involved in the conjoint analysis of hydro-sustainable pistachios.

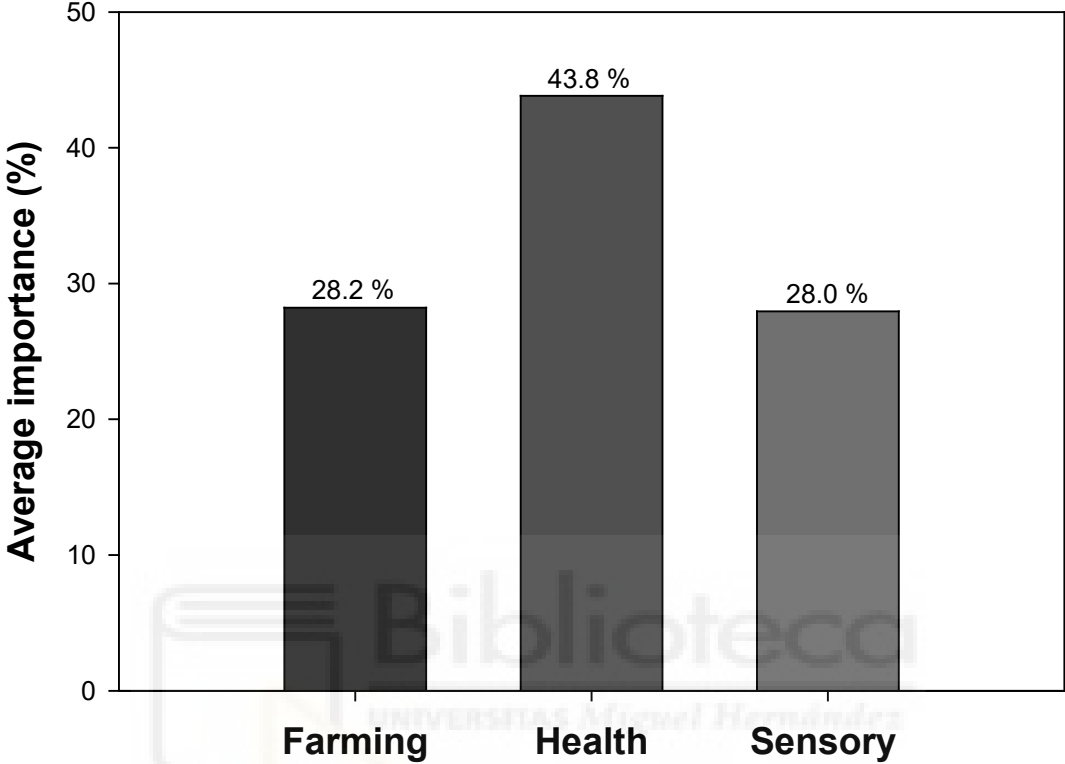
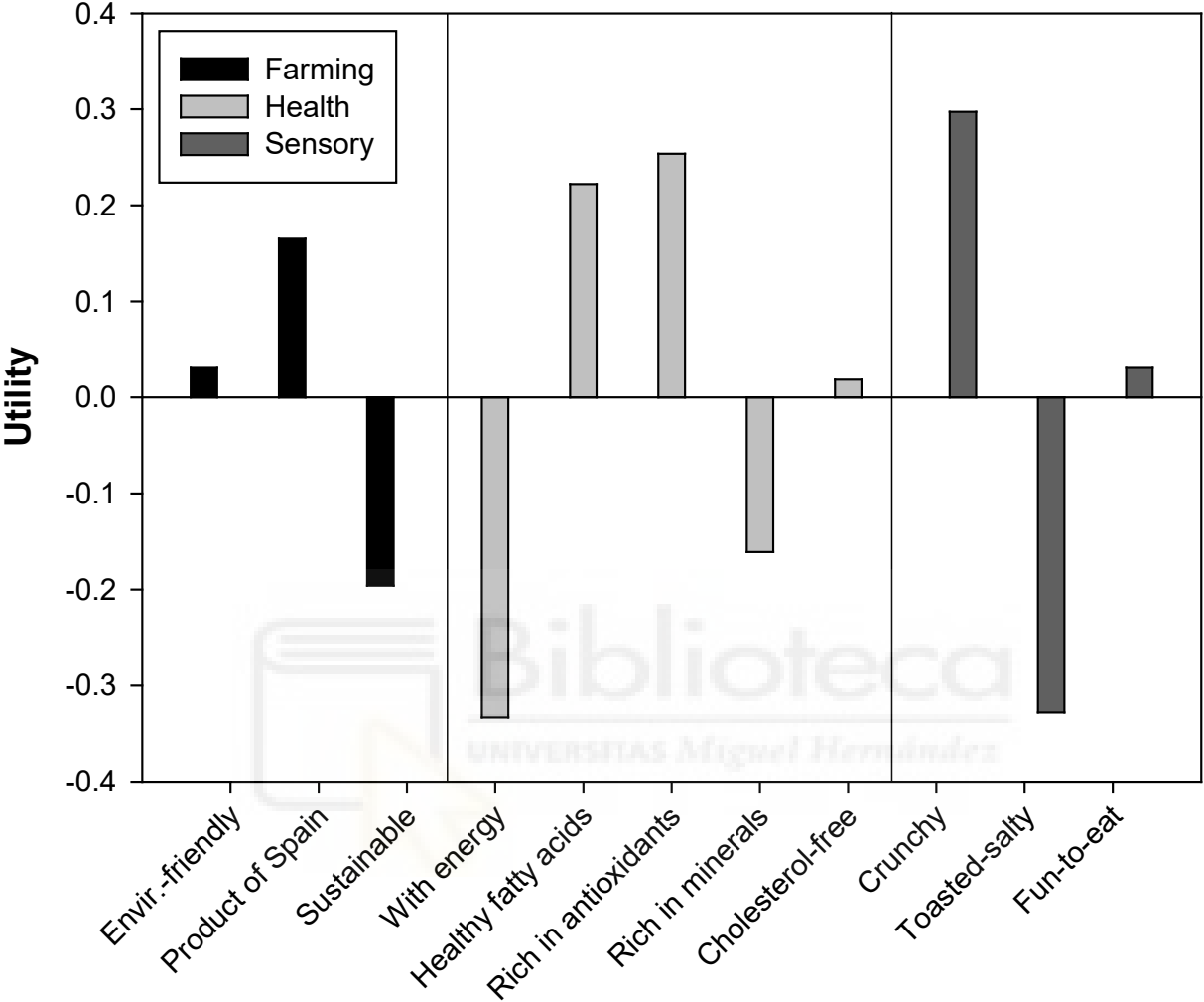


Figure 4. Part-worth utility values for concepts related to hydro-sustainable pistachios.



**INFLUENCE OF REGULATED DEFICIT IRRIGATION AND
ROOTSTOCK ON THE FUNCTIONAL, NUTRITIONAL AND
SENSORY QUALITY OF PISTACHIO NUTS**

Luis Noguera-Artiaga

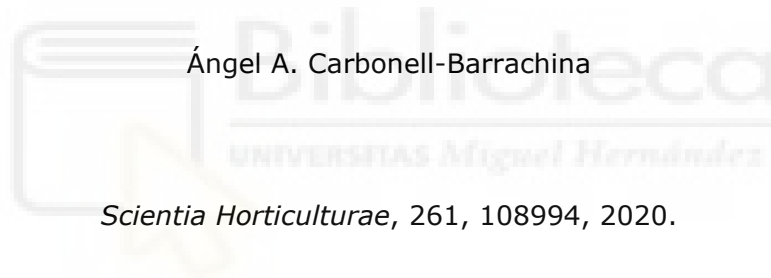
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INFLUENCE OF REGULATED DEFICIT IRRIGATION AND ROOTSTOCK ON THE FUNCTIONAL, NUTRITIONAL AND SENSORY QUALITY OF PISTACHIO NUTS

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Declaration of interest: none

ABSTRACT

The aim of this study was to determine the influence of rootstock and regulated deficit irrigation (RDI) on pistachio nuts, by studying their size, weight, color, fatty acids (gas chromatography with mass spectrometer detector), minerals (atomic absorption), total polyphenol content (TPC), antioxidant activity (ABTS, FRAP, and DPPH), and sensory properties (trained panel). Three rootstocks (*P. atlantica*, *P. integerrima*, and *P. terebinthus*) and three irrigation treatments (T0: fully irrigation, 100 % ET_c; T1: during phenological phase II the stem water potential, SWP, was maintained around -1.5 MPa; and T2: during phase II, SWP < -2.0 MPa) were used. Pistachios obtained from *P. terebinthus* had the highest size, weight and oleic acid content (main fatty acid), while *P. integerrima* nuts had the best sensory profile. The use of moderate RDI (T1) led to pistachio nuts with higher weight, smaller size, similar fatty acid profile, higher TPC (1284 and 1192 g GAE kg⁻¹ dry weigh, respectively), and similar (no statistically differences) antioxidant activity (AA) and sensory profile than control samples. Thus, moderate RDI produces nuts with a good functional quality (high values of TPC and AA), without affecting their sensory quality, but being environmentally friendly and having reduced economic cost due to a lower use of irrigation water.

KEYWORDS: Antioxidant activity; fatty acid methyl esters; hydroSOS; *Pistacia vera*; total polyphenol content.

1. INTRODUCTION

The pistachio tree (*Pistacia vera*) belongs to the Anacardiaceae family, and it is considered the only commercially edible nut among 11 species of the *Pistacia* genus (Couceiro et al., 2013). Thus, *P. vera* is the species with the greatest commercial interest and as a consequence it is widely cultivated in Mediterranean countries (Galindo et al., 2018).

Traditionally, pistachio trees are considered resistant to drought and salinity (Goldhamer, 1995), although the effect of the water stress on the quality and especially on the functionality of the nuts has not been fully characterized. The current water scarcity is forcing farmers to focus on crops that can withstand water deficit. Thus, pistachio is a good alternative for arid or semi-arid farming areas. This crop could generate important advantages for farmers, decreasing water consumption and, therefore, increasing economic benefit and income, as well as being beneficial for the biodiversity of the ecosystem (Pérez-López et al., 2018). Following this recommendation, at the end of the 1980 decade, regulated deficit irrigation (RDI) was used in pistachio trees grafted on *P. atlantica* in California (Goldhamer et al., 1987). RDI is an irrigation strategy through which the tree is subjected to water deficit at specific phenological stages, considered less sensitive, and without significantly affecting the yield or the economic benefits (Behboudian and Mills, 1997; Gijón et al., 2011). Pistachio nuts are characterized by 3 phenological stages: stage I, the nut starts its growth; stage II, the shell hardens (stage less sensitive to irrigation deficits); and stage III, the kernel grows (Fereres et al., 2003; Gijón et al., 2011).

Fruit and vegetables cultivated under RDI are marketed under the brand "hydroSOSustainable or hydroSOS" products. This brand is characterized by being environment-friendly (optimized use of irrigation water) and by a theoretical increase of secondary metabolites, which will improve the quality and functionality of the RDI products (Noguera-Artiaga et al., 2016).

On the other hand, pistachio is a species with low rooting capacity; it is not possible to propagate this species by cutting and planting because not enough roots are produced. Thus, it is necessary to use rootstocks for its vegetative propagation (Moriani et al., 2018). Studies on pistachio rootstocks are not numerous and, in general, they are focused on comparing the productive response (yield) and/or diseases resistance. Selection of the rootstock is one of the most important decisions for the development of a pistachio orchard, and the appropriate rootstock could be different for different farming areas. *P. atlantica*, *P. integerrima*, and hybrids between *P. atlantica* x *P. integerrima* are the main rootstocks in USA; *P. francs* are used in Turkey; while *P. mutica*, *P. khinjuk* and *P. francs* are the most used in Iran (Acar et al., 2017).

Considering all the above-discussed information, the aim of this work was to study the influence of regulated deficit irrigation (RDI) and the use of different rootstocks on the quality (morphological, physico-chemical, functional and sensory properties) of pistachio nuts.

2. MATERIALS AND METHODS

2.1. Plant material, growing conditions and experimental design

Pistachio nuts from trees (*P. vera*), cultivar "Kerman" were collected during 2015 from the experimental orchard "La Entresierra" located at Ciudad Real, Spain (3°56' W, 39° N; altitude 640 m above sea level). The climate of this area is Mediterranean, with an average annual rainfall of 397 mm. The soil is a shallow clay-loam (Petrocalcic Palexeralfs) with a discontinuous petrocalcic horizon located at 0.5 m with a pH about 8.1, low electrical conductivity (0.2 dS m⁻¹), 1.05 % organic matter, 0.12 % N, 17x10⁻⁴ mol kg⁻¹ K and a high cation exchange capacity (0.186 mol kg⁻¹).

A completely randomized factorial design was used for the study. There were 18 plots in the field, with 2-replicates and 2-factors: (i) irrigation treatment, and (ii)

rootstock. Each experimental plot consisted of 2 trees which were used for the measurements, surrounded by 10 trees under the same conditions (rootstock and irrigation).

Three irrigation treatments were evaluated:

- (i) T0 (control), in which trees were irrigated to ensure non-limiting water conditions in the soil (100 % ET_c of the previous week, estimated according to daily reference evapotranspiration calculated using the Penman-Monteith equation, a crop factor based on the time of the year, and taking into consideration the canopy size (Allen et al., 1998; Fereres and Goldhamer, 1990; Goldhamer, 1995);
- (ii) T1, during phase II irrigation was suppressed until pistachio trees reached a stem water potential (SWP) below -1.5 MPa; then, irrigation was managed to keep SWP below this threshold but near of it; and
- (iii) T2 had the same irrigation protocol as T1 but with a SWP threshold of -2.0 MPa.

Treatments T1 and T2 were irrigated with a threshold of -1.1 MPa, considerate as without water stress during phenological phases I and III.

Water relations were characterized according to Memmi et al. (2016): irrigation was performed daily using a drip irrigation system with 12 self-compensating emitters (each delivering 4 L h^{-1}) per tree; the irrigation water used had an electrical conductivity of $2.6\text{--}2.9 \text{ dS cm}^{-1}$. Irrigation started when the measured SWP values were lower than the targeted threshold values. The first irrigation event was always 1 mm. Then, each increase or decrease of irrigation water supply was scheduled according to the percentage of difference derived from the relationship between measured SWP (0.25 mm day^{-1} for every 10 % deviation). The soil moisture was measured using a portable capacitance probe (Diviner 2000 Sentek Pty. Ltd., Stepney South, Australia) using the default calibration supplied by the manufacturer, according to procedure described by Memmi et al. (2016).

Besides, pistachio trees were grafted over 3 different rootstocks: *P. atlantica*, *P. integerrima* and *P. terebinthus*.

Pistachio nuts were collected from the field, peeled and dried in a convection oven with hot air at 60 °C until the targeted moisture content of 5 % (~3 d) was reached. Nuts were immediately packed and ~2 kg were posted to Universidad Miguel Hernández de Elche, Orihuela (Alicante, Spain) for analyses. Finally, samples were vacuum packed and kept under refrigeration (4-5 °C) until analysis.

2.2. Morphological analysis

Twenty-five pistachio nuts from each treatment were randomly selected and the whole nut, shell and edible part were weighed (model AG204 scale; Mettler Toledo, Barcelona, Spain) with a precision of 0.1 mg. Also, length, width and height of the edible part from each pistachio taken per treatment were measured using a digital caliper (model 500-197-20 150 mm; Mitutoyo Corp., Aurora, IL, USA).

Color was measured using a colorimeter (model CR-300, Minolta, Osaka, Japan) with an illuminant D65 and an observer of 10 °. Twenty-five pistachios were ground for 10 s (Taurus Aromatic Ver II; Taurus Group, Barcelona, Spain) and placed in Petri dishes (100x15 mm). Color data were provided as CIEL*a*b* coordinates and the following coordinates were evaluated: L^* (lightness); a^* (green-red coordinate) and b^* (blue-yellow coordinate). Lightness taking values within the range 0–100, the coordinate a^* takes positive values for reddish colors and negative values for greenish ones, whereas b^* takes positive values for yellowish colors and negative values for bluish ones. Besides, C^* (Chroma, $C^* = [(a^{*2}) + (b^{*2})]^{1/2}$) was studied. Chroma 0 is at the center of a color sphere and increases according to the distance from the center. Analyses were run in triplicate (using 25 nuts for each measurement).

2.3. Fatty acids

The determination of fatty acid methyl esters (FAME) were carry out following the method described by Carbonell-Barrachina et al. (2015), with some modifications. The organic layer of the extracts was identified and quantified using a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detect or GCMS QP-5050A, equipped with a polar column, Suprawax 280, 100 % polyethylene glycol (Teknokroma S. Co. Ltd., Barcelona, Spain; 30 m x 0.25 mm x 0.25 µm film thickness). Detector and injector temperatures were 260 °C and 230 °C, respectively. Helium was used as a carrier gas at a flow rate of 1.1 mL min⁻¹ (split ratio 1:10). The oven program was as follow: initial temperature 80 °C (hold 2 min) and rate up to 160 °C (8 °C min⁻¹); then, rate up to 220 °C (rate of 4 °C min⁻¹; hold 13 min); and, to finally, rate up the oven temperature up to 260 °C (rate of 10 °C min⁻¹; hold 6 min). Identification of peaks was made by comparison with FAME standards from Sigma-Aldrich. Analysis of FAME was run in triplicate (n=3).

2.4. Mineral content

To determine the mineral content in the samples, 0.5 g of ground pistachios were digested for 2 hours in a multi-place digestion block (Digest 20, Selecta), at a temperature below 130 °C, using 5 mL of 65 % (w/v) HNO₃ as described Carbonell-Barrachina et al. (2002). Then, dilutions of 1:10 and 1:50 were prepared and stored in refrigeration (4 °C) until analysis. The quantification of minerals was carried using an atomic absorption–emission spectrometer (Solaar 969; Unicam Ltd, Cambridge, UK) using atomic absorption for Ca, Mg, Cu, Fe, Mn and Zn, whereas atomic emission was used for K, and Na. Calibration curves were used for the mineral quantification and they showed good linearity ($R^2 \geq 0.997$). Five replicates were run for the mineral analysis.

2.5. Antioxidant activity

It is necessary to use several of methods to better compare the results obtained on the analysis of the antioxidant activity, and their choice is based on the matrix of the sample and on the chemical nature of the compounds to be evaluated (Prior et al., 2005). In this work, ABTS⁺, FRAP, and DPPH* methods were used to describe the antioxidant activity of the samples.

The ABTS⁺ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation was carried out as described by Re et al. (1999), ferric reducing antioxidant power (FRAP) as described by Benzie and Strain (1996), and, the radical scavenging activity using the DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) as described by Brand-Williams et al. (1995).

For the analysis, a methanol extract was prepared for each treatment and rootstock. Ten milliliters of MeOH/water (80:20, v/v) plus 1% HCl were added to around 0.5 g of crushed pistachio. Then, the mixture was sonicated at 20 °C for 15 min and left at 4 °C for 24 h. After that, the extract was sonicated again for 15 min and centrifuged at 10,000 x g during 10 min.

Ten-μL of the supernatant was mixed with 990 μL of ABTS⁺ or FRAP. After 10 min of reaction, the absorbance was measured at 734 nm for ABTS⁺ and 593 nm for FRAP method. In the DPPH method, 10 μL of the supernatant was mixed with 40 μL of MeOH and added to 950 μL of DPPH* solution. The mixture was shaken and placed under dark conditions for 15 min. All the results were expressed as mmol Trolox kg⁻¹ dry weight, dw.

2.6. Total polyphenol content

For the total polyphenol content (TPC) determination, a methanol extract was prepared for each sample as previously described for antioxidant activity. TPC was quantified using the Folin-Ciocalteu colorimetric method described by Gao et al. (2000), with some modifications. In a sample of pistachio extracts (0.1 mL) were added 0.2 mL of Folin-Ciocalteu reagent, 2 mL of distilled water and incubated for 3

min at room temperature. Then one-mL of 20 % sodium carbonate was added and incubated again for one hour. The TPC was determined by measurement at 765 nm using an UV-visible spectrophotometer (Helios Gamma model, UVG 1002E). Quantification was carried out according to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), g kg^{-1} (dw).

2.7. Sensory analysis

For the sensory evaluation, was used a trained panel formed by nine panelists (aged 30-65 years; five women and four men) from the research group Food Quality and Safety (Universidad Miguel Hernández de Elche, Orihuela, Alicante, Spain). Each panelist had more than 500 h of testing experience with nuts.

About 10 pistachio nuts were served, to each panelist, in odor-free disposable 90 mL covered plastic cups, coded using three-digit numbers, and at room temperature. Between samples, to clean the panelists' palate were used unsalted crackers and drinking water. The testing room was at $\sim 22 \pm 2$ °C and natural illumination was used. Four 2 h sessions were done for the samples evaluation. The total number of samples under analysis was 9 and all were evaluated in each session.

The panel worked with the lexicon developed by Carbonell-Barrachina et al. (2015) with some added attributes: floral, bitter, astringent and wood. The panel used a numerical scale for quantifying the intensity of the pistachio attributes, where 0 represents none and 10 extremely strong, with 0.5 increments.

2.8. Statistical analysis

Data from the analyzes performed on the pistachios were processed by means of an analysis of variance (ANOVA) and by the Tukey's multiple range test. Percentage data were transformed by Arcosen function before statistical analysis. The software StatGraphicsPlus 5.0 Software (Manugistics, Inc., Rockville, Maryland, United States) was used. The significant difference was defined as $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Morphological analysis

The mean values for the split-open and non-split-open pistachios were 52.6 and 42.6 %, respectively; there were not statistically significant differences among treatments (**Table 1**). On the other hand, the factor "rootstock" significantly affected the percentages of split and non-split open pistachios, with *P. terebinthus* having the highest value of split open nuts (60 %) and the lowest one of non-split open ones (35 %).

In general, RDI nuts had higher weights (whole, edible nut and shell) than control, especially T2 nuts (**Table 1**). For example, both RDI treatments (T1 and T2), shared the highest value for edible nut weight (~0.7 g). However, control samples were the biggest ones (length, height and width), except in their length; control had the highest values of height and width (10.516 and 9.021 g, respectively).

Regarding rootstocks, *P. terebinthus* showed the highest values (weight and size) whereas *P. integerrima* and *P. atlantica* having the lowest values of weight and size, respectively (**Table 1**).

Color of the samples did not present statistical significant differences, in most of the parameters studied, as affected by the irrigation treatments and rootstocks. Although, lightness (L^*) and tone (*Hue*) were affected by the irrigation treatments, with T1 having the lowest L^* value (64.05), which was reflected in a slight increase, but not significant, in tone (79.92). As can be seen in **Table 1**, the differences among color coordinates for the studied samples were lower than 2-units for most of the treatments. These authors (Galindo et al. (2015) and Navarro et al. (2011) have concluded that differences smaller than 2 units were imperceptible for the human eye. Even though, differences in these determinations were statistically significant, they did not represented an appreciable color change in the final product. Therefore, it can be stated that the irrigation treatment applied and the rootstock used did not significantly affected pistachio color. Similar results were previously reported by Carbonell-Barrachina et al. (2015), who showed that neither RDI treatments nor

rootstocks did significantly affect the weight, size, split-nuts and color of pistachio nuts.

3.2. Fatty acids

Composition of fatty acids from pistachios under study are presented in **Table 2**. Of the 9 fatty acids (FAMES) identified, 4 were saturated (SFAs), 3 monounsaturated (MUFAs) and 2 polyunsaturated (PUFAs). The SFAs were myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and arachidic acid (C20:0); the MUFAs were palmitoleic acid (C16:1), oleic acid (C18:1), and eicosenoic acid (C20:1) and finally linoleic acid (C18:2) and α -linolenic acid (C18:3) were the PUFAs.

In general, the abundance of FAMES followed the order: $C18:1 > C18:2 > C16:0 \gg C18:0 > C16:1 > C18:3 > C20:1 > C20:0 > C14:0$. The mean values for these compounds in the two RDI treatments (mean of T1 and T2) were 52.97 %, 31.12 %, 12.39 %, 1.33 %, 1.06 %, 0.53 %, 0.34 %, 0.13 % and 0.08 %, respectively; RDI did not significantly affect the FAMES content.

P. terebinthus had the highest percentage (53.88 %) of the main compound, oleic acid (C18:1), while *P. atlantica* had the lowest one (52.17 %). The second most abundant fatty acid was linoleic acid (C18:2), and *P. atlantica* and *P. integerrima* had the highest contents (31.64 and 31.49 %, respectively), without significant difference between them. In case specific of *P. integerrima*, the content of linoleic acid increased proportionately to the water restriction (**Table 2**). If FAMES are grouped according to their unsaturation degree, it can be observed that *P. atlantica* had the lowest concentration of the MUFAs, 53.6 %, and the combination of RDI T2 and *P. terebinthus* had the highest concentration.

This findings agreed with those reported by Acar et al. (2017), who reported that oleic, linoleic and palmitic acids were the 3 main fatty acids in pistachios. Okay and Sevin (2011) observed that the content of oleic acid increased and the content of linoleic acid decreased in well-irrigated trees. Carbonell-Barrachina et al. (2015) showed that a moderate irrigation treatment produced a significant increase in the

linoleic acid content. In another type of nuts, particularly almonds, Zhu et al. (2015) reported that moderate deficit irrigation did not affect to the lipid content.

3.3. Mineral content

Application of RDI on pistachios influenced their mineral composition (**Table 3**). Pistachios from T2 treatment presented higher contents of Na, Ca and Cu than those of the control nuts, but simultaneously they had lower contents of K and Mn. In case specific of Cu, an increase of water restriction led to an increase of this mineral, independently of the rootstock used. The decrease in irrigation water could cause, among other actions, an accumulation of available Ca and Na contents in the soil-plant system by reducing losses by leaching, leading to a reduced absorption and accumulation of K and Mn. This could be justified by the antagonistic effect that Ca and Na can cause on the rest of minerals; for instance, the antagonism trend between Na and K is one of the most studied in plant metabolism (Carbonell-Barrachina et al., 1997). Regarding rootstock, *P. Integerrima* showed the lowest contents of Mg and of all studied micro-nutrients (Cu, Mn, Fe, and Zn). As has been shown, pistachios are a good source of minerals, especially K with $\sim 9 \text{ g kg}^{-1}$, which contributes to normal functioning of the nervous system, to normal muscle function, and to the maintenance of normal blood pressure (Commission Regulation (EU) No 432/2012). The effect of RDI on pistachio had incidence on the content of K, in case of *P. integerrima* and *P. atlantica*, reducing their content when irrigation water was restricted. In case of *P. terebinthus* the content of K increased with moderate RDI (T1).

3.4. Antioxidant activity (AA) and total polyphenol content (TPC)

In general, RDI pistachios (T1 and T2) showed higher TPC than that of the control (except in case of *P. integerrima* in which T2 had the lowest content), being those from T1 treatment the ones with the highest content ($1284 \text{ g GAE kg}^{-1} \text{ dw}$). Additionally, RDI had a significant effect on the antioxidant activity measured by both

DPPH and FRAP methods (**Table 4**). The moderate RDI treatment (T1) showed no differences regarding these two AA methods with respect to the control, while under the severe irrigation treatment (T2) the antioxidant activity decreased, especially that quantified by the DPPH method. This effect can be observed for every interaction between irrigation treatment and rootstock. Otherwise, the AA measured by ABTS did not show significant differences among the irrigation treatments. On the other hand, the rootstock did not affect neither the TPC nor the AA of pistachios.

This result indicates that under high water stress, an important stomatal regulation occurs and the CO₂ produced is used to maintain the primary metabolism. However, under a mild or soft water stress, the CO₂ is redistributed to the formation of secondary metabolites although growth is slightly limited. This behavior has been observed in fruits with a high content of bioactive compounds on which RDI has been applied (Behboudian et al., 2011). In addition, situations of water stress causing an accumulation of antioxidant substances, as a physiological response for the removal or control of the free radicals, have been widely reported (Grant, 2012). Similar results were reported in olives by Gucci et al. (2019), who found that phenolic concentration in RDI treatment was higher than in other water regimes. On the other hand, Cano-Lamadrid et al. (2017) obtained that application of RDI treatments on table olives did not influence neither the AA nor the TPC.

3.5. Sensory analysis

The irrigation treatments significantly affected 5 out of the 15 the sensory attributes under analysis: color, size, pistachio-ID, aftertaste and crunchiness (**Table 5**). With respect to the attributes related with pistachios appearance (color and size), T1 treatment showed no differences with respect to the control, while the T2 had lower color intensity and small size (2.8 and 5.1 *versus* 3.5 and 7.7, respectively). Sample of control irrigation treatment (T0) had high intensity of color in case of rootstock *P. integerrima* and *P. atlantica*, while sample T1 was the most intense in case of *P. terebinthus*. However, RDI in general did not affect the sensory quality of

the pistachio; it only led to slight differences (although statistically significant) in pistachio-ID, aftertaste and crunchiness. The T2 treatment had the lowest intensity of pistachio flavor and aftertaste (6.2 and 6.8, respectively), while it had the highest intensity of crunchiness (T0= 7.0, T1= 6.7, and T2= 7.8). The highest crunchiness intensity may be linked to the fibrousness of the nut, due to low water accumulation in these nuts (T2, the most stressed ones). Similar results were obtained by Galindo et al. (2015), who showed that the texture attributes, including crunchiness, in jujube were increased by severe RDI conditions.

On the other hand, the factor "rootstock" had more effects on the pistachio sensory profile than the factor "irrigation treatment". *P. atlantica* nuts had the highest size as compared to *P. terebinthus* and *P. integerrima*. Among the flavor attributes, nutty (global), pistachio-ID, salty, sweet and aftertaste were significantly affected by the rootstock. *P. terebinthus* showed the lowest score for the attributes nutty, pistachio-ID, salty and aftertaste (6.7, 6.6, 0.4 and 6.9, respectively) but the highest sweetness (3.2). In contrast, *P. integerrima* presented the highest values for nutty, pistachio-ID, salty and aftertaste attributes (7.7, 7.6, 0.8 and 7.6, respectively) and the lowest values for sweet (2.2). *P. atlantica* presented a sensory profile in between those of *P. integerrima* and *P. terebinthus*. Regarding texture, *P. integerrima* and *P. terebinthus* presented high values for hardness and crunchiness (*P. terebinthus* 7.6 and 7.8, respectively; *P. integerrima* 8.1 and 7.8, respectively). Regarding friability, *P. integerrima* and *P. atlantica* showed the highest values (6.6 and 5.7, respectively) and *P. integerrima* had the lowest values for adhesiveness (5.7), which was positive because it is considered as a negative attribute by pistachio consumers.

According to the information related to the interaction between the two studied factors (irrigation and rootstock), crunchiness and hardness was reduced with moderate RDI in case of *P. integerrima* and *P. terebinthus*, while for *P. atlantica* the RDI treatments (T1 and T2) led to obtain pistachios with high intensity these two attributes.

As described by Carbonell-Barrachina et al. (2015), the purchase choice of international pistachio consumers based on the sensory attributes is due to pistachio flavor, saltiness, crunchiness and toasted flavor. Although, Noguera-Artiaga et al. (2016) exposed that international consumers preferred intense crunchy but low salty nuts.

4. CONCLUSIONS

The results obtained in this study demonstrated that moderate deficit irrigation (T1) has increased the TPC without showing statistically significant effects on AA, FAMES profile, weight, number of open pistachios, color, appearance and texture (crunchiness) of pistachios. The application of a severe deficit irrigation (T2) led to decreases on size, AA, and intensity of pistachio-ID flavor and aftertaste, although it had similar values of many other parameters, such as number of open pistachios, texture, flavor, color, TPC and FAMES profile and even an increased the weight of the samples. On the other hand, the results established that the choice of the rootstock on pistachio cultivation had no significant influence on color, TPC and AA. *P. integerrima* and *P. terebinthus* resulted in an overall increase of MUFAs, size, and texture, despite showing the worst appearance. Furthermore, *P. integerrima* had the highest pistachio-ID flavor and *P. terebinthus* showed the highest weight and number of open pistachios. These results indicated that the application of moderate deficit irrigation (T1) led to an increase product functionality and quality, without jeopardizing nut yield, weight or size, consequently generating savings of water during cultivation, and resulting in less environmental and economic costs. On the other hand, *P. terebinthus* presented appropriate FAME profile, size, texture and weight; thus, it would be also a good choice for the farmers. As a final recommendation, results obtained here proved that the combination moderate deficit irrigation (T1) and *P. terebinthus* rootstock is the best one according to the nuts composition, functionality and sensory quality.

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REFERENCES

- Acar, I., Kafkas, S., Kapchina-Toteva, V., Ercisli, S., 2017. Effect of rootstock on fat content and fatty acid composition of immature pistachio kernels. *Comptes rendus de l'Académie bulgare des Sciences* 70, 1049-1056.
- Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration — guidelines for computing crop water requirements, in: Organization, F.a.A. (Ed.), *FAO Irrigation and drainage paper* 56, Rome.
- Behboudian, M.H., Marsal, J., Girona, J., Lopez, G., 2011. Quality and Yield Responses of Deciduous Fruits to Reduce Irrigation, In: Janick, J. (Ed.), *Horticultural Reviews*, pp. 149-189.
- Behboudian, M.H., Mills, T.M., 1997. Deficit Irrigation in Deciduous Orchards, In: Janick, J. (Ed.), *Horticultural Reviews*, pp. 105-131.
- Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 239, 70-76. <https://doi.org/10.1006/abio.1996.0292>.
- Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* 28, 25-30. [https://doi.org/10.1016/s0023-6438\(95\)80008-5](https://doi.org/10.1016/s0023-6438(95)80008-5).
- Cano-Lamadrid, M., Hernandez, F., Corell, M., Burlo, F., Legua, P., Moriana, A., Carbonell-Barrachina, A.A., 2017. Antioxidant capacity, fatty acids profile, and descriptive sensory analysis of table olives as affected by deficit irrigation. *J. Sci. Food Agric.* 97, 444-451. <https://doi.org/10.1002/jsfa.7744>.

- Carbonell-Barrachina, A.A., Garcia, E., Sanchez Soriano, J., Aracil, P., Burlo, F., 2002. Effects of raw materials, ingredients, and production lines on arsenic and copper concentrations in confectionery products. *J. Agric. Food Chem.* 50, 3738-3742. <https://doi.org/10.1021/jf0115591>.
- Carbonell-Barrachina, A.A., Memmi, H., Noguera-Artiaga, L., Gijon-Lopez Mdel, C., Ciapa, R., Perez-Lopez, D., 2015. Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *J. Sci. Food Agric.* 95, 2866-2873. <https://doi.org/10.1002/jsfa.7027>.
- Carbonell-Barrachina, A.A., Burló-Carbonell, F., Mataix-Beneyto, J., 1997. Effect of sodium arsenite and sodium chloride on bean plant nutrition (macronutrients). *J. Plant Nutr.* 20, 1617-1633. <https://doi.org/10.1080/01904169709365361>.
- Commision Regulation (EU) No 432/2012, Commision Regulation (EU) No 432/2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health Text with EEA relevance.
- Couceiro, J.F., Guerrero, J., Gijón, M.C., Pérez-López, D., Moriana, A., Rodriguez, M., 2013. *El Cultivo del Pistacho*. Mundi-Prensa.
- Fereres, E., Goldhamer, D., 1990. *Deciduous fruit and nut trees*. Agronomy (USA).
- Fereres, E., Goldhamer, D.A., Parsons, L.R., 2003. Irrigation water management of horticultural crops. *HortScience* 38, 1036-1042.
- Galindo, A., Collado-González, J., Griñán, I., Corell, M., Centeno, A., Martín-Palomo, M.J., Girón, I.F., Rodríguez, P., Cruz, Z.N., Memmi, H., Carbonell-Barrachina, A.A., Hernández, F., Torrecillas, A., Moriana, A., Pérez-López, D., 2018. Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. *Agric. Water Manage.* 202, 311-324. <https://doi.org/10.1016/j.agwat.2017.08.015>.
- Galindo, A., Noguera-Artiaga, L., Cruz, Z.N., Burló, F., Hernández, F., Torrecillas, A., Carbonell-Barrachina, Á.A., 2015. Sensory and physico-chemical quality attributes of jujube fruits as affected by crop load. *LWT - Food Sci. Technol.* 63, 899-905. <https://doi.org/10.1016/j.lwt.2015.04.055>.
- Gao, X., Ohlander, M., Jeppsson, N., Bjork, L., Trajkovski, V., 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *J. Agric. Food Chem.* 48, 1485-1490. <https://doi.org/10.1021/jf991072g>.
- Gijón, M.C., Gimenez, C., Pérez-López, D., Guerrero, J., Couceiro, J.F., Moriana, A., 2011. Water relations of pistachio (*Pistacia vera* L.) as affected by phenological stages and water regimes. *Sci. Hortic.* 128, 415-422. <https://doi.org/10.1016/j.scienta.2011.02.004>.

- Goldhamer, D.A., 1995. Irrigation management, In: Ferguson, L. (Ed.), Pistachio Production Manual. Fruit and Nut Research and Information Center, University of California, Davis, CA (USA), pp. 71-81.
- Goldhamer, D.A., Phene, B.C., Beede, R., Sherlin, L., Mahan, S., Rose, D., 1987. Effects of sustained deficit irrigation on pistachio tree performance, Annual Report. Crop Year 1986-1987. California Pistachio Industry, Fresno, CA (USA), pp. 61-66.
- Grant, O.M., 2012. Understanding and exploiting the impact of drought stress on plant physiology, Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability, pp. 89-104.
- Gucci, R., Caruso, G., Gennai, C., Esposto, S., Urbani, S., Servili, M., 2019. Fruit growth, yield and oil quality changes induced by deficit irrigation at different stages of olive fruit development. *Agric. Water Manage.* 212, 88-98.
<https://doi.org/10.1016/j.agwat.2018.08.022>.
- Memmi, H., Gijón, M.C., Couceiro, J.F., Pérez-López, D., 2016. Water stress thresholds for regulated deficit irrigation in pistachio trees: Rootstock influence and effects on yield quality. *Agric. Water Manage.* 164, 58-72.
<https://doi.org/10.1016/j.agwat.2015.08.006>.
- Moriana, A., Memmi, H., Centeno, A., Martín-Palomo, M.J., Corell, M., Torrecillas, A., Pérez-López, D., 2018. Influence of rootstock on pistachio (*Pistacia vera* L. cv Kerman) water relations. *Agric. Water Manage.* 202, 263-270.
<https://doi.org/10.1016/j.agwat.2017.12.026>.
- Navarro, P., Nicolas, T.S., Gabaldon, J.A., Mercader-Ros, M.T., Calin-Sanchez, A., Carbonell-Barrachina, A.A., Perez-Lopez, A.J., 2011. Effects of cyclodextrin type on vitamin C, antioxidant activity, and sensory attributes of a mandarin juice enriched with pomegranate and goji berries. *J. Food Sci.* 76, S319-S324.
<https://doi.org/10.1111/j.1750-3841.2011.02176.x>.
- Noguera-Artiaga, L., Lipan, L., Vazquez-Araujo, L., Barber, X., Perez-Lopez, D., Carbonell-Barrachina, A.A., 2016. Opinion of Spanish Consumers on Hydrosustainable Pistachios. *J. Food Sci.* 81, S2559-S2565.
<https://doi.org/10.1111/1750-3841.13501>.
- Okay, Y., Sevin, M., 2011. The effect of irrigation on protein, fat and fatty acids amounts in pistachio. *Acta Hort.* 912, 211-216.
<https://doi.org/10.17660/ActaHortic.2011.912.30>.
- Pérez-López, D., Memmi, H., Gijón-López, M.d.C., Moreno, M.M., Couceiro, J.F., Centeno, A., Martín-Palomo, M.J., Corell, M., Noguera-Artiaga, L., Galindo, A., Torrecillas, A., Moriana, A., 2018. Irrigation of Pistachios, In: García Tejero,

- I.F., Durán Zuazo, V.H. (Eds.), *Water Scarcity and Sustainable Agriculture in Semiarid Environment*. Academic Press, pp. 247-269.
- Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53, 4290-4302. 10.1021/jf0502698.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* 26, 1231-1237. [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3).
- Zhu, Y., Taylor, C., Sommer, K., Wilkinson, K., Wirthensohn, M., 2015. Influence of deficit irrigation strategies on fatty acid and tocopherol concentration of almond (*Prunus dulcis*). *Food Chem.* 173, 821-826. <https://doi.org/10.1016/j.foodchem.2014.10.108>.



Table 1. Weight, size, color and nature of pistachios as affected by deficit irrigation treatment and rootstock.

Factor	Weight (g)			Size (mm)			Color Coordinates					Number per 100 units		
	Whole	Edible nut	Shell	Length	Height	Width	L*	a*	b*	C*	Hue	Split open	Non-split open	Others [§]
ANOVA Test[†]														
Treatment	***	***	***	**	***	***	**	NS	NS	NS	*	NS	NS	NS
Rootstock	***	***	***	***	***	***	NS	NS	NS	NS	NS	***	***	NS
Treat. x Rootstock	**	**	**	***	***	***	NS	NS	NS	NS	NS	*	*	NS
Tukey's Multiple Range Test[‡]														
Treatment														
T0	1.350 c	0.704 b	0.646 b	16.635 ab	10.516 a	9.021 a	66.23 a	-6.45	33.39	34.01	79.07 ab	55	39	6
T1	1.382 b	0.728 a	0.654 b	16.476 b	10.335 b	8.798 b	64.05 b	-5.90	33.04	33.58	79.92 a	51	45	4
T2	1.408 a	0.727 a	0.681 a	16.755 a	10.110 c	8.823 b	65.44 a	-6.63	33.34	34.00	78.77 b	52	44	4
Rootstock														
<i>P. integerrima</i>	1.343 b	0.705 b	0.638 b	16.417 b	10.351 a	8.937 a	65.00	-6.39	33.89	34.50	79.39	48 b	48 a	4
<i>P. atlantica</i>	1.393 a	0.716 b	0.678 a	16.711 a	10.148 b	8.657 b	65.46	-6.37	33.19	33.30	78.98	51 b	46 a	3
<i>P. terebinthus</i>	1.404 a	0.738 a	0.666 a	16.739 a	10.463 a	9.047 a	65.25	-6.22	32.69	33.78	79.41	60 a	35 b	5
Treat. x Rootstock														
T0* <i>P. integerrima</i>	1.329 c	0.699 c	0.630 c	16.244 c	10.516 a	9.033 a	66.18	-6.57	34.13	34.76	79.11	49 b	46 a	4
T1* <i>P. integerrima</i>	1.357 bc	0.717 ab	0.639 c	16.420 bc	10.393 b	8.924 ab	63.26	-5.73	33.31	33.83	80.36	46 b	51 a	4
T2* <i>P. integerrima</i>	1.344 bc	0.699 c	0.645 b	16.587 bc	10.144 bc	8.855 b	65.56	-6.86	34.22	34.91	78.70	51 b	46 a	3
T0* <i>P. atlantica</i>	1.357 b	0.692 c	0.665 b	16.997 a	10.284 b	8.856 b	66.19	-6.31	32.65	33.26	79.06	51 b	46 a	3
T1* <i>P. atlantica</i>	1.377 b	0.717 ab	0.660 b	16.234 c	9.973 c	8.323 c	64.23	-6.38	32.64	33.26	78.95	51 b	46 a	4
T2* <i>P. atlantica</i>	1.445 a	0.737 a	0.708 a	16.902 a	10.186 bc	8.793 b	65.98	-6.42	32.77	33.40	78.91	51 b	47 a	3
T0* <i>P. terebinthus</i>	1.363 b	0.721 ab	0.642 c	16.666 bc	10.748 a	9.173 a	66.32	-6.47	33.39	34.01	79.05	62 a	34 b	4
T1* <i>P. terebinthus</i>	1.412 ab	0.749 a	0.664 b	16.774 b	10.638 a	9.147 a	64.66	-5.58	33.16	33.63	80.46	59 a	36 b	5
T2* <i>P. terebinthus</i>	1.436 a	0.744 a	0.692 a	16.777 b	10.001 c	8.821 b	64.78	-6.61	33.03	33.69	78.70	58 a	37 b	5
Pooled variance	0.022	0.012	0.018	0.014	0.012	0.024	0.53	0.78	0.64	1.35	1.48	3	3	2

[†] NS: not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test. [§] 'Others' means broken shell, unpeeled, dark color, etc.

Table 2. Fatty acid composition of pistachios as affected by deficit irrigation treatment and rootstock.

Factor	Fatty Acids (%)									SFA	MUFA	PUFA
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1			
ANOVA Test[†]												
Treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rootstock	NS	**	**	NS	*	*	*	NS	NS	NS	**	NS
Treat. x Rootstock	NS	NS	NS	NS	NS	*	NS	NS	*	NS	*	NS
Tukey's Multiple Range Test[‡]												
Treatment												
T0	0.08	12.49	1.05	1.34	52.78	31.23	0.55	0.12	0.32	14.03	54.15	31.78
T1	0.08	12.38	1.10	1.30	53.23	30.85	0.51	0.14	0.34	13.90	54.67	31.36
T2	0.08	12.29	1.04	1.36	52.90	31.29	0.54	0.13	0.36	13.86	54.30	31.83
Rootstock												
<i>P. integerrima</i>	0.08	12.17 b	1.03 b	1.29	52.91 ab	31.49 a	0.54 ab	0.13	0.34	13.67	54.88 a	32.03
<i>P. atlantica</i>	0.08	12.61 a	1.13 a	1.31	52.17 b	31.64 a	0.55 a	0.14	0.34	14.14	53.64 b	32.19
<i>P. terebinthus</i>	0.07	12.39 ab	1.03 b	1.40	53.88 a	30.24 b	0.50 b	0.12	0.34	13.98	55.25 a	31.74
Treat. x Rootstock												
T0* <i>P. integerrima</i>	0.08	12.39	1.04	1.32	53.59	30.60 b	0.57	0.10	0.29 b	13.89	54.91 b	31.17
T1* <i>P. integerrima</i>	0.08	12.20	1.07	1.27	52.94	31.42 ab	0.52	0.15	0.35 ab	13.70	54.36 bc	31.94
T2* <i>P. integerrima</i>	0.09	11.98	0.99	1.27	52.36	32.25 a	0.54	0.12	0.38 a	13.47	53.74 c	32.78
T0* <i>P. atlantica</i>	0.09	12.60	1.11	1.38	51.56	32.17 a	0.58	0.15	0.36 ab	14.22	53.03 c	32.75
T1* <i>P. atlantica</i>	0.08	12.62	1.16	1.29	53.32	30.46 b	0.53	0.15	0.30 b	14.15	54.79 b	30.99
T2* <i>P. atlantica</i>	0.07	12.59	1.09	1.26	51.63	32.28 a	0.55	0.13	0.35 ab	14.05	53.07 c	32.82
T0* <i>P. terebinthus</i>	0.08	12.52	1.01	1.31	53.42	30.67 b	0.50	0.10	0.31 ab	14.01	54.74 b	31.17
T1* <i>P. terebinthus</i>	0.07	12.33	1.03	1.34	53.31	30.95 b	0.48	0.13	0.36 ab	13.87	54.70 b	31.43
T2* <i>P. terebinthus</i>	0.07	12.31	1.05	1.55	54.70	29.33 b	0.52	0.14	0.34 ab	14.06	56.09 a	29.85
Pooled variance	0.02	0.33	0.08	0.13	1.44	1.45	0.05	0.03	0.04	0.24	1.65	1.48

[†]NS: not significant at $p < 0.05$; * and **, significant at $p < 0.05$ and 0.01 , respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 3. Mineral content of pistachios as affected by deficit irrigation treatment and rootstock.

Factor	K	Na	Ca	Mg	Cu	Mn	Fe	Zn
	Macro-elements (g kg ⁻¹)				Micro-elements (mg kg ⁻¹)			
ANOVA Test[†]								
Treatment	***	*	**	NS	***	***	NS	NS
Rootstock	NS	NS	NS	***	**	**	***	***
Treat. x Rootstock	*	NS	NS	NS	*	***	NS	NS
Tukey's Multiple Range Test[‡]								
Treatment								
T0	9.373 a	0.264 b	0.980 b	1.105	16.41 c	6.96 a	27.34	21.59
T1	9.512 a	0.279 ab	1.063 a	1.109	22.01 b	7.06 a	30.45	21.85
T2	9.050 b	0.294 a	1.047 a	1.136	28.25 a	5.88 b	30.91	22.99
Rootstock								
<i>P. integerrima</i>	9.412	0.285	1.022	1.079 b	19.40 b	6.01 b	23.54 b	16.5 b
<i>P. atlantica</i>	9.360	0.270	1.035	1.128 a	24.23 a	6.72 ab	31.83 a	24.0 a
<i>P. terebinthus</i>	9.178	0.269	1.056	1.158 a	23.91 a	7.21 a	34.53 a	25.3 a
Treat. x Rootstock								
T0* <i>P. integerrima</i>	9.471 ab	0.263	0.976	1.076	14.95 c	6.52 ab	22.01	17.35
T1* <i>P. integerrima</i>	9.301ab	0.299	1.016	1.102	23.55 b	6.13 ab	25.42	17.33
T2* <i>P. integerrima</i>	8.749 c	0.294	1.077	1.060	19.97 bc	5.31 b	23.20	15.06
T0* <i>P. atlantica</i>	9.596 ab	0.267	0.987	1.135	18.48 bc	6.67 ab	29.10	23.95
T1* <i>P. atlantica</i>	9.502 ab	0.270	1.079	1.093	23.28 b	7.26 a	32.41	22.75
T2* <i>P. atlantica</i>	9.196 bc	0.273	1.041	1.157	31.36 a	6.15 ab	33.98	25.60
T0* <i>P. terebinthus</i>	9.160 bc	0.271	1.005	1.146	17.67 c	7.40 a	34.28	23.65
T1* <i>P. terebinthus</i>	9.746 a	0.260	1.124	1.133	19.28 bc	7.82 a	33.58	26.13
T2* <i>P. terebinthus</i>	9.218 b	0.275	1.045	1.194	35.96 a	6.13 ab	35.73	26.16
Pooled variance	0.206	0.020	0.071	0.088	0.63	0.54	3.42	1.53

[†] NS: not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test.

Table 4. Total polyphenol content [g gallic acid equivalent (GAE) kg⁻¹ dry weigh, dw] and antioxidant activity (mmol Trolox kg⁻¹ dw) of pistachios as affected by deficit irrigation treatment and rootstock.

Factor	TPC	DPPH	FRAP	ABTS
	(g GAE kg ⁻¹ dw)	(mmol Trolox kg ⁻¹ dw)		
ANOVA Test[†]				
Treatment	*	***	***	NS
Rootstock	NS	NS	NS	NS
Treatment x Rootstock	*	***	NS	NS
Tukey's Multiple Range Test[‡]				
Treatment				
T0	1192 b	19.19 a	35.45 a	21.54
T1	1284 a	18.46 a	33.49 ab	22.82
T2	1211 ab	10.64 b	29.63 b	20.98
Rootstock				
<i>P. integerrima</i>	1242	14.12	32.08	21.75
<i>P. atlantica</i>	1212	16.86	32.20	21.63
<i>P. terebinthus</i>	1187	17.31	33.30	21.97
Treat. x Rootstock				
T0* <i>P. integerrima</i>	1212 ab	19.63 a	35.91	21.55
T1* <i>P. integerrima</i>	1287 a	21.79 a	35.85	24.14
T2* <i>P. integerrima</i>	1063 b	9.19 b	26.95	20.15
T0* <i>P. atlantica</i>	1158 b	18.84 a	35.58	21.83
T1* <i>P. atlantica</i>	1249 a	21.77 a	31.32	22.79
T2* <i>P. atlantica</i>	1230 ab	11.31 b	29.62	20.13
T0* <i>P. terebinthus</i>	1204 b	19.09 a	34.01	21.29
T1* <i>P. terebinthus</i>	1255 a	11.83 b	30.19	21.51
T2* <i>P. terebinthus</i>	1267 a	11.44 b	32.08	22.29
Pooled variance	76	2.80	4.64	2.20

[†]NS: not significant at p < 0.05; * and ***, significant at p < 0.05 and 0.001, respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different (p < 0.05), Tukey's least significant difference test.

Table 5. Descriptive sensory analysis of pistachios as affected by deficit irrigation treatment and rootstock.

Factor	Visual		Flavor							Texture					
	Color	Size	Nutty	Pistachio-ID	Floral	Salty	Sweet	Bitter	Astringent	Aftertaste	Wood	Hardness	Crunchiness	Friability	Adhesiveness
ANOVA Test[†]															
Treatment	*	***	NS	***	NS	NS	NS	NS	NS	**	NS	NS	*	NS	NS
Rootstock	NS	***	***	***	NS	**	*	NS	NS	**	NS	***	***	***	***
Treat. × Rootstock	***	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	***	NS	NS
Tukey's Multiple Range Test[‡]															
Treatment															
T0	3.5 a	7.7 a	7.3	7.3 a	2.9	0.7	2.9	1.0	0.8	7.4 a	5.5	7.0	7.0 b	5.0	6.7
T1	3.2 ab	7.8 a	6.9	6.9 a	1.6	0.5	2.6	1.3	0.8	7.4 a	5.8	7.0	6.7 b	5.5	7.0
T2	2.8 b	5.1 b	7.4	6.2 b	1.7	0.4	2.7	1.1	0.8	6.8 b	5.9	7.8	7.8 a	5.4	6.3
Rootstock															
<i>P. integerrima</i>	3.2	6.3 b	7.7 a	7.6 a	1.5	0.8 a	2.2 b	1.2	0.8	7.6 a	6.3	8.1 a	7.8 a	6.6 a	5.7 b
<i>P. atlantica</i>	3.3	7.5 a	7.2 ab	6.2 b	1.8	0.4 b	2.7 ab	1.1	0.8	7.1 ab	5.8	6.1 b	5.9 b	5.7 a	7.0 a
<i>P. terebinthus</i>	3.1	6.8 b	6.7 b	6.6 b	1.7	0.4 b	3.2 a	0.9	0.8	6.9 b	5.7	7.6 a	7.8 a	4.5 b	7.3 a
Treat. × Rootstock															
T0* <i>P. integerrima</i>	3.8 a	7.0 b	7.9	8.1	1.7	1.4	2.3	1.2	0.7	8.0	6.3	8.4 a	8.2 a	6.4	5.5
T1* <i>P. integerrima</i>	2.9 b	7.2 b	7.2	6.7	1.6	0.7	2.3	1.1	0.7	7.2	6.6	7.2 b	6.6 b	6.0	6.5
T2* <i>P. integerrima</i>	2.9 b	4.5 c	8.0	7.9	1.3	0.4	2.1	1.3	0.8	7.5	6.3	8.8 a	8.6 a	7.4	5.1
T0* <i>P. atlantica</i>	4.1 a	8.0 ab	6.9	6.9	1.8	0.4	3.1	0.8	0.8	7.3	6.6	4.6 c	4.6 c	4.0	7.6
T1* <i>P. atlantica</i>	2.9 b	9.0 a	7.5	6.0	1.6	0.4	2.4	1.6	0.7	6.9	4.0	7.4 b	7.0 b	5.3	6.5
T2* <i>P. atlantica</i>	2.9 b	5.5 c	7.2	5.7	2.0	0.4	2.5	1.0	0.7	7.2	4.1	6.4 b	6.1 b	4.4	7.0
T0* <i>P. terebinthus</i>	2.7 b	8.0 ab	7.0	7.0	1.7	0.4	3.3	0.9	0.9	7.0	4.9	8.0 a	8.0 a	5.5	7.0
T1* <i>P. terebinthus</i>	3.8 a	7.1 b	6.0	5.8	1.6	0.4	3.2	1.0	0.8	6.4	6.1	6.5 b	6.5 b	5.7	8.1
T2* <i>P. terebinthus</i>	2.7 b	5.3 c	7.0	7.1	1.7	0.4	3.1	1.0	0.9	7.4	6.3	8.1 a	8.8 a	5.9	6.7
Pooled variance	0.3	0.8	0.5	0.3	1.2	0.3	0.4	0.3	0.2	0.6	0.8	0.8	0.4	0.9	0.7

[†]NS: not significant at p< 0.05; *, **, and ***, significant at p< 0.05, 0.01, and 0.001, respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different (p<0.05), Tukey's least significant difference test.

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VOLATILE, SENSORY AND FUNCTIONAL PROPERTIES OF HYDROSOS PISTACHIOS

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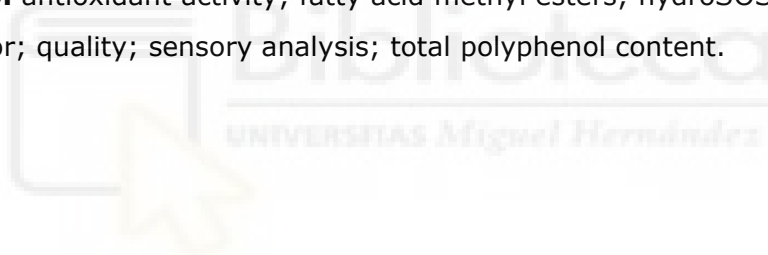
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ABSTRACT

Climate change, the increase in world population, and the intensification of urban and industrial activities, will cause a shortage of water for agriculture. This situation requires conscientious studies to manage water deficits without affecting the quality of the crops. In this study, regulated deficit irrigation (RDI) strategies and three rootstocks (*P. atlantica*, *P. integerrima*, and *P. terebinthus*) were applied to pistachio cultivation to study the quality of fruits obtained based on the morphological, functional, aroma, and their sensory properties. The results obtained demonstrated that RDI T1 (during phenological phase II of cultivation the stem water potential was maintained around -1.5 MPa) led to pistachios with same morphological properties, total polyphenol content, antioxidant activity, volatile composition, sensory properties, better profile of fatty acids, and being the favorite ones for international consumers, as compared to pistachios obtained under full irrigation treatments. On the other hand, when *P. integerrima* was used, pistachios obtained had the highest weight, the lowest content of sucrose and the best functional properties.

KEYWORDS: antioxidant activity; fatty acid methyl esters; hydroSOS; *Pistacia vera*; pistachio flavor; quality; sensory analysis; total polyphenol content.



1. INTRODUCTION

Mediterranean and South American countries, Southern California, Southern Australia and South Africa are characterized by partially wet springs and autumns, mostly rainy winters and hot dry summers. Water scarcity and water deficits in plants, mainly due to scarce rainfall, must be supplemented with irrigation treatments. In addition, different factors, such as climate change, the increase in world population, and the intensification of urban and industrial activities, will cause a shortage of water for agriculture, and it will become more and more severe in the near future [1]. This situation requires more conscientious studies to manage water deficits without affecting the quality of crops. These studies should focus on crops which are able to withstand deficit irrigation or have low water needs but without drastic impacts on production and fruit quality [2].

One of the techniques focused on the reduction of irrigation water during fruits and vegetables farming is regulated deficit irrigation (RDI). RDI consists of the imposition of water deficits in specific phenological stages, which are less sensitive to water stress without affect the crop yield or its economic benefits [3,4].

Pistachio (*Pistacia vera*) is considered the only commercially edible nut among the different species in the genus *Pistacia*, and it has been cultivated for centuries in Mediterranean areas and is considered resistant to both drought and salinity [5]. This tolerance is based mainly on crop yields, but the physicochemical, functional and sensory quality of nuts has not been fully characterized. For their vegetative propagation, pistachio trees requires the use of rootstocks, because they cannot be propagated by cutting and planting because this propagation material do not produce enough roots [6]. The main rootstocks used for pistachio cultivation are *P. atlantica* Desf., *P. integerrima* L., *P. terebinthus* L. and *P. vera* L. [7]. Cultivation of pistachio trees has become a very profitable business, because in recent years, their harvesting was fully mechanized, the inputs associated to their cultivation has decreased, and the prize paid to producers is constantly increased [2]. In the future, this trend is expected to keep increasing due to the many studies supporting the health benefits observed after pistachio consumption [8,9]. It has been proved that the pistachio antioxidant capacity, total phenolic content, monounsaturated and polyunsaturated acids, lutein, phytosterols, and another functional compounds (founded on the pistachio nuts) were responsible for the anti-inflammatory potential, helping to promote cardiovascular health, and foster protective effect against colorectal and breast cancer of this nuts [10–13].

For all the above reasons, it is necessary to establish or identify those parameters that allow characterizing the quality of pistachios. In this sense, the main objective of this study was to evaluate the quality of pistachio nuts obtained using three irrigation treatments and three rootstocks, based on their morphological properties, fatty acids

content, antioxidant properties, total polyphenol content, volatile composition and their sensory properties.

2. MATERIALS AND METHODS

2.1. Plant material, growing conditions and experimental design

Pistachio nuts from trees (*P. vera*), cultivar "Kerman" were collected during 2016 from the experimental orchard "La Entresierra" located at Ciudad Real, Spain (3°56' W, 39° N; altitude 640 m above sea level). This area is characterized by a Mediterranean climate, with an average annual rainfall of 397 mm. The soil is a shallow clay-loam (Petrocalcic Palexeralfs) with a discontinuous petrocalcic horizon located at 0.5 m with a pH about 8.1, low electrical conductivity (0.2 dS/m), 1.05% organic matter, 0.12% N, 17×10^{-4} mol/kg K and a high cation exchange capacity (0.186 mol/kg).

Eighteen plots were used for this study with a completely randomized factorial design. Each of these plots had 12 trees (2 on the center for the analyses and 10 surrounded them) with same conditions of irrigation and rootstock. Pistachio trees were grafted over 3 rootstocks: (i) *P. atlantica*, (ii) *P. integerrima* and (iii) *P. terebinthus*, and, 3 irrigation treatments: (i) T0, in which trees were irrigated to ensure non-limiting water conditions in the soil (100 % ETC of the previous week); (ii) T1, in which irrigation was suppressed (during phase II) until pistachio trees reached a stem water potential (SWP) below -1.5 MPa; and (iii) T2 with same irrigation protocol as T1 but with a SWP threshold of -2.0 MPa. Water relations were evaluated according to Memmi [14].

Pistachio nuts were collected from the field, and after being peeled and dried (convection oven with hot air at 60 °C until the moisture content of 5 %), were immediately vacuum-packed and posted to the Universidad Miguel Hernández de Elche facilities in Orihuela (Alicante, Spain). Once there, samples were kept at 4-5 °C until analysis.

2.2. Volatile compounds

The extraction of the volatile compounds of the samples of pistachios was carried out using the headspace solid-phase micro-extraction (HS-SPME) method. A sample of 1 g of ground pistachios was placed on a 50 mL vial, with a magnetic bar, and closed with an aluminum layer (foil). After equilibration time, 5 min at 45 °C, a 30/50 µm fiber (SUPELCO) covered by DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane) was exposed to the vial headspace at 45 °C, with continuous agitation (500 rpm) in a magnetic stirrer (IKA C-MAG HS 4, IKA-Werke GmbH & Co. KG, Staufen, Germany). After 25 min of exposure, fiber was put in a gas chromatograph to analyse.

The isolation and identification of the volatile compounds previously extracted by HS-SPME were performed using a Saturn 2000 Varian Chrompack gas chromatograph (Varian, Inc., Palo Alto, CA, USA) with an HP-5 column (5 % Phenyl Methylpolysiloxane) 30 m × 0.53 mm ID × 1.0 μm (Agilent, Santa Clara, CA, USA). A mass spectrometer equipped with an ion-analyzer was set to 1508 V for all analyzes, and an electronic voltage factor to 1350 V. The analysis was carried out from 39 to 400 m/z, with an electronic impact (EI) of 70 eV, in 1 scan/s mode. Helium was used as carrier gas at a flow rate 1.0 mL/min and with a split ratio of 1:20. The injector and detector temperatures were 200 and 300 °C, respectively. The oven temperature started at 40 °C and, after 3 min, was increased by 5 °C/min up to 110 °C. Then, the temperature was increased by 20 °C/min up to 270 °C. The total analysis program lasted 25 min.

The volatile compounds were identified using three analytical methods: (i) Kovats Index (KI), (ii) GC-MS retention index (original chemical compound), and (iii) mass spectrum (original chemical compound and collection of the NIST05 and Adams 2012 spectrum library). The retention indexes were calculated using standard of aliphatic hydrocarbons in the range from C-5 to C-23. For the identification and determination of volatile compounds, the MS Workstation (Version 6.5, 2005 Varian, Palo Alto, CA, USA) and MestReNova (Version 9.0.1, 2014, Mestrelab Research, Santiago de Compostela, Spain) programs were used.

2.3. Sensory analysis

The sensory analysis of samples was focused only on the analysis of the pistachios obtained under different irrigation treatments, in order to minimize the number of samples and, thus, maintain the panelists concentration to the maximum. Based on previous results [7], pistachio nuts obtained by *P. atlantica* rootstock were used on the sensory study.

To obtain information about the consumer opinion on the sensory properties of pistachios, a sensory evaluation with consumer panel was carry out in 3 countries: Mexico, Poland, and Spain. At least 60 consumers were recruited in each country. Consumers had to complete a screening questionnaire stating their age, gender, and allergies or diet restrictions. Consumers were asked about nut consumption frequency and willingness to taste pistachios. Consumers who stated that they were 18–70 years old, had no diet restrictions or allergies, ate any kind of nut at least once per week and were willing to taste pistachios were recruited for testing. In the specific case of Poland, the ballots, screeners and demographic questionnaires were translated from Spanish to Polish, and, then, back to Spanish.

Ten pistachio nuts were served, to each panelist, in odor-free disposable 60 mL covered plastic cups, coded using three-digit numbers, and at room temperature.

Unsalted crackers and drinking water were used between samples to clean the panelists' palate. Natural illumination was used during the test, and testing room was at 20 ± 2 °C.

Consumers responded using a 9-point hedonic scale, where 9 = like extremely and 1 = dislike extremely. Consumers were, then, asked to indicate their order of preference for the samples, and mark the reasons of their preference regarding the attributes under study (size, peel, color, pistachio-ID, toasted, sweet, sour, aftertaste, oiliness, hardness, crunchiness, friability and adhesiveness). Then, consumers were asked about their "global" satisfaction degree for the samples under evaluation and for their intent to purchase.

2.4. Determination of sugars and organic acids

Sugars and organic acids were identified and quantified according to Hernández [15], with some modifications. Approximately 1 g of sample was diluted in 5 mL of phosphate buffer (pH 7.8), homogenized by Ultra-Turrax™ (IKA L004640, Staufen, Germany) for 1 min, and centrifuged at $15,000 \times g$ for 10 min. Finally, samples were filtered through a 0.45 μm Millipore filter. For the determination of the content of sugars and organic acids on samples, an HPLC (high-performance liquid chromatograph) Hewlett-Packard series 1100 (Hewlett-Packard, Wilmington, DE, USA) was used. The elution buffer consisted of 0.1 % phosphoric acid with a flow rate of 0.5 mL/min.

Sugars and organic acids were isolated using a Supelco column (Supelcogel™ C-610H column 30 cm \times 7.8 mm, Supelco, Inc., Bellefonte, PA, USA) and a precolumn Supelguard (5 cm \times 4.6 mm; Supelco), and the absorbance was measured at 210 nm using a diode-array detector (DAD). Standards of sugars (glucose, fructose, sucrose, raffinose, maltitol, and sorbitol) and organic acids (oxalic, citric, tartaric, malic, quinic, shikimic, succinic and fumaric) were obtained from Sigma (Poole, UK). Calibration curves were used for the quantification of sugars and organic acids, showing good linearity ($R^2 = 0.999$). Results for both organic acids and sugars were expressed as concentrations g/L of dry weight (dw).

2.5. Total polyphenol content and antioxidant activity

For the total polyphenol content (TPC) determination and the antioxidant activity of the pistachios affected by rootstock and irrigation treatments, a methanol extract was prepared. Half a gram of pistachios (crushed with a grinder) was introduced in a test tube with 10 mL of MeOH/water (80:20, v/v) in 1 % HCl. Then, the mixture was sonicated at 20 °C for 15 min and left at 4 °C for 24 h. After that, the extract was sonicated again for 15 min and centrifuged at $10,000 \times g$ for 10 min [16].

TPC was quantified using the Folin-Ciocalteu colorimetric method described by Gao [17], with some modifications. To 0.1 mL of the methanolic extract was added 2 mL of distilled water, 0.2 mL of Folin-Ciocalteu reagent, and was incubated for 3 min at room temperature. After that, 1 mL of 20 % sodium carbonate was added and the mixture was incubated again for 1 h [16]. The absorbance was determined by measurement at 765 nm using an UV-visible spectrophotometer (Thermo Fisher Scientific Helios Gamma model, UVG 1002E, Waltham, MA, USA). Quantification was carried out according to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), mg/kg (dw).

For the analysis of the antioxidant activity, the methods ABTS⁺ [18], FRAP [19], and DPPH[•] [20] were used. Ten microliters of the supernatant of the methanolic extract was mixed with 990 µL of reagent ABTS⁺ or FRAP. After a reaction time of 10 min, the absorbance was measured at 734 nm for ABTS⁺ and 593 nm in case of FRAP method. For the DPPH method, 10 µL of the supernatant was mixed with 40 µL of MeOH and 950 µL of DPPH[•] solution. Then, the mixture was shaken, placed under dark conditions (15 min), and its absorbance was determined at 515 nm. The results obtained on the analysis of the antioxidant activity of pistachio samples were expressed as mmol Trolox/kg dw.

2.6. Fatty acids

The determination of fatty acid methyl esters (FAMES) was carried out according to Noguera-Artiaga [16]. A gas chromatograph Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (GCMS QP-5050A) was used for the analysis of the organic layer of the pistachio extracts. The chromatograph was equipped with a column (polar) Suprawax 280, 100 % polyethylene glycol (Teknokroma S. Co. Ltd., Barcelona, Spain; 30 m × 0.25 mm × 0.25 µm film thickness). Helium (flow rate of 1.1 mL/min) was used as a carrier gas (split ratio 1:10). The temperature on the injector was 230 °C; on the detector, the temperature was 260 °C. The oven program and the identification of peaks were carried out following the method described by Carbonell-Barrachina [7] and Noguera-Artiaga [16].

2.7. Morphological analysis

Twenty-five pistachio nuts from each treatment were randomly selected and used to determine the size, weight, and color. In addition, pistachios were classified as non-split open, split open, and others (uncommercial: unpeeled, broken shell, dark color, etc.). For the determination of the size, the height, width and length of the edible part from each pistachio were measured using a digital caliper (model 500-197-20 150 mm; Mitutoyo Corp., Aurora, IL, USA). In case of weight, the whole nut, shell and edible part

were weighed (model AG204 scale; Mettler Toledo, Barcelona, Spain) with a precision of 0.1 mg. For the color, these 25 pistachios were ground (Taurus Aromatic Ver II; Taurus Group, Barcelona, Spain) and placed in Petri dishes (100 mm × 15 mm). A colorimeter Minolta model CR-300 (Minolta, Osaka, Japan) with an illuminant D65 and an observer of 10° was used for the measuring of color of samples. Color data were provided as CIEL *a*b* coordinates.

2.8. Statistical analysis

The data presented in this study are the mean values of 3 replicates and was subjected to two-way analysis of variance (ANOVA). Then, data were subjected to Tukey's multiple-range test to compare the means. Percentage data were transformed by Arcosen function before statistical analysis. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were done using XLSTAT software (Addinsoft, version 2014.1, Paris, France).

3. RESULTS AND DISCUSSION

3.1. Volatile compounds

Thirty-one compounds were identified in the volatile profile of pistachios under study (**Table S1**) and were characterized according their sensory descriptors. The three most abundant compounds were α -pinene (~35 %), limonene (~14 %), and β -myrcene (~11 %), and it was demonstrated that both irrigation and rootstocks significantly affected the content these compounds (**Table 1**). These results agreed with those obtained by Hojjati [21] and Penci [22] in previous studies, who also found that the same main compounds predominated in the aromatic profile of pistachios and its essential oils, respectively.

The volatile composition of the T1 pistachios showed no significant differences with that of the control samples (T0). On the other hand, pistachios obtained under RDI T2 had lower amounts of α -pinene, dodecane and tridecane, but higher ones of β -myrcene and limonene than T0. Regarding the effect of the pistachio rootstock, *P. integerrima* had the highest content of α -pinene (the predominant compound); *P. atlantica* led to nuts with the highest content of β -myrcene, dodecane and tridecane; and, *P. terebinthus* had the highest content of limonene.

In previous studies, Carbonell-Barrachina [7] demonstrated that RDI treatments led to pistachio nuts with similar or even higher amounts of the main volatile compounds. These results are in concordance with those obtained in the current study, in the sense that both studies demonstrated that the application of RDI strategies had no negative effects on the volatile composition of pistachio nuts.

Based on the study of the interaction between the two studied factors (irrigation and rootstock), to obtain pistachios with the highest possible content of α -pinene, it is necessary to use the rootstock *P. integerrima* and the irrigation treatments T0 or T1. On the other hand, if the objective is to obtain pistachios with more citrus aroma (more limonene), the combination of *P. terebinthus* rootstock and irrigation treatment T1 will be the most successful one.

According to results obtained, the main volatile compounds found in the volatile profile of pistachios α -pinene, limonene, and β -myrcene (compounds sensory related with descriptors of woody, citrus and fruity, respectively) can be used as indicators to evaluate the pistachio aroma quality.

3.2. Sensory analysis

Around 200 consumers from Mexico, Spain and Poland (at least 60 in each country) participated in the pistachio affective sensory analysis. In Mexico, a 68 % of panelist were women, 37 % in Poland, and 55 % in case of Spain. Of the total number of consumers, 35 % were between 18 and 25 years old, 32 % were between 26 and 35, 15 % between 36 and 45 years old, 17 % between 46 and 55 years old, and 2 % were older than 55 years old.

The irrigation treatments significantly affected three out of the 13 sensory attributes under analysis (**Table 2**): pistachio-ID, oiliness, and overall. Pistachios obtained under RDI T1 obtained higher intensities of pistachio-ID (6.7) than control and T2 (6.4 and 6.4). This result was observed in each of the countries under study (Table 2).

The oiliness of T1 and T0 samples were slightly but statistically higher, 6.0 than T2 samples, 5.8. Mexican consumers liked the oiliness of the pistachio samples less than Polish and Spanish consumers; a similar trend was observed for hardness and crunchiness. The most consumed dried fruit in Mexico is peanut, so it is possible that consumers in this country are used to high intensities of these attributes and were expecting a little more oiliness, hardness and crunchiness in pistachio samples, hoping to find a texture similar to that of fried peanuts.

In addition, in case of overall liking (the attribute that define the final opinion of consumers about the overall quality of sample), the T1 treatment obtained the highest score (6.7), while T0 got 6.3; T2 was statistically related with both of them (score of 6.5). Regarding the factor country, this same trend was observed in Mexico and Spain. However, there were no statistically significant differences in the satisfaction degree of Polish consumers regarding the three irrigations treatments (**Table 2**).

When consumers were forced to choose (among the three samples studied) which was their favorite sample, T1 pistachios were the most liked ones in each of the countries

(in case of Poland, no statistically significant differences were observed between T1 and T0). On the other hand, the least liked sample in all countries was T2 (**Figure 1**). Similar trends were observed when consumers were asked about their willingness to pay for the samples under study. Consumers mentioned that the main reasons for selecting the preferred sample (the most liked one) were: (i) pistachio flavor (~83 %), (ii) crunchiness (~65 %), (iii) aftertaste (~45 %) and (iv) hardness (~30 %) (**Figure 1**). Similar results were reported in previous studies; for instance, Carbonell-Barrachina [7] studied the purchase drivers of international pistachio consumers were pistachio flavor, saltiness, crunchiness and toasted flavor. Although, Noguera-Artiaga [23] concluded that international consumers preferred intense crunchy but low salty nuts.

3.3. Sugars and organic acids

Three sugars (maltitol, raffinose and sucrose) and three organic acids (fumaric, oxalic, and shikimic) were identified and quantified in the pistachio samples under study (**Table 3**). In a previous study, Luh [24], identified four main sugars present in pistachios: sucrose, fructose, glucose and raffinose, being sucrose the main sugar, representing ~40% of the total content.

T1 samples had lower concentration of fumaric acid (0.287 g/L) than control pistachios (T0 = 0.315 g/L), while T2 nuts (0.287 g/L) were statistically related with both T0 and T1. In the rest of organic acids and sugars, no statistically differences were found among pistachios obtained under different irrigation treatments (**Table 3**).

Regarding rootstocks, *P. integerrima* led to pistachios with lower sucrose concentration (19.77 g/L) than *P. terebinthus* and *P. atlantica* (22.51 and 24.98 g/L, respectively). In the analysis of organic acids, *P. integerrima* was the rootstock having the highest concentrations of the three studied acids, while *P. terebinthus* had the lowest amount of oxalic and fumaric acids (**Table 3**).

According to interaction of the two factors studied, pistachios obtained under *P. integerrima* and irrigation T1 had the lowest concentration of sucrose, while rootstock *P. atlantica* and T2 led to pistachios with the highest concentration of this sugar (Table 3).

Similar results were obtained by Lipan [25] in almonds affected by different treatments of regulated deficit irrigation (with sucrose, the predominant sugar showing no differences due to irrigation treatments).

According to our acknowledgement, these are the first results published regarding the effect of deficit irrigation on the acid and sugar composition of pistachios.

3.4. Antioxidant activity (AA) and total polyphenol content (TPC)

Results obtained on the study of AA and TPC are shown in **Table 4**. In general, pistachios have high functional potential based on their high total polyphenol content (~1350 mg GAE/kg, dw) and their antioxidant activity (~22 mmol Trolox/kg, dw, on the three methods studied). Similar results of TPC were obtained by Hojjati [21] in roasted pistachios, Lipan [25] in almonds, and Suárez [26] in chestnut.

The application of moderate regulated irrigation treatments (T1) on the cultivation of pistachios had no statistically incidence on the AA and TPC of nuts. On the contrary, when the water restriction was severe (T2), the AA of pistachios was reduced (according to DPPH and FRAP methods). Under situations of moderate water stress, plants redistribute the CO₂ to the formation of secondary metabolites as a physiological response for the removal the free radicals formed; while under high stress, this CO₂ is mainly used by primary metabolism [27,28]. Same results were found, in previous studies under similar conditions, by Noguera-Artiaga [16] who obtained that hydroSOS pistachios had same or even higher TPC than control samples. Similar results of AA and TPC were found in previous studies with pistachios affected by different irrigation treatments [4,7,29].

In case of the study of rootstocks, *P. integerrima* led to obtain pistachio nuts with the highest concentrations of TPC and AA than rootstocks *P. atlantica* and *P. terebinthus* (**Table 4**). In previous studies, no significant differences were found in the functional composition of pistachios obtained through these same rootstocks.

3.5. Fatty acids

Nine fatty acids (FAMES) were identified by GC-MS in pistachio samples (**Table 5**): two were polyunsaturated (PUFAs) [α -linolenic acid (C18:3) and linoleic acid (C18:2)]; three monounsaturated (MUFAs) [eicosenoic acid (C20:1), oleic acid (C18:1), and palmitoleic acid (C16:1)]; and, four saturated (SFAs) [arachidic acid (C20:0), stearic acid (C18:0), palmitic acid (C16:0), and myristic acid (C14:0)]. The three predominant compounds were C18:1 (~53 % of the total), C18:2 (~31 %), and C16:0 (~12 %).

Pistachios obtained under moderate RDI (T1) had the highest content of oleic acid and the lowest one of α -linolenic as compared to those of control (T0) and T2 treatment (**Table 5**). In case of rootstocks, no statistically differences were observed on the fatty acid composition except on the content of α -linolenic acid, where *P. atlantica* had the lowest values (**Table 5**). Regarding the interaction between the two factors studied, rootstock and irrigation, pistachios obtained by *P. integerrima* and T1 had the highest content of oleic acid (**Table 5**).

The application of treatment T1 affected the unsaturated fatty acid composition of pistachios, increasing the content of MUFAs, and decreasing that of PUFAs. The use of

different rootstocks had no significant effect on the composition of the SFAs, MUFAs or PUFAs (**Figure 2**).

In previous studies, carried out under similar conditions, Carbonell-Barrachina [7] obtained that moderate RDI increased the content of linoleic acid, while in this study the one that has been increased was that of the oleic acid. Acar [30] reported that main fatty acids found in pistachio were oleic, linoleic and palmitic acids as has been shown in the current study.

3.6. Morphological analysis

On the analysis of split-open and non-split open pistachios (**Table 6**) no statistically significant differences were observed among samples obtained under different irrigation treatments, being the mean values 54 % and 42 %, respectively. In case of effect of rootstock, *P. terebinthus* had the highest number of split pistachios (60 %) and, consequently, the lowest number of non-split open pistachios (35 %); while data on *P. atlantica* and *P. integerrima* were statistically equivalent.

The moderated reduction of water during the phenological phase II of pistachios (T1) had no effect on the weight and size of the commercial nuts (**Table 6**). On the other hand, high reduction of water during this same phase (T2), led to pistachios with the lowest weight of their edible nut (T0 = 0.692 g and T2 = 0.673 g).

Regarding rootstocks, no statistically significant differences were found on the weight of whole nut and shell, and on the length and height of pistachio nuts. However, samples of *P. integerrima* and *P. atlantica* had the highest weight of the edible nut (**Table 6**).

The color of the samples had statistically significant differences in the parameters L* and a*, in case of irrigation and rootstock. These differences were minimum, and some authors have concluded that differences smaller than two units, as it is the current case (**Table 6**), are imperceptible for the human eye [31,32].

Similar results were previously reported by Carbonell-Barrachina [7] and Noguera-Artiaga [16], who showed that neither rootstocks nor RDI treatments significantly affected the morphological parameters of pistachios.

4. CONCLUSIONS

The results obtained in this study demonstrated that the application of a moderate deficit irrigation during pistachio cultivation (T1) led to pistachios with same morphological properties, total polyphenol content, antioxidant activity, volatile composition and sensory properties than pistachios obtained using full irrigation (T0). Moreover, T1 led to pistachios with better profile of fatty acids and being the sample

preferred by international consumers. On the contrary, when the RDI was severe (T2), pistachio nuts had the lowest antioxidant activity, the lowest total polyphenols content, and the least preferred samples by consumers. In case of pistachios obtained using different rootstocks, *P. integerrima* led to pistachio nuts with the highest weight, the lowest content of sucrose and better functional properties than *P. atlantica* and *P. terebinthus*. These results demonstrated that it is possible to save irrigation water in pistachio farming, with low environmental and economic cost, and leading to pistachio nuts with same or even better quality attributes.

Supplementary Material: The following are available online at www.mdpi.com/xxx/TableS1. Table S1. Identification and sensory descriptors of volatile compounds on pistachios affected by regulated deficit irrigation and rootstock.

Author Contributions: L.N.A. and P.S.B contributed to experimental design, collected data, interpreted the results and drafted the manuscript. A.S. contributed to experimental design of analysis of volatile compounds. D.P.L contributed to experimental design of irrigation treatments. Á.A.C.B, A.B.H., and Á.C.S. interpreted the all results and revised the manuscript. All authors discussed and approved the final manuscript.

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REFERENCES

1. Galindo, A.; Collado-González, J.; Griñán, I.; Corell, M.; Centeno, A.; Martín-Palomo, M.J.; Girón, I.F.; Rodríguez, P.; Cruz, Z.N.; Memmi, H.; Carbonell-Barrachina, A.A.; Hernández, F.; Torrecillas, A.; Moriana, A.; Pérez-López, D., Deficit irrigation and

- emerging fruit crops as a strategy to save water in mediterranean semiarid agrosystems. *Agric. Water Manage.* **2018**, *202*, 311-324.
2. Pérez-López, D.; Memmi, H.; Gijón-López, M.d.C.; Moreno, M.M.; Couceiro, J.F.; Centeno, A.; Martín-Palomo, M.J.; Corell, M.; Noguera-Artiaga, L.; Galindo, A.; Torrecillas, A.; Moriana, A., Irrigation of pistachios. In *Water scarcity and sustainable agriculture in semiarid environment*, García Tejero, I.F.; Durán Zuazo, V.H., Eds. Academic Press: 2018, pp 247-269.
 3. Gijón, M.C.; Gimenez, C.; Pérez-López, D.; Guerrero, J.; Couceiro, J.F.; Moriana, A., Water relations of pistachio (*pistacia vera* L.) as affected by phenological stages and water regimes. *Sci. Hortic.* **2011**, *128*, 415-422.
 4. Noguera-Artiaga, L.; Pérez-López, D.; Burgos-Hernández, A.; Wojdyło, A.; Carbonell-Barrachina, Á.A., Phenolic and triterpenoid composition and inhibition of α -amylase of pistachio kernels (*pistacia vera* L.) as affected by rootstock and irrigation treatment. *Food Chem.* **2018**, *261*, 240-245.
 5. Goldhamer, D.A., Irrigation management. In *Pistachio production manual*, Ferguson, L., Ed. Fruit and Nut Research and Information Center: University of California, Davis, CA (USA), 1995, pp 71-81.
 6. Moriana, A.; Memmi, H.; Centeno, A.; Martín-Palomo, M.J.; Corell, M.; Torrecillas, A.; Pérez-López, D., Influence of rootstock on pistachio (*pistacia vera* L. Cv kerman) water relations. *Agric. Water Manage.* **2018**, *202*, 263-270.
 7. Carbonell-Barrachina, A.A.; Memmi, H.; Noguera-Artiaga, L.; Gijon-Lopez Mdel, C.; Ciapa, R.; Perez-Lopez, D., Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *J. Sci. Food Agric.* **2015**, *95*, 2866-2873.
 8. Kay, C.D.; Gebauer, S.K.; West, S.G.; Kris-Etherton, P.M., Pistachios increase serum antioxidants and lower serum oxidized-ldl in hypercholesterolemic adults. *J. Nutr.* **2010**, *140*, 1093-1098.

9. Tomaino, A.; Martorana, M.; Arcoraci, T.; Monteleone, D.; Giovinazzo, C.; Saija, A., Antioxidant activity and phenolic profile of pistachio (*pistacia vera* L., variety bronte) seeds and skins. *Biochimie* **2010**, *92*, 1115-1122.
10. Alasalvar, C.; Bolling, B.W., Review of nut phytochemicals, fat-soluble bioactives, antioxidant components and health effects. *British Journal of Nutrition* **2015**, *113*, S68-S78.
11. Dreher, M.L., Pistachio nuts: Composition and potential health benefits. *Nutr. Rev.* **2012**, *70*, 234-240.
12. Mandalari, G.; Bisignano, C.; Filocamo, A.; Chessa, S.; Sarò, M.; Torre, G.; Faulks, R.M.; Dugo, P., Bioaccessibility of pistachio polyphenols, xanthophylls, and tocopherols during simulated human digestion. *Nutrition* **2013**, *29*, 338-344.
13. Saitta, M.; La Torre, G.L.; Potortì, A.G.; Di Bella, G.; Dugo, G., Polyphenols of pistachio (*pistacia vera* L.) oil samples and geographical differentiation by principal component analysis. *J. Am. Oil Chem. Soc.* **2014**, *91*, 1595-1603.
14. Memmi, H.; Gijón, M.C.; Couceiro, J.F.; Pérez-López, D., Water stress thresholds for regulated deficit irrigation in pistachio trees: Rootstock influence and effects on yield quality. *Agric. Water Manage.* **2016**, *164*, 58-72.
15. Hernández, F.; Noguera-Artiaga, L.; Burló, F.; Wojdyło, A.; Carbonell-Barrachina, A.A.; Legua, P., Physico-chemical, nutritional, and volatile composition and sensory profile of spanish jujube (*ziziphus jujuba* mill.) fruits. *J. Sci. Food Agric.* **2016**, *96*, 2682-2691.
16. Noguera-Artiaga, L.; Sánchez-Bravo, P.; Hernández, F.; Burgos-Hernández, A.; Pérez-López, D.; Carbonell-Barrachina, Á.A., Influence of regulated deficit irrigation and rootstock on the functional, nutritional and sensory quality of pistachio nuts. *Sci. Hort.* **2020**, *261*, 108994.
17. Gao, X.; Ohlander, M.; Jeppsson, N.; Bjork, L.; Trajkovski, V., Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*hippophae rhamnoides* L.) during maturation. *J. Agric. Food Chem.* **2000**, *48*, 1485-1490.

18. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C., Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231-1237.
19. Benzie, I.F.; Strain, J.J., The ferric reducing ability of plasma (frap) as a measure of "antioxidant power": The frap assay. *Anal. Biochem.* **1996**, *239*, 70-76.
20. Brand-Williams, W.; Cuvelier, M.E.; Berset, C., Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* **1995**, *28*, 25-30.
21. Hojjati, M.; Calín-Sánchez, Á.; Razavi, S.H.; Carbonell-Barrachina, Á.A., Effect of roasting on colour and volatile composition of pistachios (*pistacia vera* l.). *Int. J. Food Sci. Tech.* **2013**, *48*, 437-443.
22. Penci, M.C.; Martinez, M.L.; Fabani, M.P.; Feresin, G.E.; Tapia, A.; Ighani, M.; Ribotta, P.D.; Wunderlin, D.A., Matching changes in sensory evaluation with physical and chemical parameters. *Food and Bioprocess Technology* **2013**, *6*, 3305-3316.
23. Noguera-Artiaga, L.; Lipan, L.; Vazquez-Araujo, L.; Barber, X.; Perez-Lopez, D.; Carbonell-Barrachina, A.A., Opinion of spanish consumers on hydrosustainable pistachios. *J. Food Sci.* **2016**, *81*, S2559-S2565.
24. Luh, B.S.; Wong, W.S.; El-Shimi, N.E., Effect of processing on some chemical constituents of pistachio nuts. *Journal of Food Quality* **1982**, *5*, 33-41.
25. Lipan, L.; Moriana, A.; López Lluch, D.B.; Cano-Lamadrid, M.; Sendra, E.; Hernández, F.; Vázquez-Araújo, L.; Corell, M.; Carbonell-Barrachina, Á.A., Nutrition quality parameters of almonds as affected by deficit irrigation strategies. *Molecules* **2019**, *24*, 2646.
26. Suárez, M.; Galdón, B.; Mesa, D.; Díaz, C.; Rodríguez-Rodríguez, E.M., Sugars, organic acids and total phenols in varieties of chestnut fruits from tenerife (Spain). *Food and Nutrition Sciences* **2012**, *03*.
27. Behboudian, M.H.; Marsal, J.; Girona, J.; Lopez, G., Quality and yield responses of deciduous fruits to reduce irrigation. In *Horticultural reviews*, Janick, J., Ed. 2011, pp 149-189.

28. Grant, O.M., Understanding and exploiting the impact of drought stress on plant physiology. In *Abiotic stress responses in plants: Metabolism, productivity and sustainability*, 2012, pp 89-104.
29. Zhu, Y.; Taylor, C.; Sommer, K.; Wilkinson, K.. Wirthensohn, M., Influence of deficit irrigation strategies on fatty acid and tocopherol concentration of almond (*prunus dulcis*). *Food Chem.* **2015**, *173*, 821-826.
30. Acar, I.; Kafkas, S.; Kapchina-Toteva, V.. Ercisli, S., Effect of rootstock on fat content and fatty acid composition of immature pistachio kernels. *Comptes rendus de l'Académie bulgare des Sciences* **2017**, *70*, 1049-1056.
31. Galindo, A.; Noguera-Artiaga, L.; Cruz, Z.N.; Burló, F.; Hernández, F.; Torrecillas, A.. Carbonell-Barrachina, Á.A., Sensory and physico-chemical quality attributes of jujube fruits as affected by crop load. *LWT - Food Sci. Technol.* **2015**, *63*, 899-905.
32. Navarro, P.; Nicolas, T.S.; Gabaldon, J.A.; Mercader-Ros, M.T.; Calin-Sanchez, A.; Carbonell-Barrachina, A.A.. Perez-Lopez, A.J., Effects of cyclodextrin type on vitamin c, antioxidant activity, and sensory attributes of a mandarin juice enriched with pomegranate and goji berries. *J. Food Sci.* **2011**, *76*, S319-324.

Table 1. Relative content (%) of volatile compounds on pistachios affected by regulated deficit irrigation and rootstocks.

Compound	ANOVA test [†]			Irrigation (%)			Rootstock (%)			Irrigation x Rootstock (%)								
	Irrig.	Root.	Irrig. x Root.	T0	T1	T2	AT	IN	TE	ATxT0	ATxT1	ATxT2	INxT0	INxT1	INxT2	TExT0	TExT1	TExT2
Acetic acid	NS	NS	NS	0.46	0.32	0.30	0.33	0.36	0.39	0.33	0.26	0.39	0.59	0.17	0.32	0.45	0.53	0.19
Ethyl acetate	NS	NS	NS	1.04	0.39	0.46	0.69	0.75	0.45	0.85	0.63	0.59	1.48	0.20	0.58	0.79	0.35	0.21
Pentanone	NS	NS	NS	0.25	0.20	0.17	0.19	0.21	0.22	0.20	0.18	0.19	0.33	0.13	0.16	0.21	0.28	0.18
1-Methyl-1H-pyrrole	NS	NS	NS	3.52	4.57	2.82	2.72	2.92	5.26	1.95	3.84	2.39	3.04	3.28	2.44	5.58	6.58	3.62
1-Pentanol	NS	NS	NS	0.92	0.55	0.43	0.67	0.47	0.76	0.90	0.72	0.39	0.67	0.27	0.45	1.18	0.65	0.46
(Z)-3-Octene	**	NS	**	0.26 b [‡]	0.20 b	0.50 a	0.35	0.27	0.35	0.18 b	0.15 b	0.71 a	0.27 ab	0.13 b	0.39 ab	0.31 ab	0.33 ab	0.41 ab
Hexanal	NS	NS	NS	1.38	0.70	0.94	0.98	0.74	1.30	1.17	0.84	0.91	0.80	0.39	1.03	2.16	0.86	0.88
2-Octene	NS	NS	NS	0.18	0.20	0.27	0.22	0.18	0.24	0.17	0.17	0.31	0.15	0.15	0.24	0.22	0.27	0.24
1-Hexanol	NS	NS	NS	4.39	2.63	2.64	3.98	2.70	2.97	5.75	3.66	2.55	2.86	1.98	3.27	4.56	2.26	2.09
(E)-4-Nonene	NS	NS	**	0.21	0.21	0.30	0.27	0.20	0.24	0.18 b	0.20 ab	0.42 a	0.19 ab	0.14 b	0.26 ab	0.24 ab	0.27 ab	0.21 ab
(Z)-4-Nonene	NS	NS	NS	0.22	0.18	0.22	0.22	0.15	0.24	0.20	0.18	0.28	0.15	0.11	0.19	0.31	0.24	0.18
Nonane	NS	NS	NS	0.25	0.23	0.27	0.29	0.21	0.24	0.30	0.23	0.35	0.20	0.17	0.26	0.26	0.29	0.19
α -Pinene	**	**	**	36.90 a	35.08 a	33.54 b	30.19 b	42.41 a	32.92 b	31.29 c	33.13 c	26.15 d	49.27 a	46.25 a	31.71 c	30.14 c	25.86 d	42.76 b
2-Pentanol	NS	NS	NS	0.37	0.40	0.60	0.56	0.41	0.41	0.23	0.47	0.98	0.43	0.24	0.55	0.45	0.50	0.26
1-Decene	NS	NS	NS	0.83	0.40	0.47	0.75	0.36	0.60	1.10	0.53	0.62	0.47	0.30	0.31	0.94	0.38	0.47
Sabinene	NS	NS	NS	0.48	0.40	0.40	0.38	0.42	0.48	0.43	0.32	0.38	0.54	0.47	0.26	0.47	0.41	0.57
3-Decene	NS	NS	NS	0.62	0.35	0.49	0.51	0.34	0.61	0.44	0.47	0.61	0.41	0.16	0.43	1.00	0.41	0.42
β -Myrcene	**	**	NS	8.39 b	9.58 b	14.89 a	13.21 a	9.79 b	9.86 b	6.77	12.66	20.22	8.16	6.12	15.09	10.26	9.97	9.37
Decane	NS	NS	NS	2.07	2.53	2.74	2.92	2.26	2.16	2.13	3.27	3.34	2.09	1.96	2.73	1.99	2.35	2.14
3-Carene	NS	NS	NS	0.24	0.39	0.39	0.29	0.34	0.39	0.34	0.26	0.26	0.19	0.52	0.31	0.18	0.39	0.61
Limonene	***	**	**	12.19 b	13.72 b	15.01 a	11.19 b	12.24 b	17.49 a	14.87 c	8.11 de	10.60 d	7.91 e	12.73 cd	16.07 bc	13.80 c	20.32 a	18.36 b
(E)-3-Hexenol	NS	NS	NS	0.92	0.94	1.06	1.17	0.82	0.92	0.84	1.23	1.43	0.81	0.64	1.01	1.10	0.94	0.72
2-Octen-1-ol	NS	NS	NS	0.74	0.97	1.11	1.21	0.85	0.75	0.76	1.32	1.54	0.77	0.67	1.12	0.67	0.93	0.65
2-Methyl-decane	NS	NS	NS	0.68	0.45	0.49	0.68	0.46	0.48	0.83	0.60	0.61	0.48	0.31	0.58	0.72	0.45	0.26
Terpinolene	NS	NS	NS	0.66	0.95	0.76	0.81	0.84	0.72	1.06	0.79	0.57	0.40	1.43	0.70	0.52	0.62	1.02
Undecane	NS	NS	NS	8.59	9.40	8.77	9.53	8.77	8.46	9.87	7.73	11.00	7.67	9.17	9.47	8.22	11.32	5.84
2-Nonen-1-ol	NS	NS	NS	1.70	0.90	0.94	1.46	1.04	1.04	2.51	0.81	1.06	1.03	1.19	0.90	1.57	0.69	0.88
1-Nonanol	NS	NS	NS	1.54	1.20	1.01	1.72	0.90	1.13	2.34	1.68	1.13	0.75	1.02	0.94	1.55	0.90	0.95
Dodecane	**	**	**	6.25 ab	8.02 a	5.29 b	8.32 a	5.71 b	5.54 b	7.73 ab	10.51 a	6.71 ab	5.19 b	6.45 ab	5.46 ab	5.83 ab	7.09 ab	3.70 b
Decanal	NS	NS	NS	0.41	0.51	0.46	0.54	0.45	0.39	0.50	0.56	0.56	0.42	0.49	0.45	0.31	0.46	0.38
Tridecane	**	**	**	2.85 ab	3.45 a	2.26 b	3.66 a	2.44 b	2.47 b	3.75 ab	4.50 a	2.73 ab	2.26 ab	2.76 ab	2.28 ab	2.54 ab	3.09 ab	1.76 b

[†] NS: not significant at $p < 0.05$; ** and ***: significant at $p < 0.01$ and 0.001 , respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same volatile compound and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test. P. atlantica (AT), P. integerrima (IN) and P. terebinthus (TE).

Table 2. Sensorial affective test of hydroSOS pistachios with consumers of Spain, Mexico, and Poland.

Factor	Liking													
	Size	Peel	Color	Pistachio ID	Toasted	Sweet	Sour	Aftertaste	Oiliness	Hardness	Crunchiness	Friability	Adhesiveness	Overall
ANOVA test[†]														
Irrigation	NS	NS	NS	**	NS	NS	NS	NS	*	NS	NS	NS	NS	**
Country	NS	NS	NS	NS	NS	NS	NS	NS	*	*	**	NS	NS	NS
Country x Irrigation	NS	NS	NS	*	NS	NS	NS	NS	*	NS	NS	NS	NS	*
Tukey's multiple range test[‡]														
Irrigation														
T0	6.6	6.8	6.4	6.4 b	6.4	5.8	5.8	6.3	6.0 a	6.5	6.4	6.0	5.7	6.3 b
T1	6.7	6.9	6.5	6.7 a	6.3	6.0	5.6	6.1	6.0 a	6.6	6.4	6.0	5.7	6.7 a
T2	6.5	6.7	6.3	6.4 b	6.5	5.8	5.9	5.9	5.8 b	6.5	6.5	6.2	5.4	6.5 ab
Country														
Mexico	6.5	6.8	6.3	6.3	6.5	5.8	5.8	5.8	5.6 b	6.2 b	6.0 b	5.9	5.5	6.4
Poland	6.9	6.9	6.5	6.7	6.4	5.9	5.8	6.3	6.0 a	6.8 a	6.7 a	6.2	5.9	6.7
Spain	6.5	6.6	6.3	6.5	6.2	5.9	5.6	6.3	6.2 a	6.6 ab	6.7 a	6.1	5.6	6.5
Country x Irrigation														
Mexico x T0	6.7	6.9	6.4	6.2 c	6.4	5.9	6.0	6.1	5.6 b	6.1	6.1	5.7	5.5	6.0 b
Mexico x T1	6.5	6.9	6.4	6.6 ab	6.5	5.8	5.5	5.9	5.7 b	6.4	5.9	6.0	5.7	6.6 a
Mexico x T2	6.2	6.6	6.2	6.2 c	6.7	5.7	5.8	5.4	5.6 b	6.2	5.9	6.1	5.1	6.4 ab
Poland x T0	6.7	6.9	6.5	6.6 ab	6.5	5.7	5.8	6.2	6.2 a	6.8	6.5	6.2	5.9	6.9 a
Poland x T1	6.9	6.9	6.4	6.8 a	6.3	6.3	5.8	6.1	6.1 a	6.8	6.7	6.1	6.0	6.7 a
Poland x T2	6.9	6.9	6.7	6.7 a	6.5	5.8	6.0	6.4	5.8 ab	6.8	6.8	6.2	5.7	6.6 a
Spain x T0	6.4	6.6	6.3	6.4 b	6.2	5.8	5.5	6.5	6.3 a	6.7	6.5	6.0	5.8	6.1 b
Spain x T1	6.8	6.8	6.5	6.8 a	6.0	6.0	5.6	6.3	6.2 a	6.5	6.7	5.9	5.5	6.8 a
Spain x T2	6.3	6.5	6.1	6.3 bc	6.4	5.8	5.8	6.0	6.0 ab	6.5	6.9	6.3	5.4	6.5 ab

[†] NS: not significant at $p < 0.05$; ** and ***: significant at $p < 0.01$ and 0.001 , respectively. [‡] Values (mean of 200 replications) followed by the same letter, within the same sensory attribute and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test.

Table 3. Sugars (g/L) and organic acids (g/L) on pistachios obtained under regulated deficit irrigation and different rootstocks.

Factor	Sugars (g/L dw)			Organic acids (g/L dw)		
	Raffinose	Sucrose	Maltitol	Oxalic	Shikimic	Fumaric
ANOVA test[†]						
Irrigation	NS	NS	NS	NS	NS	***
Rootstock	NS	***	NS	***	***	***
Irrigation x Rootstock	NS	***	NS	NS	NS	***
Tukey's multiple range test[‡]						
Irrigation						
T0	9.793	22.831	4.111	0.156	0.535	0.315 a
T1	9.077	20.449	4.848	0.127	0.581	0.287 b
T2	10.351	23.972	5.796	0.148	0.555	0.296 ab
Rootstock						
<i>P. atlantica</i>	10.345	24.977 a	5.772	0.165 a	0.467 b	0.260 b
<i>P. integerrima</i>	9.517	19.770 b	5.257	0.151 a	0.737 a	0.328 a
<i>P. terebinthus</i>	9.359	22.505 a	3.727	0.116 b	0.468 b	0.309 a
Irrigation x Rootstock						
T0 x <i>P. atlantica</i>	9.091	23.692 ab	4.441	0.189	0.456	0.273 bc
T1 x <i>P. atlantica</i>	8.963	21.574 ab	4.447	0.145	0.418	0.266 bc
T2 x <i>P. atlantica</i>	12.982	29.666 a	8.427	0.160	0.527	0.241 c
T0 x <i>P. integerrima</i>	10.418	20.981 ab	3.911	0.137	0.701	0.339 a
T1 x <i>P. integerrima</i>	9.237	18.240 b	6.503	0.140	0.832	0.299 ab
T2 x <i>P. integerrima</i>	8.895	20.090 ab	5.355	0.175	0.678	0.347 a
T0 x <i>P. terebinthus</i>	9.868	23.821 ab	3.982	0.141	0.448	0.332 a
T1 x <i>P. terebinthus</i>	9.032	21.534 ab	3.595	0.096	0.494	0.295 abc
T2 x <i>P. terebinthus</i>	9.176	22.160 ab	3.605	0.112	0.461	0.300 ab

[†] NS: not significant at $p < 0.05$; ***, significant at $p < 0.001$. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test. dw: dry weight.

Table 4. Total polyphenol content [mg gallic acid equivalents (GAE)/kg dry weigh, dw] and antioxidant activity (mmol Trolox/kg dw) of pistachios as affected by deficit irrigation treatment and rootstock.

Factor	TPC	DPPH	FRAP	ABTS
	(mg GAE/kg dw)	(mmol Trolox/kg dw)		
ANOVA test[†]				
Irrigation	***	***	***	NS
Rootstock	***	***	***	***
Irrigation x Rootstock	***	***	***	***
Tukey's multiple range test[‡]				
Irrigation				
T0	1390 ab	21.70 a	23.89 a	23.5
T1	1409 a	20.50 ab	24.45 a	23.3
T2	1297 b	18.77 b	19.66 b	22.0
Rootstock				
<i>P. atlantica</i>	1310 b	19.02 b	20.08 b	21.53 b
<i>P. integerrima</i>	1522 a	22.09 a	25.47 a	28.08 a
<i>P. terebinthus</i>	1265 b	19.87 b	22.44 ab	19.07 c
Irrigation x Rootstock				
T0 x <i>P. atlantica</i>	1294 bcd	20.06 ab	22.07 abc	22.78 bc
T1 x <i>P. atlantica</i>	1450 abc	19.51 ab	21.80 abc	22.85 bc
T2 x <i>P. atlantica</i>	1184 d	17.48 b	16.37 c	18.95 c
T0 x <i>P. integerrima</i>	1615 a	24.28 a	26.57 ab	29.01 a
T1 x <i>P. integerrima</i>	1460 ab	22.53 ab	30.02 a	28.26 a
T2 x <i>P. integerrima</i>	1489 ab	19.46 ab	19.82 bc	26.96 ab
T0 x <i>P. terebinthus</i>	1260 bcd	20.76 ab	23.03 abc	18.56 c
T1 x <i>P. terebinthus</i>	1317 bcd	19.47 ab	21.51 abc	18.71 c
T2 x <i>P. terebinthus</i>	1216 cd	19.37 ab	22.77 abc	19.93 c

[†] NS: not significant at $p < 0.05$; ***: significant at $p < 0.001$. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test.

Table 5. Fatty acid composition of pistachios as affected by deficit irrigation treatment and rootstock.

Factor	Fatty Acids (%)								
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
ANOVA test[†]									
Irrigation	NS	NS	NS	NS	**	NS	**	NS	NS
Rootstock	NS	NS	NS	NS	NS	NS	*	NS	NS
Irrigation x Rootstock	NS	NS	NS	NS	*	*	NS	NS	NS
Tukey's multiple range test[‡]									
Irrigation									
T0	0.07	12.03	1.28	1.28	51.94 b	32.18	0.73 a	0.16	0.32
T1	0.07	11.60	1.21	1.52	54.95 a	29.54	0.62 b	0.16	0.33
T2	0.07	11.86	1.19	1.32	52.05 b	32.39	0.67 ab	0.15	0.30
Rootstock									
<i>P. atlantica</i>	0.07	11.90	1.20	1.38	53.34	31.00	0.63 b	0.18	0.31
<i>P. integerrima</i>	0.08	11.63	1.19	1.37	52.95	31.63	0.67 ab	0.15	0.33
<i>P. terebinthus</i>	0.07	11.96	1.29	1.36	52.66	31.48	0.73 a	0.15	0.31
Irrigation x Rootstock									
T0 x <i>P. atlantica</i>	0.07	12.13	1.26	1.28	52.09 b	32.01 ab	0.64	0.20	0.31
T1 x <i>P. atlantica</i>	0.07	11.59	1.17	1.29	52.95 b	31.87 ab	0.61	0.17	0.28
T2 x <i>P. atlantica</i>	0.06	11.97	1.17	1.58	54.98 ab	29.12 ab	0.63	0.16	0.33
T0 x <i>P. integerrima</i>	0.08	11.95	1.30	1.28	50.96 b	33.18 ab	0.78	0.14	0.33
T1 x <i>P. integerrima</i>	0.07	11.27	1.10	1.69	58.44 a	26.33 b	0.58	0.19	0.33
T2 x <i>P. integerrima</i>	0.08	11.66	1.18	1.16	49.45 b	35.38 a	0.66	0.11	0.33
T0 x <i>P. terebinthus</i>	0.07	12.01	1.28	1.28	52.76 b	31.36 ab	0.78	0.15	0.31
T1 x <i>P. terebinthus</i>	0.06	11.93	1.35	1.58	53.47 b	30.41 ab	0.68	0.14	0.37
T2 x <i>P. terebinthus</i>	0.08	11.93	1.23	1.23	51.73 b	32.65 ab	0.73	0.17	0.25

[†] NS: not significant at $p < 0.05$; * and **, significant at $p < 0.05$ and 0.01 , respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test.

Table 6. Weight, size, and nature of pistachios as affected by deficit irrigation treatment and rootstock.

Factor	Weight (g)			Size (mm)			Number per 100 units			Color coordinates				
	Whole	Edible Nut	Shell	Length	Height	Width	Split open	Non-split open	Others [§]	L	a*	b*	C*	Hue
ANOVA test[†]														
Irrigation	NS	***	NS	NS	***	***	NS	NS	NS	***	NS	NS	NS	***
Rootstock	NS	***	NS	NS	NS	***	***	***	NS	***	***	NS	NS	***
IrrigationxRootstock	NS	***	NS	NS	***	***	*	*	NS	***	***	NS	NS	***
Tukey's multiple range test[‡]														
Irrigation														
T0	2.062	0.692 a	1.370	17.10	10.78 a	9.48 ab	55	42	4	67.74 a	-5.93	32.60	33.15	100.28 a
T1	2.051	0.695 a	1.356	17.23	10.74 a	9.56 a	53	43	4	66.37 b	-5.63	32.98	33.47	99.69 ab
T2	2.009	0.673 b	1.335	17.06	10.49 b	9.35 b	54	42	4	66.44 b	-5.54	32.67	33.16	99.61 b
Rootstock														
<i>P. atlantica</i>	2.036	0.686 ab	1.350	17.16	10.62	9.43 b	50 b	46 a	4	66.95 ab	-5.39 a	32.86	33.31	99.27 b
<i>P. integerrima</i>	2.047	0.695 a	1.352	17.18	10.68	9.61 a	52 b	46 a	2	67.32 a	-6.34 b	32.51	33.13	101.02 a
<i>P. terebinthus</i>	2.038	0.679 b	1.359	17.05	10.71	9.36 b	60 a	35 b	5	66.29 b	-5.38 a	32.88	33.33	99.29 b
Irrigation x Rootstock														
T0 x <i>P. atlantica</i>	2.061	0.691 ab	1.369	17.16	10.71 abc	9.37 bc	50 b	46 a	4	66.68 bc	-5.79 ab	33.16	33.68	99.87 ab
T1 x <i>P. atlantica</i>	2.029	0.688 ab	1.340	17.28	10.73 ab	9.41 abc	49 b	47 a	4	66.90 bc	-5.61 ab	32.79	33.27	99.69 ab
T2 x <i>P. atlantica</i>	2.018	0.678 ab	1.339	17.04	10.42 c	9.47 abc	51 b	44 a	5	67.26 b	-4.75 a	32.61	32.97	98.24 b
T0 x <i>P. integerrima</i>	2.062	0.702 a	1.360	17.15	10.91 a	9.67 ab	53 b	45 a	4	68.75 a	-6.59 b	32.56	33.23	101.43 a
T1 x <i>P. integerrima</i>	2.061	0.702 a	1.358	17.16	10.61 abc	9.74 a	51 b	46 a	4	66.49 c	-5.84 ab	32.64	33.16	100.15 a
T2 x <i>P. integerrima</i>	2.017	0.681 ab	1.336	17.24	10.54 bc	9.41 abc	51 b	47 a	3	66.74 bc	-6.58 b	32.34	33.01	101.48 a
T0 x <i>P. terebinthus</i>	2.062	0.682 ab	1.379	16.99	10.75 ab	9.37 bc	62 a	34 b	4	67.79 ab	-5.41 ab	32.07	32.53	99.52 ab
T1 x <i>P. terebinthus</i>	2.062	0.693 ab	1.368	17.25	10.88 a	9.54 ab	60 a	36 b	4	65.74 cd	-5.44 ab	33.51	33.96	99.23 ab
T2 x <i>P. terebinthus</i>	1.990	0.661 b	1.329	16.91	10.51 bc	9.17 c	59 a	36 b	5	65.34 d	-5.29 ab	33.07	33.51	99.11 ab

[†]NS: not significant at $p < 0.05$; * and ***: significant at $p < 0.05$ and 0.001 , respectively. [‡] Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test. [§] Others' means unpeeled, broken shell, dark color, etc. L, a*, b*: CIEL*a*b* color coordinates.

Figure 1. Preference of Mexican, Polish, and Spanish consumers about pistachios obtained using different irrigation treatments (T0, control; T1, moderate RDI regulated deficit irrigation); T2, severe RDI), together with their willingness to pay and the main reasons behind their election. Factors with the same letter were not significantly different ($p < 0.05$), Tukey's least significant difference test.

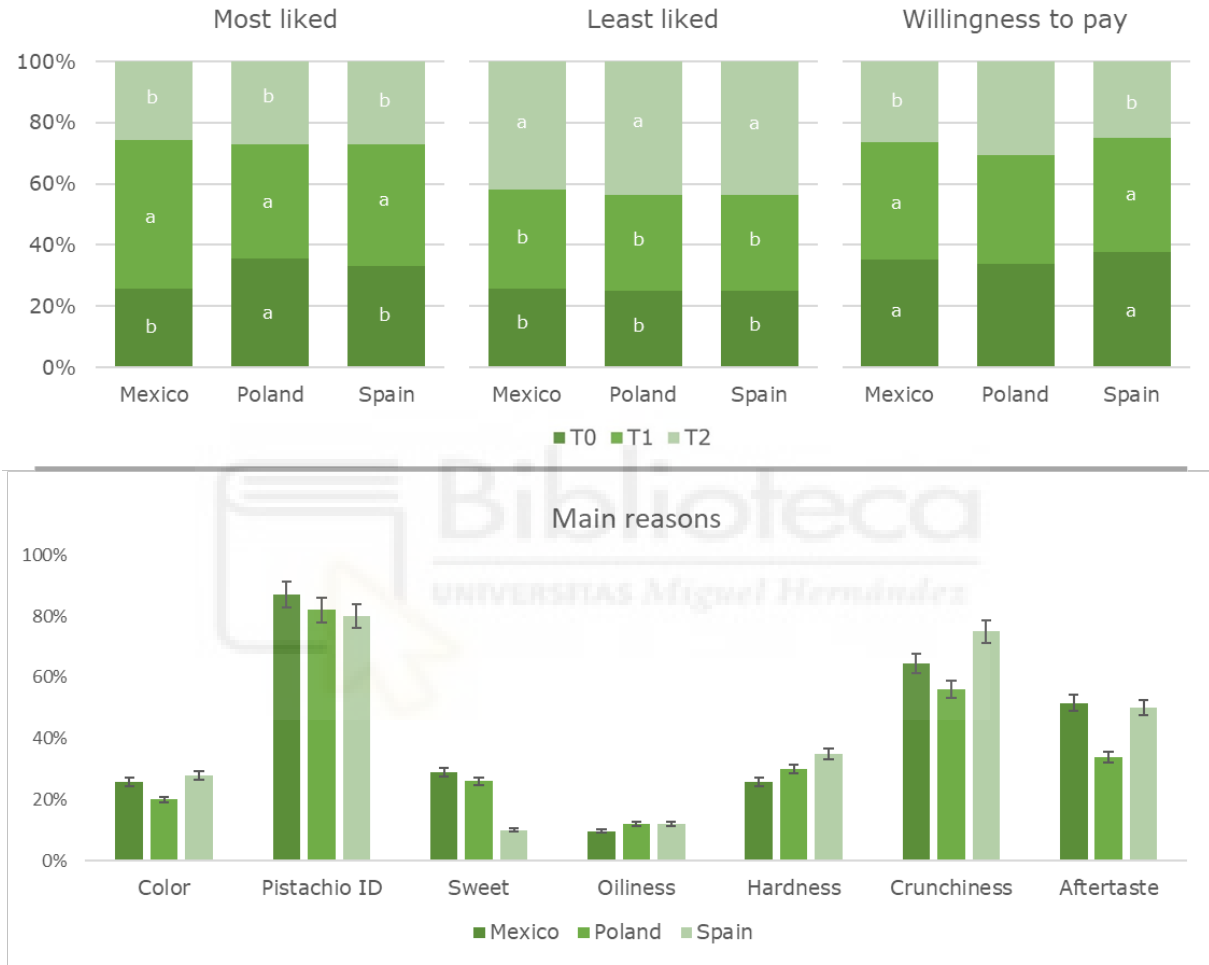


Figure 2. Fatty acid composition of pistachios as affected by deficit irrigation treatment and rootstock, grouped according to their saturation. Values (mean of 3 replications) followed by the same letter, within the same factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test. SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.

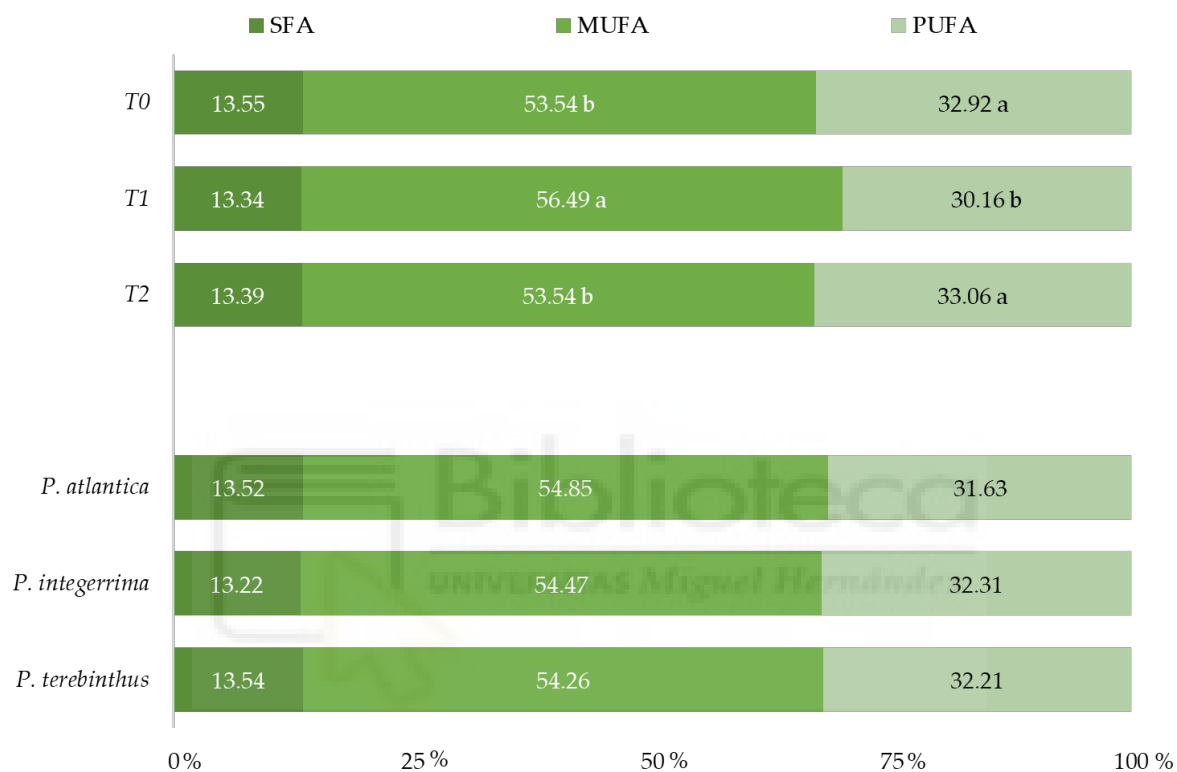


Table S1. Identification and sensory descriptors of volatile compounds on pistachios affected by regulated deficit irrigation and rootstock

#	Compound	Rt (min)	KI (exp.)	KI (lit.)	Sensory descriptor
1	Acetic acid	2.21	603	605	Vinegar
2	Ethyl acetate	2.44	601	612	Pineapple; Anise
3	Pentanone	3.52	702	705	Fruity; Cheese; Chocolate
4	1-Methyl-1H-pyrrole	4.38	748	743	Woody; Smoky; Herbal
5	1-Pentanol	5.03	774	765	Sweet; Vanilla; Fusel
6	(Z)-3-Octene	5.56	792	790	nf
7	Hexanal	5.85	801	800	Fatty; Green
8	2-Octene	6.26	818	815	nf
9	1-Hexanol	7.95	874	868	Green; Herbaceous; Woody
10	(E)-4-Nonene	8.51	890	890	nf
11	(Z)-4-Nonene	8.64	893	893	nf
12	Nonane	8.90	900	900	Gasoline
13	α -Pinene	9.99	938	937	Woody
14	2-Pentanol	10.30	948	950	Oily; Green
15	1-Decene	11.21	975	981	nf
16	Sabinene	11.38	980	975	Woody; Pine; Spicy
17	3-Decene	11.71	989	988	nf
18	β -Myrcene	11.84	992	991	Fruity; Herbaceous; Sweet
19	Decane	12.15	1000	1000	Alkane
20	3-Carene	12.48	1012	1011	Lemon
21	Limonene	13.06	1032	1031	Citrus; Sweet
22	(E)-3-Hexenol	13.29	1040	1038	Green
23	2-Octen-1-ol	13.86	1057	1061	Citrus; Fruity; Green; Vegetable
24	2-Methyl-decane	14.19	1068	1064	nf
25	Terpinolene	14.95	1090	1089	Plastic
26	Undecane	15.31	1100	1100	Alkane
27	2-Nonen-1-ol	15.43	1106	1105	Melon; Waxy
28	1-Nonanol	17.39	1180	1176	Citrus; Rose
29	Dodecane	18.00	1201	1200	Alkane
30	Decanal	18.11	1205	1206	Floral; Citrus; Sweet; Waxy
31	Tridecane	19.48	1301	1300	Alkane

Rt = Retention time; KI (exp.) = Kovat's index experimental; KI (lit.) = Kovat's index literature; nf = not found.

PUBLICACIÓN 5 (Transcripción literal)

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PHENOLIC AND TRITERPENOID COMPOSITION AND INHIBITION OF A-AMYLASE OF PISTACHIO KERNELS (*PISTACIA VERA* L.) AS AFFECTED BY ROOTSTOCK AND IRRIGATION TREATMENT

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Running title: Phenolics in hydroSOS pistachios

ABSTRACT

The current water scarcity forces farmers to adopt new irrigation strategies to save water without jeopardizing the fruit yield and quality. In this study, the influence of 3 regulated deficit irrigation (RDI) treatments and 3 rootstocks on the functional quality of pistachios were studied. The functional parameters studied included, polyphenols, triterpenoids, and inhibition of α -amylase. The results showed that *P. terebinthus* and *P. atlantica* rootstocks led to pistachio kernels with higher contents of polyphenols and triterpenoids (mainly betulinic acid with 111 and 102 $\mu\text{g g}^{-1}$, respectively) than pistachios obtained using *P. integerrima* rootstock (81 $\mu\text{g g}^{-1}$). On the other hand, the use of moderate RDI (T1 treatment) increased the total content of polyphenols ($\sim 10\%$), quercetin-*O*-galloyl-hexoside ($\sim 15\%$), keampferol-3-*O*-glucoside ($\sim 19\%$), and polymeric procyanidins ($\sim 20\%$), as compared to the control trees, resulting in pistachios with a better functional profile, lower economic cost and with a lesser environmental impact.

KEYWORDS: polymeric procyanidins; hydroSOS; regulated deficit irrigation; functional properties; LC-PDA-MS-Qtof.



1. INTRODUCTION

Pistachio (*Pistacia vera* L.) is a popular and delicious nut and due to a highly favorable taste, nutritional value, and health promoting composition is widely consumed worldwide as a snack, either fresh or roasted and salted. Furthermore, pistachio is normally used as an ingredient in cakes, confectionery products, biscuits, candies, ice creams, chocolates, sausages, and sauces (Hojjati, Noguera-Artiaga, Wojdyło, & Carbonell-Barrachina, 2015; Kahyaoglu, 2008).

A pistachio nut consists of a fleshy hull (epicarp and mesocarp) surrounding the nut shell, which encloses the nutmeat. As the nutmeat grows, it fills the shell and at the end, its growth exceeds the shell size, and cracks the shell. In general, the hull remains intact during this entire process, protecting the nutmeat from insects and pathogens (Grace, Esposito, Timmers, Xiong, Yousef, Komarnytsky, et al., 2016).

The world food production heavily depends on water availability, and this resource is getting more and more scarce, especially for agricultural purposes. Thus, it is absolutely necessary to improve farming efficiency through the use of drought-resistant species and/or through techniques that reduce the volume of irrigation water (Grant, 2012). Regulated deficit irrigation (RDI) is a system of managing water supply by imposing some water deficits at specific phenological stages, which have been found to be less sensitive, with no or low reduction of yield and consequently economic benefits (Gijón, Gimenez, Perez-López, Guerrero, Couceiro, & Moriana, 2011). Fruits and vegetables cultivated under RDI are called hydrosustainable or hydroSOS products and have a solid identity based on (i) an increase of their secondary metabolites, that improves their functionality and quality of these products, and (ii) environmental respect because they optimize the use of water (Noguera-Artiaga, Lipan, Vázquez-Araújo, Barber, Pérez-López, & Carbonell-Barrachina, 2016).

During the phenological development of pistachio nut there are differentiated 3 different stages (Carbonell-Barrachina, Memmi, Noguera-Artiaga, del Carmen Gijón-López, Ciapa, & Pérez-López, 2015; Goldhamer, 1995):

- *stage I* starts at the beginning of nut growth and finishes when maximum nut size is reached;
- *stage II* is the period in which the shell hardens; and,
- *stage III* is the period in which the edible part grow.

It is well known that stage II in pistachio nut development is the less sensitive to water stress (Gijón, Gimenez, Perez-López, Guerrero, Couceiro, & Moriana, 2011;

Goldhamer, 1995); thus, the deficit irrigation strategies used in the current experiment were based on restricting irrigation water only during stage II.

Pistachio cultivation requires the use of rootstocks, and grafting is the only form of vegetative propagation. Thus, it is essential to study the influence of the rootstock on the nut yield, and the functionality and quality of the edible kernels. The main pistachio rootstocks are *Pistacia vera* L. in Iran, *P. integerrima* L. and a hybrid between *P. integerrima* Steward ex Brandis and *P. atlantica* Desf. (UCB-I) in the USA, and *P. terebinthus* L. in the Mediterranean basin (Carbonell-Barrachina, Memmi, Noguera-Artiaga, del Carmen Gijón-López, Ciapa, & Pérez-López, 2015; Moriana, Memmi, Centeno, Martín-Palomo, Corell, Torrecillas, et al., 2018).

The beneficial effects of pistachio consumption on decreasing the levels of the main risk factors of cardiovascular disease (CVD), such as lipids, endothelial function, inflammation, blood pressure, and oxidative status are well known (Bisignano, Filocamo, Faulks, & Mandalari, 2013). Phytochemicals previously identified from pistachios include fatty acids, phytosterols, and polyphenols; all of them have been identified as protective agents against CVD and even cancer (Liu, 2004). Furthermore, the bioactive constituents of nuts have cardioprotective, antiobesity, anticancer, and antioxidant properties (Casas-Agustench, López-Uriarte, Ros, Bulló, & Salas-Salvadó, 2011; Martínez-González & Bes-Rastrollo, 2011; Urpi-Sarda, Casas, Chiva-Blanch, Romero-Mamani, Valderas-Martínez, Arranz, et al., 2012; Yang, Fletcher, & Reilly, 2009).

To the best of our knowledge, there have been no reports in the literature comparing the bioactive compounds (polyphenols and triterpenoids) of pistachio kernels obtained after grafting on different rootstocks and being cultivated under different irrigation treatments. Thus, the aim of this work was to evaluate the profile of bioactive compounds (polyphenol identification and quantification, polymeric procyanidins, triterpenoids content and inhibitory effects of α -amylase) of pistachio kernels obtained using 3 rootstocks and 3 RDI treatments. The results will help in deciding the best rootstock for Kerman pistachio trees and whether the application of regulated deficit irrigation have any potential benefits for edible pistachio kernels regarding their content of bioactive compounds.

2. MATERIALS AND METHODS

2.1 Chemicals

Methanol, oleanolic, betulinic, and ursolic acids, 3,5-dinitrosalicylic acid, dipotassium hydrogen, phloroglucinol, p-nitrophenyl- α -D-glucopyranoside, orthophosphate dihydrogen, potassium sodium tartrate tetrahydrate, sodium phosphate

monobasic, and α -amylase from porcine pancreas (type VI-8) were purchased from Sigma-Aldrich (Steinheim, Germany). (+)-Catechin, and (-)-epicatechin, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, procyanidin B1 and B2, eriodictyol-3-*O*-glucoside and quercetin and keampferol-3-*O*-glucoside were purchased from Extrasynthese (Lyon Nord, France). Both, ascorbic acid and acetonitrile for ultra-pressure liquid chromatography (UPLC, gradient grade), were purchased from Merck (Darmstadt, Germany). UPLC grade water, prepared by HPL SMART 1000s system (Hydrolab, Gdańsk, Poland), sample was filtrated through a 0.20 μm membrane filter (Millex Smplicity® Filters Membrane) immediately before use.

2.2 Plant material and experimental design

Pistachio trees, cultivar Kerman, were grown during 2013 and 2014 in an area with a Mediterranean climate (experimental farm "El Chaparrillo", Ciudad Real (Spain), 3°56' W and 39°0' N, altitude 640 m above sea level), with an average annual rainfall of 397 mm, mostly distributed outside a four-month summer drought period. The soil can be classified as shallow clay-loam (*Petrocalcic Palexeralfs*) of 0.5 m depth, and a discontinuous *petrocalcic* horizon of around 0.5 m. Tree spacing was set at 7 × 6 m (238 trees ha⁻¹). Male trees of the cultivar Peter were used and distributed evenly throughout the field, in a proportion of 10 %.

The study was conceived using a factorial design with two replicates and with two factors: (i) type of rootstock and (ii) irrigation treatment. Pistachio trees (*Pistacia vera*) were grafted over 3 different rootstocks: *P. terebinthus* L. (TE), *P. integerrima* L. (INT), and *P. atlantica* Desf (AT). Three irrigation treatments were evaluated: (i) T0 or control, in which trees were irrigated with enough water volume to completely avoid hydric stress; (ii) T1, in which irrigation was suppressed until trees reached a stem water potential (SWP) below -1.5 MPA during phase II, then, irrigation was managed to keep SWP below this threshold but as near to it as possible; and, (iii) T2, in which the same strategy as in T1 was used, but with a SWP threshold of -2.0 MPa. In both deficit irrigation treatments (T1 and T2), pistachio trees were rehydrated when phase III started. The irrigation protocol for stressed treatments was derived from the methodology proposed by Moriana, Pérez-López, Prieto, Ramírez-Santa-Pau, and Pérez-Rodríguez (2012) for olive trees and later adapted to pistachio trees by Memmi, Gijón, Couceiro, and Pérez-López (2016).

2.3 Polyphenols extraction

The extraction of polyphenols was conducted as described previously by Wojdyło, Oszmiański, and Bielicki (2013) in quinces, and El-Zaeddi, Calín-Sánchez, Nowicka, Martínez-Tomé, Noguera-Artiaga, Burló, et al. (2017) in herbs. Briefly, grounded pistachio

(~0.6 g) was weighed, 5 mL of 30 % of aqueous methanol (v/v) with 1 % of ascorbic acid and 3 mL of hexane were added, and the suspension was stirred, sonicated for 15 min in a ultrasonic bath JP Selecta S.A, model 3000512 (Barcelona, Spain) with constant frequency (40 kHz), left for 24 h at room temperature in darkness, and, then, centrifuged for 10 min ($20.878 \times g$ at 4 °C); finally, supernatants were analyzed within the same day. The extraction procedure for the analysis of α -amylase activity was the same previously described for polyphenols but using a different solvent, 80 % of aqueous methanol (v/v) with 1 % of HCl (v/v).

2.4 Identification and quantification of polyphenols by LC-PDA-MS-QToF (liquid chromatography photodiode array quadrupole time-off flight mass spectrometry)

The samples were analyzed by using an Acquity UPLC, ultra performance liquid chromatography, system (Waters, Milford, MA), and a Micromass QToF spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative mode were used for the identification in pistachio extracts of polyphenols. The UPLC system was used for the separation of individual polyphenols using a UPLC BEH C18 column (1.7 μm , 2.1 \times 100 mm, Waters Corp.; Milford, USA) at 30 °C. The flow rate was 0.45 mL min^{-1} and 10 μL of the samples were injected, and only 15 min were needed to complete the elution of the sample. Solvent A (2.5 % formic acid, v/v) and solvent B (100 % acetonitrile) were the mobile phase. The elution process was as follows: (i) 0 - 1 min, isocratic elution with 99 % A; (ii) linear gradient was used until 12 min, lowering A to 0 %; and, (iii) from 12.5 to 13.5 min, the initial composition (99 % A) was used, and was held constant to re-equilibrate the column. Full scan from m/z 100 to 1500 was used for the analysis of the samples. The internal reference compound (leucine encephalin) was introduced via the LockSpray channel and the lock mass correction was ± 1.000 for the mass window. All QToF-MS chromatograms are presented as the base peak intensity (BPI) chromatograms and scaled to 12.400 counts per second (cps) (=100 %). The eluent was passed to the electrospray source with the following conditions: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 130 °C, desolvation temperature of 350 °C and desolvation gas (nitrogen) flow rate 300 L h^{-1} .

Polyphenolic compounds were monitored at the following wavelengths, 520 nm (anthocyanins), 360 nm (flavonols and flavanones), 320 nm (phenolic acids), and 280 nm (flavan-3-ols). Quantification was done by injecting standard solutions of known concentrations according to Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016). This analysis was run in triplicate, and results were expressed as mg *per* 100 g dry matter (dm) of pistachio kernel.

2.5 Analysis of triterpenoids by the LC-MS-PDA (liquid chromatography-mass spectrometry-photodiode array)

Sample extraction was performed as described by Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016) in figs, and Kolniak-Ostek (2016) in pears. The identification and quantification of oleanolic, betulinic, and ursolic acids were performed using an ACQUITY Ultra Performance LC system with binary solvent manager (Waters Corp.), a UPLC BEH C18 column (1.7 μm , 2.1 \times 150 mm, Waters Corp.), a QToF mass spectrometer (Waters Corp.) equipped with an ESI source operating in negative mode, and methanol-acetonitrile (15:85, v/v) as an elution solvent. The compounds were monitored at 210 nm; the m/z for betulinic, ursolic, and oleanolic acids were 455.3452, 455.3365, 455.3496, respectively, and their retention times were 6.80, 7.50, and 7.85 min, respectively. This analysis was run in triplicate, and results were expressed as $\mu\text{g g}^{-1}$ dm.

2.6 Polymeric procyanidins (PP)

The protocol described previously by Kennedy and Jones (2001) and later modified by Wojdyło, Carbonell-Barrachina, Legua, and Hernández (2016) was used to produce the phloroglucinolysis of the pistachio samples. These samples were treated with a solution of HCl/MeOH/phloroglucinol/ascorbic acid (solution of 0.3 M HCl in MeOH containing 75 g L^{-1} phloroglucinol and 10 g L^{-1} ascorbic acid) and after 30 min, 1.2 mL of aqueous sodium acetate was added to stop the reaction. Polymeric proanthocyanidins were analyzed by UPLC-FL (ACQUITY UPLC™; Waters Corporation; Milford, USA), and using wavelengths of excitation/emission of 278/330 nm. Sample was injected into a BEH RP Shield C18 column (2.1 mm \times 50 mm; 1.7 μm) with precolumn (Waters Corp., Milford, MA, USA) operated at 15 $^{\circ}\text{C}$; the injection volume was 5 μL , and flow rate was 0.45 mL min^{-1} . The elution solvents were 2.5 % aqueous acetic acid (v/v) (A) and 100 % acetonitrile (B). Samples were eluted using the following solvent program: 0–0.6 min, 2 % B; 0.6–2.17 min, 2–3 % B; 2.17–3.22 min, 3–10 % B; 3.22–5.00 min, 10–15 % B; 5.00–6.00 min, 100 % B; and finally, washing and reconditioning of the column for next 1.50 min. Procyanidin B1 and B2 were used as reference compounds and were subjected to phloroglucinolysis degradation as described above, and the conversion rate of (–)-epicatechin and (+)-catechin was calculated. The mean degree of polymerization (DP) was obtained by calculating the molar ratio of both extension and terminal flavan-3-ol units to terminal units (Wojdyło, Oszmiański, & Bielicki, 2013). This analysis was run in triplicate and results were expressed as *mg per 100 g dm* of pistachio nut.

2.7 α -Amylase inhibition

The α -amylase inhibition was measured according to the procedure described initially by González-Muñoz, Quesille-Villalobos, Fuentealba, Shetty, and Gálvez Ranilla

(2013), and later modified by Nowicka, Wojdyło, and Samoticha (2016). The α -amylase inhibitory effect of pistachio was assayed by measuring the absorbance at 540 nm using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). This analysis was run in triplicate and results were expressed as IC_{50} (mg of dried nut mL^{-1}).

2.8 Statistical analyses

The data was subjected to two-way analysis of variance (ANOVA) and later to Tukey's multiple-range test to compare the means. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were done using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD).

3. RESULTS AND DISCUSSION

3.1. Qualitative analysis of phenolic compounds

Seventeen compounds were identified in the methanolic extract of pistachio samples using LC-PDA-MS-QToF: 8 flavonols, 4 flavan-3-ols, 2 anthocyanins, 2 phenolic acids, and 1 flavanone (**Table 1**). The phenolic compounds were identified according to their retention times, molecular masses, fragmentation patterns, characteristic spectra, and literature references (Fabani, Luna, Baroni, Monferran, Ighani, Tapia, et al., 2013; Grace, et al., 2016; Gültekin-Özgüven, Davarci, Pasli, Demir, & Özçelik, 2015; Mandalari, Bisignano, Filocamo, Chessa, Sarò, Torre, et al., 2013; Regueiro, Sánchez-González, Vallverdú-Queralt, Simal-Gándara, Lamuela-Raventós, & Izquierdo-Pulido, 2014). The combination of LC-PDA-MS-QToF analysis and electrospray ionization (ESI) mass spectrometry allowed efficient identification of all polyphenols found in the pistachio samples under analysis.

Flavonoids have a C₆-C₃-C₆ general structural backbone in which the two C₆ units (rings A and B) are of phenolic nature. Due to the hydroxylation pattern and variations in the chromane ring (ring C), flavonoids can be further divided into sub-groups, such as anthocyanins, flavan-3-ols, flavones, flavanones, and flavonols (Tsao, 2010). In case of the pistachio under analysis, the predominant sub-group was flavan-3-ols being the main compound identified as (+)-catechin (peak at 2.65 min), with a (M-H)⁻ ion at m/z 289.0727 and MS/MS fragments at m/z 245, 205, and 179. Also it was possible to identify (-)-epicatechin with the same (M-H)⁻ ion as (+)-catechin, but different retention time (peak at 3.55 min). Apart from these compounds, two procyanidins dimers were identified (peaks at 3.22 and 9.00 min) with m/z 577 and a characteristic fragmentation pattern for a monomer of a negatively charged molecular ion [M-H]⁻ at m/z 289. In the case of flavonols, there were two types of derivatives, with a fragment at m/z 285 and 301, characteristic

for kaempferol and quercetin, respectively (**Table 1**). Furthermore, two anthocyanins with a fragment at m/z 287 were found, cyanidin-3-*O*-galactoside and cyanidin-3-*O*-glucoside (peaks at 4.21 and 4.27 min, respectively). Finally, there was one flavanone, eriodictyol-3-*O*-glucoside ($[M-H]^-$ at m/z 449), and one sugar derivative, deoxydihexoside, at retention time 2.87 min and with a characteristic fragmentation pattern $[M-H]^-$ at m/z 309 (Kitrytė, Kraujalienė, Šulniūtė, Pukalskas, & Venskutonis, 2017).

3.2. Quantification of phenolic compounds

The main phenolic compound found in pistachio samples was (+)-catechin with values ranging 97 and 143 mg (100 g)⁻¹ dm, followed by (-)-epicatechin with values from 25.5 to 42.6 mg (100 g)⁻¹ dm. These two compounds have been also reported as the predominant phenolic compound in the skin and kernels of pistachios by others researchers (Fabani, et al., 2013; Gültekin-Özgüven, Davarci, Pasli, Demir, & Özçelik, 2015; Mandalari, et al., 2013; Tomaino, Martorana, Arcoraci, Monteleone, Giovinazzo, & Saija, 2010).

The use of different rootstocks proved to have a significant effect on the individual contents of caffeic acid hexoside and kaempferol-*O*-dihexoside; with the rootstock *P. terebinthus* getting the highest contents 9.18 and 1.91 mg (100 g)⁻¹ dm, respectively (**Table 2**). However, no significant differences were found in the rest of phenolic compounds.

The use of regulated deficit irrigation in pistachios caused a decrease in the concentration of the main compound, (+)-catechin; its content was directly related to the amount of water reduced during cultivation, with T0 pistachios having the highest concentration [143 mg (100 g)⁻¹ dm], followed by T1 nuts [119 mg (100 g)⁻¹ dm], and T2 [97.6 mg (100 g)⁻¹ dm]. On the other hand, application of moderate regulated deficit irrigation (T1) had a significant effect on the concentration of kaempferol-3-*O*-glucoside and quercetin-*O*-galloyl-hexoside, increasing their contents from 1.6 (T0 and T2) to 1.8 mg (100 g)⁻¹ dm (T1), and from ~6.8 (T0 and T2) to 8.1 mg (100 g)⁻¹ dm (T1), respectively (**Table 2**).

The concentration of polymeric procyanidins (PP) in pistachio samples ranged from 323 to 443 mg (100 g)⁻¹ dm and statistically significant differences were found for both of the factors under study, rootstock and irrigation treatment (**Table 2**). Pistachios obtained with *P. terebinthus* and *P. atlantica* rootstocks [443 and 415 mg (100 g)⁻¹ dm, respectively] had higher PP content than those obtained using the *P. integerrima* rootstock [323 mg (100 g)⁻¹ dm]. In case of pistachios obtained by different irrigation treatments, the application of RDI treatments caused a significant increase of the PP contents, increasing

from 352 mg (100 g)⁻¹ dm in control samples up to 427 and 404 mg (100 g)⁻¹ dm in T1 and T2 samples, respectively (**Table 2**).

If the total content of phenolic compounds is considered, the pistachios obtained with *P. terebinthus* and *P. atlantica* rootstocks had higher concentration [680 and 636 mg (100 g)⁻¹, respectively] than those obtained using *P. integerrima* rootstock [554 mg (100 g)⁻¹ dm]. On the other hand, the application of a severe RDI treatment, T2, did not have a significant incidence as compared to the control treatment; [T0= 603 mg (100 g)⁻¹ dm; T2 = 604 mg (100 g)⁻¹ dm], while the application of a moderate deficit irrigation (T1) increased the total concentration of phenolic compounds up to 664 mg (100 g)⁻¹ dm (about 10 % of the total content) (**Table 2**).

This increase in the total content of phenolic compounds and especially the increase of PP in pistachio kernels obtained under moderate water deficit (T1) agreed with results obtained by Sun, Liang, Shan, Viernstein, and Unger (2011) and Guo, Duan, Tang, Qian, Zhu, Qian, et al. (2011) for jujube fruits, and Galindo, Calín-Sánchez, Griñán, Rodríguez, Cruz, Girón, et al. (2017) for pomegranates. They postulated that polyphenolic compounds, especially PP, increased under harsh growing conditions, i.e. water deficit. Therefore, in plants under water deficit (T1 and T2), when carbohydrates exceed the amount used for growth concentrations, the excess of CO₂ assimilated could increase the biosynthesis of carbon-based secondary metabolites (Horner, 1990). Moreover, the increase in the procyanidin content through a water stress effect could also be related with the fact that water deficit can lead to an increase in the levels of free phenylalanine (Chalker-Scott & Fuchigami, 1989), a precursor in the PP synthesis, and an increase in L-phenylalanine ammonia lyase (PAL) activity and, probably PAL synthesis (Chalker-Scott & Fuchigami, 1989; Jess Tovar, Paz Romero, Girona, & Jos Motilva, 2002).

3.3. Quantification of triterpenoids in pistachio kernels

Triterpenoids are known for their antioxidant, anti-inflammatory, and anticancer properties (Claude, Morin, Lafosse, & Andre, 2004). **Table 3** shows the results obtained after quantification of triterpenoids in pistachio as affected by rootstock and irrigation treatment. The detected compounds were identified as betulinic, oleanolic, and ursolic acids based on MS profiles with the fragmentation pathways, UV spectra, and the retention times of authentic standards.

In pistachio samples, the average total of triterpenoids content was ~644 µg g⁻¹ dm and it was not significantly affected by neither the rootstock nor the irrigation treatment. Even if the individual contents of each triterpenoid are studied, no significant effect of the irrigation treatment was found. In this way, the mean values for the contents of the three

triterpenoids identified were 98, 60, and 486 $\mu\text{g g}^{-1}$ dm for betulinic, oleanolic, and ursolic acids, respectively. Thus, the predominant compound in this chemical family in pistachio kernels was the ursolic acid.

In case of pistachios obtained under different rootstocks, *P. terebinthus* and *P. Atlantica* had the highest content of betulinic acid (111 and 102 $\mu\text{g g}^{-1}$, respectively) while *P. integerrima* had the lowest one (81 $\mu\text{g g}^{-1}$). In the other two studied triterpenoids (oleanolic and ursolic acid), the use of different rootstocks had no significant effect on their concentration (**Table 3**).

3.4. α -Amylase activity in pistachio kernels

Dietary carbohydrates are hydrolyzed in human body by pancreatic α -amylase, one of the enzymes responsible for the breakdown of oligosaccharides and disaccharides into absorbable monosaccharides. Inhibition of that enzyme, by controlling glucose absorption, may be effective in the regulation of type 2 diabetes (Gironés-Vilaplana, Baenas, Villaño, Speisky, García-Viguera, & Moreno, 2014; Wojdyło, Nowicka, Carbonell-Barrachina, & Hernández, 2016). In this study, the α -amylase inhibition was measured in pistachios obtained using different rootstocks and irrigation treatments, and the results are presented as IC_{50} (mg of dried nut mL^{-1}) (**Table 4**). No significant effect was found in pistachios obtained using different rootstocks; however, pistachio samples obtained under RDI (T1 and T2) had significantly higher values of inhibition (IC_{50} = 26.4 and 25.2, respectively) than control nuts (IC_{50} = 19.7). Consequently, results showed that the application of RDI treatments did not eliminate the α -amylase inhibitory activity in pistachio. The α -amylase inhibitory activity in pistachios might be related to their higher phenolic contents (**Table 2**), as triterpenoids have not being proved to have any significant effect on this enzyme; in addition, this effect could be linked to the antioxidant capacity of the phenolic compounds. Recent studies suggested that fruits with high concentration of procyanidins, such as the case of pistachios, are effective inhibitors of α -amylase activity by the formation of enzyme-tannin complexes, which prevented the enzyme from interacting with the starch (Boath, Stewart, & McDougall, 2012; Nowicka, Wojdyło, & Samoticha, 2016). This inhibitory effect on α -amylase demonstrated that pistachio nuts could possess possible beneficial effects in the treatment of diabetes, which must be further confirmed by *in vivo* studies.

To the best of our knowledge, the α -amylase inhibitory activity of pistachios, obtained under different irrigation treatments, has not been previously reported elsewhere. Therefore, the data presented on this study could be assumed as the first report in the literature stating this point.

4. CONCLUSIONS

This was the first study establishing quantitatively the phenolic profiles, triterpenoid compounds, and α -amylase inhibition in pistachio kernels as affected by rootstock and regulated irrigation treatments. After comparing the results obtained using 3 rootstocks (*P. atlantica*, *P. integerrima*, and *P. terebinthus*), it has been demonstrated that the use of the *P. terebinthus* and *P. atlantica* rootstocks led to pistachio kernels with higher functional quality, by increasing the contents of betulinic acid and total polyphenols as compared to the fruits obtained with *P. integerrima* rootstock. On the other hand, reducing the irrigation water volume (regulated deficit irrigation) during the cultivation of pistachio (stage II, shell hardening) has been shown to have a significant impact on the functionality of pistachios. The application of a moderate water reduction treatment (T1) increased the total polyphenol content of the samples, as well as the contents of quercetin-*O*-galloyl-hexoside, keampferol-3-*O*-glucoside, and polymeric procyanidins. When the water reduction was severe (T2), the concentration of polymeric procyanidins was also increased, but the total polyphenolic content was not significantly affected. In case of the α -amylase inhibition, the application of RDI treatments does not eliminate inhibitory activity in pistachio. Therefore, the final conclusion of this study was that the application of a T1 treatment led to pistachios with a better functional profile than the control ones besides reducing the water consumption during its cultivation; this fact is essential because it is linked to a lower economic cost and environmental impact by saving water.

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REFERENCES

- Bisignano, C., Filocamo, A., Faulks, R. M., & Mandalari, G. (2013). In vitro antimicrobial activity of pistachio (*Pistacia vera* L.) polyphenols. *FEMS Microbiology Letters*, 341(1), 62-67.
- Boath, A. S., Stewart, D., & McDougall, G. J. (2012). Berry components inhibit α -glucosidase in vitro: Synergies between acarbose and polyphenols from black currant and rowanberry. *Food Chemistry*, 135(3), 929-936.

- Carbonell-Barrachina, A. A., Memmi, H., Noguera-Artiaga, L., del Carmen Gijón-López, M., Ciapa, R., & Pérez-López, D. (2015). Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *Journal of the Science of Food and Agriculture*, 95(14), 2866-2873.
- Casas-Agustench, P., López-Uriarte, P., Ros, E., Bulló, M., & Salas-Salvadó, J. (2011). Nuts, hypertension and endothelial function. *Nutrition, Metabolism and Cardiovascular Diseases*, 21, S21-S33.
- Chalker-Scott, L., & Fuchigami, L. H. (1989). The role of phenolic compounds in plant stress responses. In H. L. Paul (Ed.), *Low-Temperature Stress Physiology in Crops*, (pp. 27-40). Boca Raton, FL: CRC Press.
- Claude, B., Morin, P., Lafosse, M., & Andre, P. (2004). Evaluation of apparent formation constants of pentacyclic triterpene acids complexes with derivatized β - and γ -cyclodextrins by reversed phase liquid chromatography. *Journal of Chromatography A*, 1049(1), 37-42.
- El-Zaiedi, H., Calín-Sánchez, Á., Nowicka, P., Martínez-Tomé, J., Noguera-Artiaga, L., Burló, F., Wojdyło, A., & Carbonell-Barrachina, Á. A. (2017). Preharvest treatments with malic, oxalic, and acetylsalicylic acids affect the phenolic composition and antioxidant capacity of coriander, dill and parsley. *Food Chemistry*, 226, 179-186.
- Fabani, M. P., Luna, L., Baroni, M. V., Monferran, M. V., Ighani, M., Tapia, A., Wunderlin, D. A., & Feresin, G. E. (2013). Pistachio (*Pistacia vera* var Kerman) from Argentinean cultivars. A natural product with potential to improve human health. *Journal of Functional Foods*, 5(3), 1347-1356.
- Galindo, A., Calín-Sánchez, Á., Griñán, I., Rodríguez, P., Cruz, Z. N., Girón, I. F., Corell, M., Martínez-Font, R., Moriana, A., Carbonell-Barrachina, A. A., Torrecillas, A., & Hernández, F. (2017). Water stress at the end of the pomegranate fruit ripening stage produces earlier harvest and improves fruit quality. *Scientia Horticulturae*, 226, 68-74.
- Gijón, M. D. C., Gimenez, C., Perez-López, D., Guerrero, J., Couceiro, J. F., & Moriana, A. (2011). Water relations of pistachio (*Pistacia vera* L.) as affected by phenological stages and water regimes. *Scientia Horticulturae*, 128(4), 415-422.
- Gironés-Vilaplana, A., Baenas, N., Villaño, D., Speisky, H., García-Viguera, C., & Moreno, D. A. (2014). Evaluation of Latin-American fruits rich in phytochemicals with biological effects. *Journal of Functional Foods*, 7, 599-608.
- Goldhamer, D. A. (1995). Irrigation management. *Pistachio production*, 71-81.
- González-Muñoz, A., Quesille-Villalobos, A. M., Fuentealba, C., Shetty, K., & Gálvez Ranilla, L. (2013). Potential of Chilean Native Corn (*Zea mays* L.) Accessions as Natural Sources of Phenolic Antioxidants and in Vitro Bioactivity for Hyperglycemia and Hypertension Management. *Journal of Agricultural and Food Chemistry*, 61(46), 10995-11007.

- Grace, M. H., Esposito, D., Timmers, M. A., Xiong, J., Yousef, G., Komarnytsky, S., & Lila, M. A. (2016). Chemical composition, antioxidant and anti-inflammatory properties of pistachio hull extracts. *Food Chemistry*, *210*, 85-95.
- Grant, O. M. (2012). Understanding and exploiting the impact of drought stress on plant physiology. In *Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability*, (pp. 89-104).
- Gültekin-Özgüven, M., Davarci, F., Pasli, A. A., Demir, N., & Özçelik, B. (2015). Determination of phenolic compounds by ultra high liquid chromatography-tandem mass spectrometry: Applications in nuts. *LWT - Food Science and Technology*, *64*(1), 42-49.
- Guo, S., Duan, J. A., Tang, Y., Qian, D., Zhu, Z., Qian, Y., Shang, E., & Su, S. (2011). UHPLC-TOFMS coupled with chemometric method as a powerful technique for rapid exploring of differentiating components between two *Ziziphus* species. *Journal of Separation Science*, *34*(6), 659-666.
- Hojjati, M., Noguera-Artiaga, L., Wojdyło, A., & Carbonell-Barrachina, Á. A. (2015). Effects of microwave roasting on physicochemical properties of pistachios (*Pistacia vera* L.). *Food Science and Biotechnology*, *24*(6), 1995-2001.
- Horner, J. D. (1990). Nonlinear effects of water deficits on foliar tannin concentration. *Biochemical Systematics and Ecology*, *18*(4), 211-213.
- Jess Tovar, M., Paz Romero, M., Girona, J., & Jos Motilva, M. (2002). L-phenylalanine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L. cv Arbequina) fruit grown under different irrigation regimes. *Journal of the Science of Food and Agriculture*, *82*(8), 892-898.
- Kahyaoglu, T. (2008). Optimization of the pistachio nut roasting process using response surface methodology and gene expression programming. *LWT - Food Science and Technology*, *41*(1), 26-33.
- Kennedy, J. A., & Jones, G. P. (2001). Analysis of Proanthocyanidin Cleavage Products Following Acid-Catalysis in the Presence of Excess Phloroglucinol. *Journal of Agricultural and Food Chemistry*, *49*(4), 1740-1746.
- Kitrytė, V., Kraujalienė, V., Šulniūtė, V., Pukalskas, A., & Venskutonis, P. R. (2017). Chokeberry pomace valorization into food ingredients by enzyme-assisted extraction: Process optimization and product characterization. *Food and Bioprocess Processing*, *105*(Supplement C), 36-50.
- Kolniak-Ostek, J. (2016). Content of bioactive compounds and antioxidant capacity in skin tissues of pear. *Journal of Functional Foods*, *23*, 40-51.
- Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *Journal of Nutrition*, *134*(12 SUPPL.), 3479S-3485S.

- Mandalari, G., Bisignano, C., Filocamo, A., Chessa, S., Sarò, M., Torre, G., Faulks, R. M., & Dugo, P. (2013). Bioaccessibility of pistachio polyphenols, xanthophylls, and tocopherols during simulated human digestion. *Nutrition*, *29*(1), 338-344.
- Martínez-González, M. A., & Bes-Rastrollo, M. (2011). Nut consumption, weight gain and obesity: Epidemiological evidence. *Nutrition, Metabolism and Cardiovascular Diseases*, *21*, S40-S45.
- Memmi, H., Gijón, M. C., Couceiro, J. F., & Pérez-López, D. (2016). Water stress thresholds for regulated deficit irrigation in pistachio trees: Rootstock influence and effects on yield quality. *Agricultural Water Management*, *164*, 58-72.
- Moriana, A., Memmi, H., Centeno, A., Martín-Palomo, M. J., Corell, M., Torrecillas, A., & Pérez-López, D. (2018). Influence of rootstock on pistachio (*Pistacia vera* L. cv Kerman) water relations. *Agricultural Water Management*, In press.
- Moriana, A., Pérez-López, D., Prieto, M. H., Ramírez-Santa-Pau, M., & Pérez-Rodríguez, J. M. (2012). Midday stem water potential as a useful tool for estimating irrigation requirements in olive trees. *Agricultural Water Management*, *112*, 43-54.
- Noguera-Artiaga, L., Lipan, L., Vázquez-Araújo, L., Barber, X., Pérez-López, D., & Carbonell-Barrachina, Á. A. (2016). Opinion of Spanish Consumers on Hydrosustainable Pistachios. *Journal of Food Science*, *81*(10), S2559-S2565.
- Nowicka, P., Wojdyło, A., & Samoticha, J. (2016). Evaluation of phytochemicals, antioxidant capacity, and antidiabetic activity of novel smoothies from selected *Prunus* fruits. *Journal of Functional Foods*, *25*, 397-407.
- Regueiro, J., Sánchez-González, C., Vallverdú-Queralt, A., Simal-Gándara, J., Lamuela-Raventós, R., & Izquierdo-Pulido, M. (2014). Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap–Orbitrap mass spectrometry. *Food Chemistry*, *152*(Supplement C), 340-348.
- Sun, Y. F., Liang, Z. S., Shan, C. J., Viernstein, H., & Unger, F. (2011). Comprehensive evaluation of natural antioxidants and antioxidant potentials in *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou fruits based on geographical origin by TOPSIS method. *Food Chemistry*, *124*(4), 1612-1619.
- Tomaino, A., Martorana, M., Arcoraci, T., Monteleone, D., Giovinazzo, C., & Saija, A. (2010). Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie*, *92*(9), 1115-1122.
- Tsao, R. (2010). Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients*, *2*(12), 1231-1246.
- Uрпи-Sarda, M., Casas, R., Chiva-Blanch, G., Romero-Mamani, E. S., Valderas-Martínez, P., Arranz, S., Andres-Lacueva, C., Llorach, R., Medina-Remón, A., Lamuela-Raventós, R. M., & Estruch, R.

- (2012). Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomarkers related to atherosclerosis. *Pharmacological Research*, 65(6), 577-583.
- Wojdyło, A., Carbonell-Barrachina, Á. A., Legua, P., & Hernández, F. (2016). Phenolic composition, ascorbic acid content, and antioxidant capacity of Spanish jujube (*Ziziphus jujube* Mill.) fruits. *Food Chemistry*, 201, 307-314.
- Wojdyło, A., Nowicka, P., Carbonell-Barrachina, Á. A., & Hernández, F. (2016). Phenolic compounds, antioxidant and antidiabetic activity of different cultivars of *Ficus carica* L. fruits. *Journal of Functional Foods*, 25, 421-432.
- Wojdyło, A., Oszmiański, J., & Bielicki, P. (2013). Polyphenolic Composition, Antioxidant Activity, and Polyphenol Oxidase (PPO) Activity of Quince (*Cydonia oblonga* Miller) Varieties. *Journal of Agricultural and Food Chemistry*, 61(11), 2762-2772.
- Yang, J., Fletcher, J. E., & Reilly, J. O. (2009). A series dynamic resistor based converter protection scheme for doubly-fed induction generator during various fault conditions. In *2009 IEEE Power & Energy Society General Meeting*, (pp. 1-8).



Table 1. Identification of phenolic compounds found in pistachios using LC-MS-QToF/PDA.

Compounds	Retention time (min)	λ_{\max} (nm)	MS [M-H] ⁻ (m/z)	MS/MS (m/z)
(+)-Catechin	2.65	278	289.0727	
Deoxydihexoside	2.87	280	309.1236	
Procyanidin dimer	3.22	280	577.1375	289.0727
(-)-Epicatechin	3.55	275	289.0727	
Caffeic acid hexoside	3.84	270	465.1021	325.0945/303.0502/285.0395
Cyanidin-3- <i>O</i> -galactoside	4.21	278/525	447.0887	287.0313
Cyanidin-3- <i>O</i> -glucoside	4.27	278/517	447.0887	287.0245
Eriodictyol-3- <i>O</i> -glucoside	4.81	285/350	449.1078	389.1755/339.0400/287.0612
Quercetin- <i>O</i> -galloyl-hexoside	6.05	270/352	615.2206	365.1801/301.0255
Keampferol-3- <i>O</i> -glucoside	6.20	287/352	447.0502	285.0361
Quercetin-3- <i>O</i> -rutinoside	6.23	237/353	609.1484	301.0297
Quercetin-3- <i>O</i> -galactoside	6.35	237/352	463.0870	301.0255
Quercetin-3- <i>O</i> -glucoside	6.51	237/350	463.0914	301.0255
Protocatechuic acid derivatives	7.14	269	425.0900	197/153/109
Keampferol- <i>O</i> -dihexoside	7.60	350	609.2394	285.0395
Procyanidin dimer	9.00	284	577.2555	289.0578
Quercetin-triglucoside	9.84	238/353	625.2783	301.1220

Table 2. Quantification of phenolic compounds [mg (100 g)⁻¹ dm] found in pistachios as affected by rootstock (*P. integerrima* = INT, *P. atlantica* = AT, and *P. terebinthus* = TE) and irrigation treatment.

Compounds	ANOVA test [†]			Concentration [mg (100 g) ⁻¹ dm]					
	Rootstock	Irrigation	Root. x Irrig.	Rootstock			Irrigation treatment		
				INT	AT	TE	T0	T1	T2
(+)-Catechin	NS	***	NS	114	118	127	143 a [‡]	119 b	97.64 c
(-)-Epicatechin	NS	NS	NS	42.61	25.51	30.98	30.49	38.10	30.51
Deoxydihexoside	NS	NS	NS	9.96	11.41	11.25	11.29	11.28	10.06
Caffeic acid hexoside	**	NS	NS	7.71 b	8.28 b	9.18 a	8.20	8.83	8.23
Protocatechuic acid derivatives	NS	NS	NS	7.76	8.67	8.13	8.37	8.75	7.43
Eriodictyol-3- <i>O</i> -glucoside	NS	NS	NS	2.40	2.23	2.10	2.19	2.27	2.27
Quercetin- <i>O</i> -galloyl-hexoside	NS	**	*	7.22	6.78	7.70	6.83 b	8.11 a	6.74 b
Keampferol-3- <i>O</i> -glucoside	NS	***	*	1.60	1.71	1.74	1.60 b	1.84 a	1.60 b
Quercetin-3- <i>O</i> -rutinoside	NS	NS	NS	3.97	4.30	4.40	3.92	4.69	4.06
Quercetin-3- <i>O</i> -galactoside	NS	NS	NS	4.24	4.22	4.37	4.12	4.34	4.37
Quercetin-3- <i>O</i> -glucoside	NS	NS	NS	4.70	5.45	4.98	5.05	5.14	4.94
Keampferol- <i>O</i> -dihexoside	***	NS	***	1.26 b	1.19 b	1.91 a	1.24	1.34	1.14
Quercetin-triglucoside	NS	NS	*	1.39	1.60	2.90	2.75	1.58	1.56
Cyanidin-3- <i>O</i> -galactoside	NS	NS	NS	16.35	15.51	14.59	14.67	15.92	15.87
Cyanidin-3- <i>O</i> -glucoside	NS	NS	NS	3.31	3.88	3.51	3.39	3.75	3.56
Polymeric procyanidins	***	***	***	323 b	415 a	443 a	352 b	427 a	404 a
Total	**	***	**	554 b	636 a	680 a	603 b	664 a	604 b

[†]NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001 respectively. [‡]Values (mean of 3 replications) followed by the same letter, within the same row and factor (rootstock or irrigation treatment), were not significantly different ($p < 0.05$), according to the Tukey's least significant difference test.

Table 3. Determination of triterpenoids ($\mu\text{g g}^{-1} \text{dm}$) found in pistachios as affected by rootstock and irrigation treatment.

Factors	Triterpenoids ($\mu\text{g g}^{-1} \text{dm}$)			
	Betulinic acid	Oleanolic acid	Ursolic acid	Total
	ANOVA test[†]			
Rootstock	*	NS	NS	NS
Irrigation	NS	NS	NS	NS
Root. x Irrigat.	NS	NS	NS	NS
	Tukey's multiple range test[‡]			
Rootstock				
<i>P. integerrima</i>	80.9 b	62.5	472	616
<i>P. atlantica</i>	102 b	57.6	456	616
<i>P. terebinthus</i>	111 a	60.5	528	700
Irrigation				
T0	108	63.6	551	723
T1	93.3	73.0	451	617
T2	92.9	44.1	455	592

[†]NS = not significant at $p < 0.05$; * significant at $p < 0.05$. [‡]Values (mean of 3 replications) followed by the same letter, within the same column and factor (rootstock or irrigation treatment), were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

Table 4. Determination of α -amylase inhibition IC_{50} (mg dried nut mL^{-1}) in pistachios as affected by rootstock and irrigation treatment.

Factors	α -Amylase inhibition IC_{50} (mg dried nut mL^{-1})
	ANOVA test[†]
Rootstock	NS
Irrigation	*
Root. x Irrigat.	NS
	Tukey's multiple range test[‡]
Rootstock	
<i>P. integerrima</i>	24.00
<i>P. atlantica</i>	23.90
<i>P. terebinthus</i>	23.42
Irrigation	
T0	19.73 b
T1	26.42 a
T2	25.17 a

[†]NS = not significant at $p < 0.05$; * significant at $p < 0.05$. [‡]Values (mean of 3 replications) followed by the same letter, within the same column and factor (rootstock or irrigation treatment), were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

**ANTIOXIDANT, ANTIMUTAGENIC AND CYTOPROTECTIVE
PROPERTIES OF HYDROSOS PISTACHIO NUTS**

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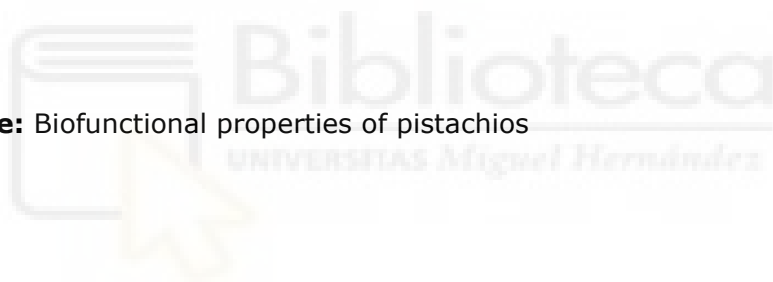
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Running title: Biofunctional properties of pistachios



ABSTRACT

Pistachio nuts are included among the foods with the highest antioxidant capacity. Stressed cultivating conditions, such as the use of regulated deficit irrigation (RDI), are expected to create a plant response that might increase the production of secondary metabolites. Fruits that are obtained under RDI treatments are commonly called hydroSOS products. The aim of this work was to study the influence of using different rootstocks (*P. atlantica*, *P. integerrima*, and *P. terebinthus*) and two RDI treatments on the antioxidant (ABTS, ferric reducing antioxidant power (FRAP), and DPPH), antimutagenic (Ames test), and cytotoxicity (MTT assay in five human cell lines) activities of pistachios. *P. terebinthus* showed the best antioxidant activity, and the RDI treatments maintained and improved the antioxidant properties of pistachios. Neither the rootstock nor the RDI had significant impact on the antimutagenic potential of pistachios. The nut extracts had no toxic effect on non-cancerous cells and the application of RDI did not reduce their cytoprotective capacity. Furthermore, neither rootstock nor RDI treatments affected the ability of the pistachio extracts of preventing the oxidative damage by H₂O₂. The application of RDI strategies, in addition to allowing irrigation water saving, led to obtaining pistachios with the same or even better biofunctional characteristics as compared to fully irrigated pistachios.

KEYWORDS: Ames test; cancer; rootstock; MTT assay; *Pistacia vera*.

1. INTRODUCTION

The genus *Pistacia* belongs to the Anacardiaceae, which is a family that comprises about 70 genera and over 600 species. *Pistacia vera* is the only species of the genus commercially cultivated, and the rest of the species are mostly used as rootstocks [1]. As pistachio cultivation requires the use of rootstock, it is essential to study its influence on nut quality and functional activity. The main pistachio rootstocks are (i) *P. terebinthus* L. in the Mediterranean basin, (ii) *P. integerrima* L., and (iii) a hybrid between *P. integerrima* Steward ex Brandis and *P. atlantica* Desf. (UCB-I) in the USA, and *P. vera* L. in Iran [2,3].

Water is a scarce commodity and all productive sectors depend on it, especially agriculture. Therefore, it is necessary to improve the efficiency of its use. Regulated deficit irrigation (RDI) is a system of managing water supply by imposing different levels of water deficit at specific phenological stages, which have been found to be less sensitive, with no or low yield reduction and, consequently, low or no loss of economic benefits to farmers [4]. Vegetables and fruit that are cultivated under RDI are marketed under the brand "hydroSOSustainable or hydroSOS" products. This food category is characterized by its environmental respect (optimized use of irrigation water) and a theoretical increase of secondary metabolites, which will improve the functionality and quality [5]. To be capable of categorizing a product as hydroSOS, it is necessary that it meets strict water control requirements during cultivation. This characterization is based on 16 hydric indicators (each one providing different marks or scores), and their sum allows for classifying orchard management as a hydroSOS one or not [6].

The application of RDI depends on the phenological phases of each crop. In the case of pistachio, three different phases can be easily distinguished during fruit development: (i) the nut grows up to its maximum size, (ii) the shell hardens, and (iii) the growth of the edible part occurs. The second stage is the one at which the pistachio nut is less sensitive to water stress; thus, strategies that aimed at reducing water supply during the cultivation of this nut must be applied at this specific time period.

Cancer is one of the leading causes of death in the world. Environmental factors, such as carcinogens, viruses, chemicals, and radiation, as well as by a genetic history, cause it [7]. Exposure to mutagens is one of the main causes of cancer development, causing the mutation of genes that are directly involved in the regulation of the cell cycle. Lung, colorectal, stomach, liver, and breast are the five types of cancer that cause the highest number of deaths [8]. Many natural products have the potential to trigger apoptosis in numerous human cancer cell types. Nowadays, a number of plant-based agents are clinically used as a support therapy in cancer treatment,

proving the goodness of these type of products [9]. Hence, it is necessary to search for new plant-derived products as apoptosis inducers [10,11].

Pistachio nuts have been recently ranked among the first 50 highest antioxidant food products and they are a rich source of phenolic compounds [12]. Pistachio contains epicatechin, quercetin, kaempferol, cyaniding-3-O-galactoside, cyanindin-3-O-glucoside, among other polyphenols [13]. It has been shown that pistachio polyphenols are bioaccessible during simulated human digestion, releasing more than 90% of its total content in the gastric compartment [14]. Consequently, pistachios have a high number of bioactive compounds, and the human body can assimilate and use these.

The main aims of this work were i) to study the antioxidant, antimutagenic, and cytoprotective properties of pistachios and ii) the influence of the rootstock ($n = 3$) and a decrease in the application of irrigation water during cultivation on their main functional activity.

2. RESULTS AND DISCUSSION

2.1. Antioxidant test

The ABTS+ method, which is based on the capability of presumptive antioxidant to reduce the ABTS+• radical, is one of the most widely used methods, because it uses a stable reaction, is practical, highly sensitive, and fast to be carried out. Additionally, this method allows confirmation of antiradical capacity of either hydrophilic and lipophilic antioxidants [15]. The free radical scavenging capacities of ABTS+• showed that pistachios that were using the *P. terebinthus* rootstock had the highest antioxidant activity (3.67 mM TE g⁻¹), followed by *P. integerrima* (3.23 mM TE g⁻¹), and finally *P. atlantica* (2.80 mM TE g⁻¹) (Table 1). In the case of pistachios that were obtained under different irrigation treatments, nuts from T2-trees had a higher antioxidant activity than samples from trees that were subjected to the other two irrigation treatments (T0 and T1), with an antioxidant activity of 3.92 mM TE g⁻¹ versus 2.86 and 2.96 mM TE g⁻¹, respectively (Table 1). In a study evaluating key characteristics of eight pistachio cultivars by Noguera-Artiaga [16], similar values of antioxidant activity for the Kerman cultivar were found, the same cultivar used in the current experiment.

The antioxidant activity of the hydrophilic fraction of pistachios was measured while using the ferric reducing antioxidant power (FRAP) method, which is based on the ability of an antioxidant to reduce Fe⁺³ in the presence of 2,4,6-Tripyridyl-s-triazine (TPTZ), forming a Fe⁺²-TPTZ complex [17]. After the analysis of samples that were obtained while using different rootstocks (Table 1), samples *P. integerrima* and *P. terebinthus* had the highest antioxidant activity with values of 1.37 and 1.32 mM TE

g⁻¹, respectively, while the nuts from *P. atlantica* had 1.23 mM TE g⁻¹. Taking the irrigation treatment factor into account, the T1-sample had the highest antioxidant activity, with 1.37 mM TE g⁻¹, followed by a group consisting of the samples T0 and T2 (1.29 mM TE g⁻¹). Taghizadeh [18] obtained similar results after analyzing the antioxidant activity by the FRAP method of pistachio extracted while using different solvents.

As shown in Table 1, statistical differences were also found after the analysis of the antioxidant activity of pistachio extracts while using the DPPH• method. Pistachios that were obtained on *P. terebinthus* rootstock showed the highest antioxidant activity (5.63 mM TE g⁻¹), while the *P. integerrima* samples had the lowest value (5.07 mM TE g⁻¹). Regarding the irrigation treatments, the DPPH method was the most effective one in differentiating among samples. The antioxidant activity increased with water restriction (deficit irrigation); the control sample (T0) showed the lowest antioxidant activity (4.59 mM TE g⁻¹), followed by T1 samples (5.07 mM TE g⁻¹), and finally T2 samples had the highest value (6.41 mM TE g⁻¹).

In view of above described results, it can be concluded that the use of the *P. terebinthus* rootstock led to the highest antioxidant activity (according to the three methods used). This can be associated to the fact that *P. terebinthus* rootstock is, amongst the three rootstocks studied, the one with the highest polyphenolic content and highest amount of betulinic acid, according to Noguera-Artiaga [13]. Besides, it can also be concluded that deficit irrigation treatments (T1 and especially T2) maintained or improved the antioxidant (hydrophilic) capacity of pistachios. In plants that were cultivated at water deficit conditions, an excess of CO₂ assimilated could have increased the biosynthesis of carbon-based secondary metabolites when carbohydrates exceed the amount used for growth concentrations [19].

2.2. Antimutagenicity test

The number of histidine+ revertant colonies obtained was 120 ± 5 (spontaneous revertants), while, in the presence of the mutagen (sodium azide, 30 µg plate⁻¹), 1652 ± 33 revertants plate⁻¹ were counted. Samples inhibited an average of 8% of the mutagenicity (92% revertants) induced by SA, without significant differences with control (Figure 1). No differences were observed for irrigation treatment and rootstock. The pistachio extracts were neither toxic nor mutagenic to the bacteria at the tested concentrations, and bacterial growth was normal. Rajaei [20] obtained similar results when the antimutagenic activity of pistachio green hull extract was assayed, since they found that hydrophilic pistachio extracts (raw, unpurified) did not show antimutagenic activity, although, in this case the study was carried out while using 2-nitrofluorene as a mutagen control instead of sodium azide.

These results showed that the phenolic compounds of pistachio extracts had no activity against sodium azide. The application of different irrigation treatments or different rootstocks had no significant impact on the antimutagenic potential of pistachios.

2.3. Cytotoxic test

In vitro cytotoxicity assessment is being increasingly recognized as an effective indicator of the toxic potential of compounds against cancerous cells. The MTT test assesses cellular viability measuring intracellular reduction of MTT reagent (water soluble and yellow) to a formazan salt (water insoluble and purple), which can be colorimetrically detected [21].

The effect that regulated deficit irrigation and rootstocks may have had on the functional properties of pistachios was studied by evaluating the cytotoxic effect of pistachio extracts on four cancerous and one non-cancerous cell lines (Table 2). The results obtained from testing samples were compared to those that were obtained with cisplatin, a positive cytotoxic control agent, which is a genotoxic drug that is used in chemotherapy. In two of the cancerous cell lines under study (HCT-116 and MDA-MB-231), no statistically differences were found to be associated to neither rootstock nor irrigation treatment factors. In the case of the HCT-116 cell line, the pistachio samples analyzed caused an inhibition of ~60% (~40% viability), achieving the same effect as a cisplatin dose that 50 $\mu\text{g mL}^{-1}$ did. The same effect was observed in the MDA-MB-231 cell line with a cellular viability of ~60% (Table 2).

In A549 and HeLa cell lines, no statistically differences were observed among the samples from different irrigation treatments (Table 2). However, rootstock had significant differences when compared to the control (cisplatin, 50 mg mL^{-1}), with pistachio samples having statistically higher viability (average ~68 and ~61% of viability in A549 and HeLa lines, against ~61 and ~57%, respectively) than when cisplatin was used.

In the ARPE-19 non-cancerous cell line, no differences were found among the samples that were obtained under different irrigation treatments, but significant differences were found when the rootstock factor was analyzed (Table 2); the *P. atlantica* rootstock led to the highest cellular viability (94%). In addition, the three rootstock samples had higher cellular viability than that observed for the control treatment. An important issue to point out is that none of the pistachio extracts reduced cellular viability of ARPE-19 below 85%; thus, they cannot be considered to be cytotoxic up to 100 $\mu\text{g mL}^{-1}$.

These results showed that the compounds that were present in pistachio extracts had no toxic effect on ARPE-19 non-cancerous cells. On the contrary, they

could significantly affect all four human cancer lines, with the A549 cell line being the most resistant and HeLa the most sensitive. Similar results have been previously reported by Seifaddinipour [22] on extracts from pistachio hulls on six cancerous cell lines and normal fibroblast, in which the same methodology used in the present study was followed. On the other hand, the application of the regulated deficit irrigation treatments had not diminished the original cytotoxic capacity that control pistachios have on the carcinoma cell lines studied.

It has been shown in other studies [13] that neither regulated deficit irrigation nor the use of different rootstocks affected the total concentration of triterpenoids. Pistachios are characterized by having triterpenoids, especially betulinic, oleanolic, and ursolic acids. Several studies have established relationships between phenolic compounds and their activity against tumor cell lines, as tested in vitro [23,24].

2.4. Cellular viability and oxidative damage tests

The lowest H₂O₂ concentrations (0.062 and 0.125 mM) tested had no significant effect on the ARPE-19 cells as compared to the control (0 mM of H₂O₂), obtaining values that were close to 100% cellular viability (Figure 2). Subsequently, a significant negative relationship between the H₂O₂ concentration and cellular viability was observed ($R^2 = 0.85$), with the viability reaching values of ~10%. Based on these results, the concentration of 10 mM of H₂O₂ was chosen for performing the cellular damage test. This selection was based on the fact that this concentration led to a cellular viability close to 20%, which caused obvious damage to the cells, but allowed enough cell survival to guarantee that a test would be performed at optimal conditions for its full development.

Figure 3 shows the results that were obtained after incubation of ARPE-19 cells during 4 and 24 h with the pistachio extracts. As previously discussed, when 10 mM of H₂O₂ was used, cellular viabilities of ~20% were obtained, without having significant differences among the studied samples. No statistically differences were found for the irrigation treatments when the cells were incubated with the pistachio extracts, leading to ~95% cellular viability. However, the *P. atlantica* rootstock led to a higher cellular viability (~100%) as ~95% for *P. integerrima* and *P. terebinthus*. When H₂O₂ (10 mM) was added to the cells for 30 min. (after 4 h of incubation with the pistachio extracts), a viability of ~30% was obtained in all of the samples. This suggests that the compounds that were present in pistachio extracts were able to reduce the oxidative damage that was observed in ARPE-19 cells by approximately 10% after 4 h (Figure 3a).

The results were similar to those obtained after 4 h of incubation by repeating the same process but incubating ARPE-19 cells with the pistachio extracts for a longer time, 24 h. There were no significant differences among irrigation treatments but *P. atlantica* showed a better behavior (~95% cellular viability) as compared to ~85% for *P. integerrima* and *P. terebinthus* (Figure 3b). When H₂O₂ (10 mM) was added to the cells for 30 min. (after 24 h of incubation with the pistachio extracts), a viability of ~55% was obtained in all of the samples (without statistically differences among them). In this case, the pistachio extracts were able to protect ARPE-19 cells from oxidative damage by approximately 35%. The cellular internalization depends on exposure time of cell to pistachio extracts. Increasing the exposure time increases the amount of pistachio compounds into the cell, so that greater protection against oxidative stress caused by peroxide radicals (formed from H₂O₂ by Fenton or Haber-Weiss reactions) was achieved.

Based on the above, neither rootstock nor regulated deficit irrigations significantly affected the original protective level against oxidative damage by H₂O₂ of the control pistachio extracts. On the other hand, it is necessary to mention that the effect of protection against oxidative stress that is caused by hydrogen peroxide does not always have a linear behavior and, therefore, it is necessary to carry out further research in this regard [25].

3. RESULTS AND DISCUSSION

3.1. Plant material and experimental design

The experiment was conducted on the experimental farm "El Chaparrillo", Ciudad Real, Spain (L 3°56'W; L 39°0'N; altitude 640 m) during the crop season 2016–2017. The plant material consisted of pistachio trees *P. vera* L. cultivar Kerman was budded on three rootstocks (i) *P. terebinthus* L., (ii) *P. atlantica* Desf., and (iii) *P. integerrima* L. The tree-spacing was set at 7 × 6 m (238 trees ha⁻¹). Peter cv. was used as male tree and it was evenly distributed throughout the field, in a proportion of 10%. The climate of the area had an average annual rainfall of 397 mm, mostly distributed outside a four-month summer drought period, and the surface is a shallow 1.3 m deep clay-loam (Alfisol Xeralf Petrocalcic Palexeralfs) soil, and a discontinuous petrocalcic horizon of around 0.75 m. The orchard was managed under no tillage conditions; the weeds were controlled with post-emergence herbicides. Pest control and fertilization practices were those usually followed by local growers.

The control plants (T₀) were irrigated at 100% of crop irrigation requirements (ET_c) of the previous week, according to daily reference evapotranspiration (ET_o), a crop factor, and while taking the canopy size into consideration [26]. In addition to

T0, two RDI treatments (T1 and T2) were applied during stage II (fruit growth), the non-critical period. In these treatments, the water deficit was increased and the threshold values were -1.5 MPa (T1) and -2.0 MPa (T2) [4].

3.2. Extraction of functional compounds

The preparation of pistachio extracts was conducted, as previously described by Noguera-Artiaga [13]. One gram of grounded pistachio was weighed and combined with 5 mL of hexane and 8 mL of 30% of aqueous methanol (v/v), amended with 1% of ascorbic acid. The suspension was stirred, sonicated during 15 min. in an ultrasonic bath (JP Selecta S.A, model 3000512 Barcelona, Spain) with constant frequency (40 kHz), left overnight at room temperature at darkness, and then centrifuged for 10 min. ($20.878 \times g$ at 4 °C). The hydrophilic phase was separated, dried under N₂ stream, re-dissolved with sterile DMSO (solution of 100 mg mL⁻¹), and stored (-24 °C) until the analyses. This extraction methodology has been shown to be one of the most effective ones when it comes to extracting the greatest number of phenolic compounds [20]. A negative control (5 mL of hexane and 8 mL of 30% of aqueous methanol (v/v) amended with 1% of ascorbic acid) was used in all determinations in order to obtain reliable results. The results shown by this control were subtracted from the results that were obtained by the samples to obtain the final result.

3.3. Antioxidant tests

Free radical scavenging capacities were determined by ABTS [27], DPPH [28], and FRAP [29] assays, with some modifications to be used in 96-well microplates.

The evaluation of antioxidant activity with ABTS reagent [2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was carried out according the method that was previously described by Loarca-Piña [30]. Briefly, 20 μ L of pistachio extracts were mixed with 230 μ L of ABTS solution, and, after reaction, absorbance was recorded at 734 nm in a Beckman Coulter AD 340 Microplate reader (Beckman Coulter, CA, USA).

A 150 μ M DPPH solution was prepared in 80% methanol in order to perform the DPPH assay. A 200- μ L aliquot of this solution was combined with 100 μ L of pistachio extract, placed into each well, and the plate was covered and left at darkness at room temperature for 6 min. After reaction, absorbance was determined at 520 nm in a Beckman Coulter AD 340 Microplate reader [31].

The ferric reducing antioxidant power (FRAP) assay measures the capability of reducing ferric ions. FRAP reagent was freshly prepared by mixing 100 mL of a sodium acetate buffer (0.3 M, pH 3.6) solution, 10 mL of 2,4,6-tri(2-pyridyl)-1,3,5-triazine

(TPTZ) solution (10 mM in 40 mM HCl), and 10 mL of FeCl₃ 6H₂O (20 mM) solution. A 200- μ L aliquot of the pistachio extract was mixed with the FRAP solution for 30 min. at darkness to obtain a proper and even reaction. The absorbance of samples was recorded at 593 nm while using a Beckman Coulter AD 340 Microplate reader [32]. Trolox was used as a standard antioxidant. All of the analyses were run in triplicate and the results were expressed as mM Trolox equivalents g⁻¹.

3.4. Antimutagenicity test

The antimutagenic activity of pistachio extract was evaluated while using the standard mutagenicity assay, as described by Maron and Ames [33], with *Salmonella typhimurium* TA100 as a tester strains, in the presence of sodium azide (SA) as positive control. The pistachio extracts were diluted in sterile DMSO and spiked with sufficient SA to reach 20.0, 2.0, and 0.2 μ g mL⁻¹. All of the assays were performed in triplicate. Antimutagenic activity was reported as the percentage of SA-induced revertants per plate inhibited due to the presence of pistachio extract.

3.5. Cell lines

Human cell lines A-549 (lung carcinoma), HeLa (epithelial cervix adenocarcinoma), MDA-MB-231 (breast adenocarcinoma), HCT 116 (colon carcinoma), and ARPE-19 (retinal non-cancerous epithelial) were used. The cell lines were obtained from the American Type Culture Collection (Rockville, MD). All cell lines were maintained in Dulbecco's modified Eagle's medium and RPMI-1640 Medium (Sigma Aldrich, St. Louis, MO, USA), supplemented with 10 and 15% heat-inactivated fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), and grown at 37 °C in an atmosphere of 5% CO₂.

3.6. Cytotoxic test

MTT assay (Roche, cell proliferation kit I, Roche, Cat. No. 11-465-007-001) was used to study the cytotoxic effect of pistachio extracts on human cancer cell lines. In a 96-flat-well plate, 1 \times 10⁴ cells well⁻¹ were seeded and suspended in 100 μ L of medium. After 24 h of incubation at 37 °C and 5% CO₂ atmosphere, the cell cultures were maintained for another 24 h at same conditions with the addition of 100 μ L of culture medium without FBS and the pistachio extracts re-suspended in DMSO (0.5%) at final a concentration of 100 μ g mL⁻¹. The control cell cultures did not show any evidence of cell damage. Cisplatin [cis-Diamminedichloroplatinum (II)] was used as a positive control for cytotoxicity at a concentration of 50 μ g mL⁻¹ for all of the studied cell lines, except for MDA-MB-231, in which case the concentration was 100 μ g mL⁻¹. The plates were read the next day while using an ELISA plate reader (Benchmark Microplate Reader; Bio-Rad, Hercules, CA, USA) [34].

3.7. Cytoprotection against H₂O₂-induced cell damage activity

A dose response curve to determine the cellular viability in the presence of H₂O₂ was prepared in order to find the H₂O₂ concentration to be used to cause oxidative cell damage. The ARPE-19 cells were seeded (1 x 10⁴ cells well⁻¹) into 96-well plates, suspended in 100 µL of DMEM, and then incubated for 24 h at 37 °C and 5% CO₂ atmosphere. Subsequently, cells were treated with different concentrations of H₂O₂ (0.062, 0.125, 0.250, 0.5, 1.0, 10.0, and 20.0 mM) for 30 min. After the treatment, the cell medium was replaced (by an equal volume of DMEM serum-free medium) and the original MTT protocol was followed.

The analysis of the protection against reactive oxygen species was conducted when the concentration of H₂O₂ to be used was known. ARPE-19 cells, 1 x 10⁴ cells well⁻¹, were plated in 96-well plates for 24 h (suspended in 100 µL of DMEM, at temperature of 37 °C, and controlled atmosphere with 5% CO₂). Afterwards, cells were incubated with extracts obtained from pistachios cultivated under different irrigation treatments and while using different rootstocks, at a concentration of 100 µg mL⁻¹, during 4 and 24 h. After that, the medium was retrieved from wells and they were incubated with 10 mM of H₂O₂ for 30 min. (at same temperature and atmosphere). After treatment, an equal volume of DMEM serum-free medium replaced the cell medium and MTT assay was performed [35,36].

3.8. Statistical analysis

The data that are presented in this study are the mean values of, at least, three replicates, and were subjected to three-way (in case of antimutagenicity test) and two-way (rest of determinations) analysis of variance (ANOVA). Subsequently, the data were subjected to Tukey's multiple-range test to compare the means. Differences were considered to be statistically significant at $p < 0.05$. All of the statistical analyses were done while using XLSTAT software (ADDINSOFT XLSTAT version 2014.1, Paris, France).

4. CONCLUSIONS

The experimental results have demonstrated that the use of the *P. terebinthus* rootstock led to pistachios with higher antioxidant activity, while application of RDI strategies maintained or improved pistachios antioxidant capacity. Neither the use of different rootstocks nor the application of RDI during pistachio cultivation had a significant impact on the antimutagenic potential and cytoprotective activity of pistachios. Of the four cancer cell lines studied, A549 was the most resistant and the

HeLa cell line was the most sensitive to pistachio extracts, which successfully protected ARPE-19 cells from oxidative damage that was caused by H₂O₂ at a level of 10% when incubated during 4 h, and at 35% after 24 h of exposure. Thus, the application of RDI strategies in pistachios allows for the saving of water during pistachio cultivation and led to producing pistachio nuts with the same or better functional characteristics than the control samples.

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REFERENCES

1. Bozorgi, M.; Memariani, Z.; Mobli, M.; Salehi Surmaghi, M.H.; Shams-Ardekani, M.R.; Rahimi, R. Five pistacia species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*): A review of their traditional uses, phytochemistry, and pharmacology. *Sci. World J.* 2013, 2013, 33.
2. Gijón, M.d.C.; Gimenez, C.; Perez-López, D.; Guerrero, J.; Couceiro, J.F.; Moriana, A. Rootstock influences the response of pistachio (*Pistacia vera* L. Cv. Kerman) to water stress and rehydration. *Sci. Hortic.* 2010, 125, 666–671.
3. Moriana, A.; Memmi, H.; Centeno, A.; Martín-Palomo, M.J.; Corell, M.; Galindo, A.; Torrecillas, A.; Pérez-López, D. Corrigendum to “influence of rootstock on pistachio (*Pistacia vera* L.) water relations”. *Agric. Water Manag.* 2019, 216, 497.
4. Gijón, M.d.C.; Gimenez, C.; Perez-López, D.; Guerrero, J.; Couceiro, J.F.; Moriana, A. Water relations of pistachio (*Pistacia vera* L.) as affected by phenological stages and water regimes. *Sci. Hortic.* 2011, 128, 415–422.

5. Noguera-Artiaga, L.; Lipan, L.; Vázquez-Araújo, L.; Barber, X.; Pérez-López, D.; Carbonell-Barrachina, Á.A. Opinion of spanish consumers on hydrosustainable pistachios. *J. Food Sci.* 2016, 81, S2559–S2565.
6. Corell, M.; Martín-Palomo, M.J.; Sánchez-Bravo, P.; Carrillo, T.; Collado, J.; Hernández-García, F.; Girón, I.; Andreu, L.; Galindo, A.; López-Moreno, Y.E.; Centeno, A.; et al. Evaluation of growers' efforts to improve the sustainability of olive orchards: Development of the hydrosustainable index. *Sci. Hortic.* 2019, 257, 108661.
7. Namvar, F.; Mohamed, S.; Fard, S.G.; Behravan, J.; Mustapha, N.M.; Alitheen, N.B.M.; Othman, F. Polyphenol-rich seaweed (*Eucheuma cottonii*) extract suppresses breast tumour via hormone modulation and apoptosis induction. *Food Chem.* 2012, 130, 376–382.
8. World Health Organization. Cancer, key facts. Available online: <https://www.who.int/news-room/fact-sheets/detail/cancer> (accessed on 16 July 2019).
9. Acquaviva, R.; Sorrenti, V.; Santangelo, R.; Cardile, V.; Tomasello, B.; Malfa, G.; Vanella, L.; Amodeo, A.; Genovese, C.; Mastrojeni, S.; et al. Effects of an extract of *Celtis aetnensis* (tornab.) strobil twigs on human colon cancer cell cultures. *Oncol. Rep.* 2016, 36, 2298–2304.
10. Cragg, G.M.; Newman, D.J. Plants as a source of anti-cancer agents. *J. Ethnopharmacol* 2005, 100, 72–79.
11. Khazir, J.; Mir, B.A.; Pilcher, L.; Riley, D.L. Role of plants in anticancer drug discovery. *Phytochem. Lett.* 2014, 7, 173–181.
12. Dreher, M.L. Pistachio nuts: Composition and potential health benefits. *Nutr. Rev.* 2012, 70, 234–240.
13. Noguera-Artiaga, L.; Pérez-López, D.; Burgos-Hernández, A.; Wojdyło, A.; Carbonell-Barrachina, Á.A. Phenolic and triterpenoid composition and inhibition of α -amylase of pistachio kernels (*Pistacia vera* L.) as affected by rootstock and irrigation treatment. *Food Chem.* 2018, 261, 240–245.
14. Mandalari, G.; Bisignano, C.; Filocamo, A.; Chessa, S.; Sarò, M.; Torre, G.; Faulks, R.M.; Dugo, P. Bioaccessibility of pistachio polyphenols, xanthophylls, and tocopherols during simulated human digestion. *Nutrition* 2013, 29, 338–344.
15. Rakholiya, K.D.; Kaneria, M.J.; Chanda, S.V. Isolation, validation and characterization of major bioactive constituents by various techniques from mango ripe seed. In *Food Technology: Applied Research and Production*; Meghwal, M., Goyal, M.R., Kaneria, M., Eds.; Apple Academic Press: Waretown, NJ, USA, 2017; pp. 273–314.

16. Noguera-Artiaga, L.; Salvador, M.D.; Fregapane, G.; Collado-Gonzalez, J.; Wojdylo, A.; Lopez-Lluch, D.; Carbonell-Barrachina, A.A. Functional and sensory properties of pistachio nuts as affected by cultivar. *J. Sci. Food Agric.* 2019, 99, 6696-6705. doi:10.1002/jsfa.9951.
17. Kaneria, M.; Rakholiya, K., Sonagara, J.; Chanda, S. Comparative assessment of antioxidant activity and phytochemical analysis of facultative halophyte *Salvadora oleoides* decne. and *Salvadora persica* L. *Am. J. Biochem. Mol. Biol.* 2017, 7, 102-110.
18. Taghizadeh, S.F.; Rezaee, R.; Davarynejad, G.; Karimi, G.; Nemati, S.H.; Asili, J. Phenolic profile and antioxidant activity of *Pistacia vera* var. *Sarakhs* hull and kernel extracts: The influence of different solvents. *J. Food Meas. Charact.* 2018, 12, 2138-2144.
19. Horner, J.D. Nonlinear effects of water deficits on foliar tannin concentration. *Biochem. Syst. Ecol.* 1990, 18, 211-213.
20. Rajaei, A.; Barzegar, M.; Mobarez, A.M.; Sahari, M.A.; Esfahani, Z.H. Antioxidant, anti-microbial and antimutagenicity activities of pistachio (*Pistachia vera*) green hull extract. *Food Chem. Toxicol.* 2010, 48, 107-112.
21. Pacifico, S.; Gallicchio, M.; Fiorentino, A.; Fischer, A.; Meyer, U.; Stintzing, F.C. Antioxidant properties and cytotoxic effects on human cancer cell lines of aqueous fermented and lipophilic quince (*Cydonia oblonga* mill.) preparations. *Food Chem. Toxicol.* 2012, 50, 4130-4135.
22. Seifaddini-pour, M.; Farghadani, R.; Namvar, F.; Mohamad, J.; Abdul Kadir, H. Cytotoxic effects and anti-angiogenesis potential of pistachio (*Pistacia vera* L.) hulls against mcf-7 human breast cancer cells. *Molecules* 2018, 23, 110.
23. Novotný, L.; Vachálková, A.; Biggs, D. Ursolic acid: An anti-tumorigenic and chemopreventive activity. *Neoplasma* 2001, 48, 241-246.
24. Pięt, M.; Paduch, R. Ursolic and oleanolic acids as potential anticancer agents acting in the gastrointestinal tract. *Mini-Rev. Org. Chem.* 2019, 16, 78-91.
25. Halliwell, B. Free radicals and antioxidants: Updating a personal view. *Nutr. Rev.* 2012, 70, 257-265.
26. Memmi, H.; Gijón, M.C.; Couceiro, J.F.; Pérez-López, D. Water stress thresholds for regulated deficit irrigation in pistachio trees: Rootstock influence and effects on yield quality. *Agric. Water Manag.* 2016, 164, 58-72.
27. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radic. Biol. Med.* 1999, 26, 1231-1237.
28. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* 1995, 28, 25-30.

29. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (frap) as a measure of "antioxidant power": The frap assay. *Anal. Biochem.* 1996, 239, 70–76.
30. Loarca-Piña, G.; Mendoza, S.; Ramos-Gómez, M.; Reynoso, R. Antioxidant, antimutagenic, and antidiabetic activities of edible leaves from *Cnidoscolus chayamansa* Mc. Vaugh. *J. Food Sci.* 2010, 75, H68–H72.
31. Cardador-Martínez, A.; Loarca-Piña, G.; Oomah, B.D. Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* 2002, 50, 6975–6980.
32. Bolanos De La Torre, A.A.S.; Henderson, T.; Nigam, P.S.; Owusu-Apenten, R.K. A universally calibrated microplate ferric reducing antioxidant power (frap) assay for foods and applications to manuka honey. *Food Chem.* 2015, 174, 119–123.
33. Maron, D.M.; Ames, B.N. Revised methods for the salmonella mutagenicity test. *Mutat. Res. Environ. Mutagenesis Relat. Subj.* 1983, 113, 173–215.
34. Fathalizadeh, J.; Bagheri, V.; Khorramdelazad, H.; Kazemi Arababadi, M.; Jafarzadeh, A.; Mirzaei, M.R.; Shamsizadeh, A.; Hajizadeh, M.R. Induction of apoptosis by pistachio (*Pistacia vera* L.) hull extract and its molecular mechanisms of action in human hepatoma cell line hepg2. *Cell. Mol. Biol.* 2015, 61, 128–134.
35. Hu, X.; Liang, Y.; Zhao, B.; Wang, Y. Thymoquinone protects human retinal pigment epithelial cells against hydrogen peroxide induced oxidative stress and apoptosis. *J. Cell. Biochem.* 2019, 120, 4514–4522.
36. Liu, H.; Liu, W.; Zhou, X.; Long, C.; Kuang, X.; Hu, J.; Tang, Y.; Liu, L.; He, J.; Huang, Z.; et al. Protective effect of lutein on arpe-19 cells upon h2o2-induced g2/m arrest. *Mol. Med. Rep.* 2017, 16, 2069–2074.

Table 1. Antioxidant activity [mM Trolox equivalents (TE) g⁻¹] of pistachio extracts, as affected by rootstock and irrigation treatment.

Factor	ABTS	FRAP	DPPH
	(mM TE g ⁻¹)		
	ANOVA¹		
Rootstock	***	***	***
Irrigation	***	**	***
Rootstock x Irrigation	***	**	**
	Tukey multiple range test²		
Rootstock			
<i>P. atlantica</i>	2.80 c	1.23 b	5.44 ab
<i>P. integerrima</i>	3.23 b	1.37 a	5.07 b
<i>P. terebinthus</i>	3.67 a	1.32 a	5.63 a
Irrigation			
T0	2.86 b	1.29 b	4.59 c
T1	2.96 b	1.37 a	5.13 b
T2	3.92 a	1.29 b	6.41 a
Rootstock x Irrigation			
<i>P. atlantica</i> x T0	2.37 d	1.24 ab	4.83 d
<i>P. atlantica</i> x T1	2.61 d	1.34 a	6.07 b
<i>P. atlantica</i> x T2	3.41 b	1.10 b	5.42 c
<i>P. integerrima</i> x T0	2.95 cd	1.36 a	4.43 e
<i>P. integerrima</i> x T1	2.90 cd	1.41 a	4.42 e
<i>P. integerrima</i> x T2	3.94 ab	1.37 a	6.36 b
<i>P. terebinthus</i> x T0	3.24 bc	1.28 a	4.51 e
<i>P. terebinthus</i> x T1	3.36 bc	1.39 a	4.89 d
<i>P. terebinthus</i> x T2	4.40 a	1.26 a	7.47 a
Pooled variance	0.32	0.15	0.22

¹NS: not significant at $p < 0.05$; **, and ***, significant at $p < 0.01$, and 0.001, respectively.

²Values (mean of three replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test.

Table 2. Viability (%) of pistachio extracts as affected by rootstock and irrigation treatment, at 100 µg mL⁻¹ on human cancerous and non-cancerous cell lines.

Factor	HCT-116	A549	HeLa	MDA-MB-231	ARPE-19
	(% viability)				
	ANOVA¹				
Rootstock	NS	***	**	NS	***
Irrigation	NS	NS	NS	NS	***
Rootstock x Irrigation	NS	***	***	NS	***
	Tukey multiple range test²				
Rootstock					
<i>P. atlantica</i>	38.9	67.4 a	30.1 a	61.3	94.2 a
<i>P. integerrima</i>	39.0	68.3 a	30.1 a	61.9	87.9 b
<i>P. terebinthus</i>	43.4	68.7 a	32.3 a	60.3	86.6 b
CISP ³	40.2	61.1 b	28.2 b	57.2	69.2 c
Irrigation					
T0	39.7	65.3	28.3	58.0	91.7 a
T1	39.8	64.4	27.0	60.8	87.7 a
T2	40.9	61.1	28.2	61.0	89.3 a
CISP ³	40.2	61.1	28.2	57.2	69.2 b
Rootstock x Irrigation					
<i>P. atlantica</i> x T0	36.1	73.2 a	30.1 a	62.5	99.1 a
<i>P. atlantica</i> x T1	35.8	66.2 a	29.3 a	60.9	91.1 a
<i>P. atlantica</i> x T2	44.3	64.6 ab	30.4 a	59.6	90.9 a
<i>P. integerrima</i> x T0	36.3	74.5 a	28.4 ab	55.5	84.9 ab
<i>P. integerrima</i> x T1	41.0	67.2 a	31.5 a	64.6	88.3 ab
<i>P. integerrima</i> x T2	38.8	65.1 ab	29.6 a	64.9	90.6 a
<i>P. terebinthus</i> x T0	46.7	63.7 ab	34.8 a	58.9	90.1 a
<i>P. terebinthus</i> x T1	42.7	75.3 a	28.8 ab	59.8	83.8 ab
<i>P. terebinthus</i> x T2	39.9	69.1 a	33.3 a	61.3	86.2 ab
CISP ³	40.2	61.1 b	28.2 b	57.2	69.2 b
Pooled variance	4.1	2.3	1.8	4.8	4.5

¹NS: not significant at $p < 0.05$; **, and ***, significant at $p < 0.01$, and 0.001, respectively. ²Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), Tukey's least significant difference test. ³Cisplatin (CISP) concentration was 50 µg mL⁻¹ for all the cell lines studied, except for MDA-MB-231 in which case the concentration was 100 µg mL⁻¹.

Figure 1. Antimutagenicity test of pistachio extracts (% inhibition of sodium azide (SA) mutation) at different concentrations (0.2, 2.0, and 20.0 mg mL⁻¹). Values with same letters within a same factor were not significantly different ($p < 0.005$), Tukey's least significant difference test. Spontaneous revertants 120 ± 5 and SA control ($30 \mu\text{g plate}^{-1}$) induced 1652 ± 33 revertants plate⁻¹.

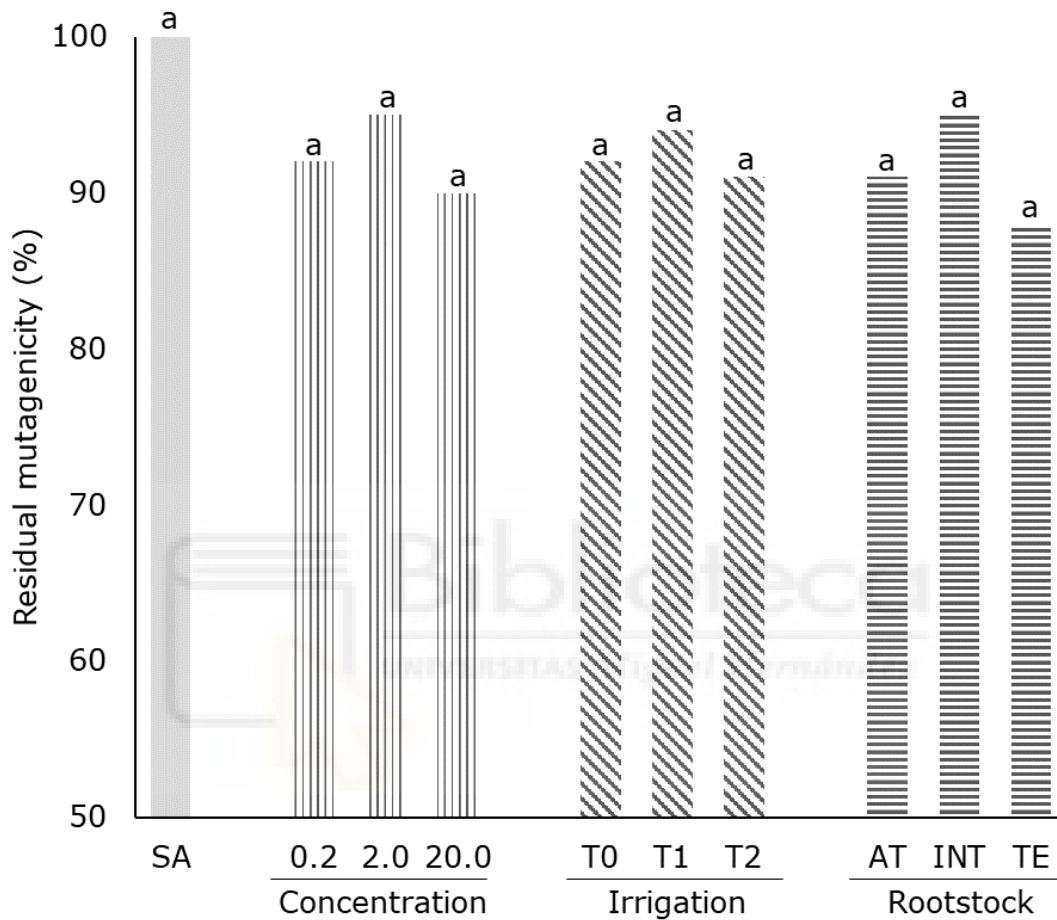


Figure 2. Cellular viability evaluated by MTT method in ARPE-19 cells exposed to different concentration of H₂O₂ during 30 min.

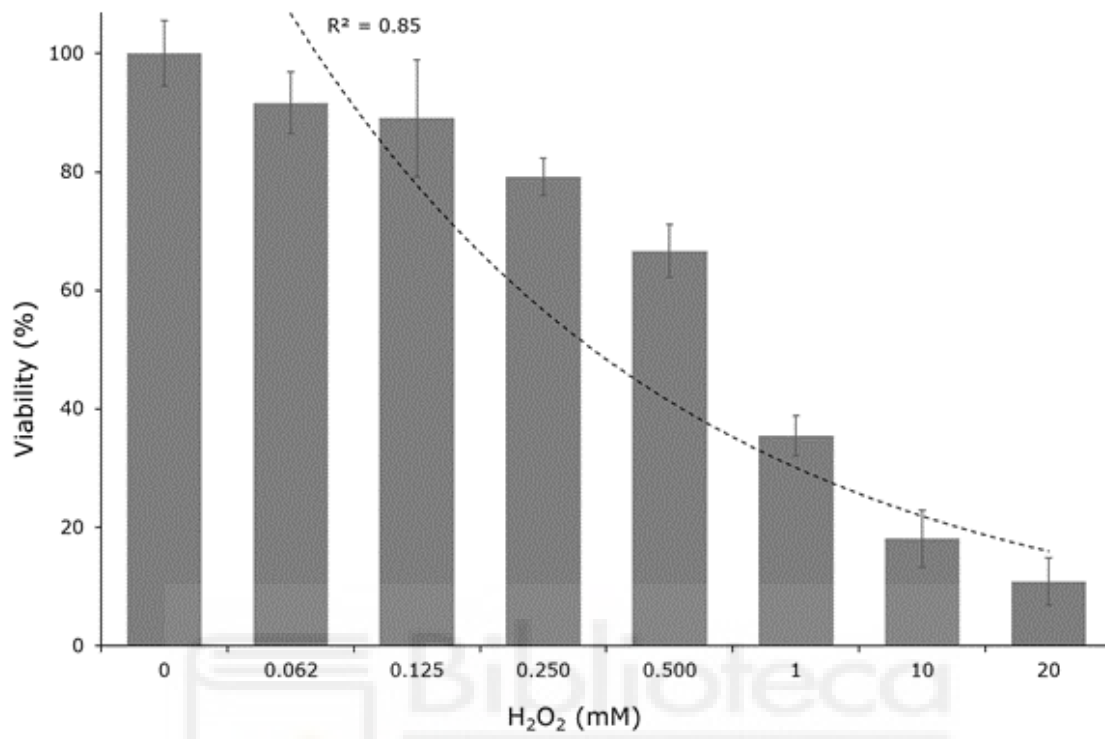
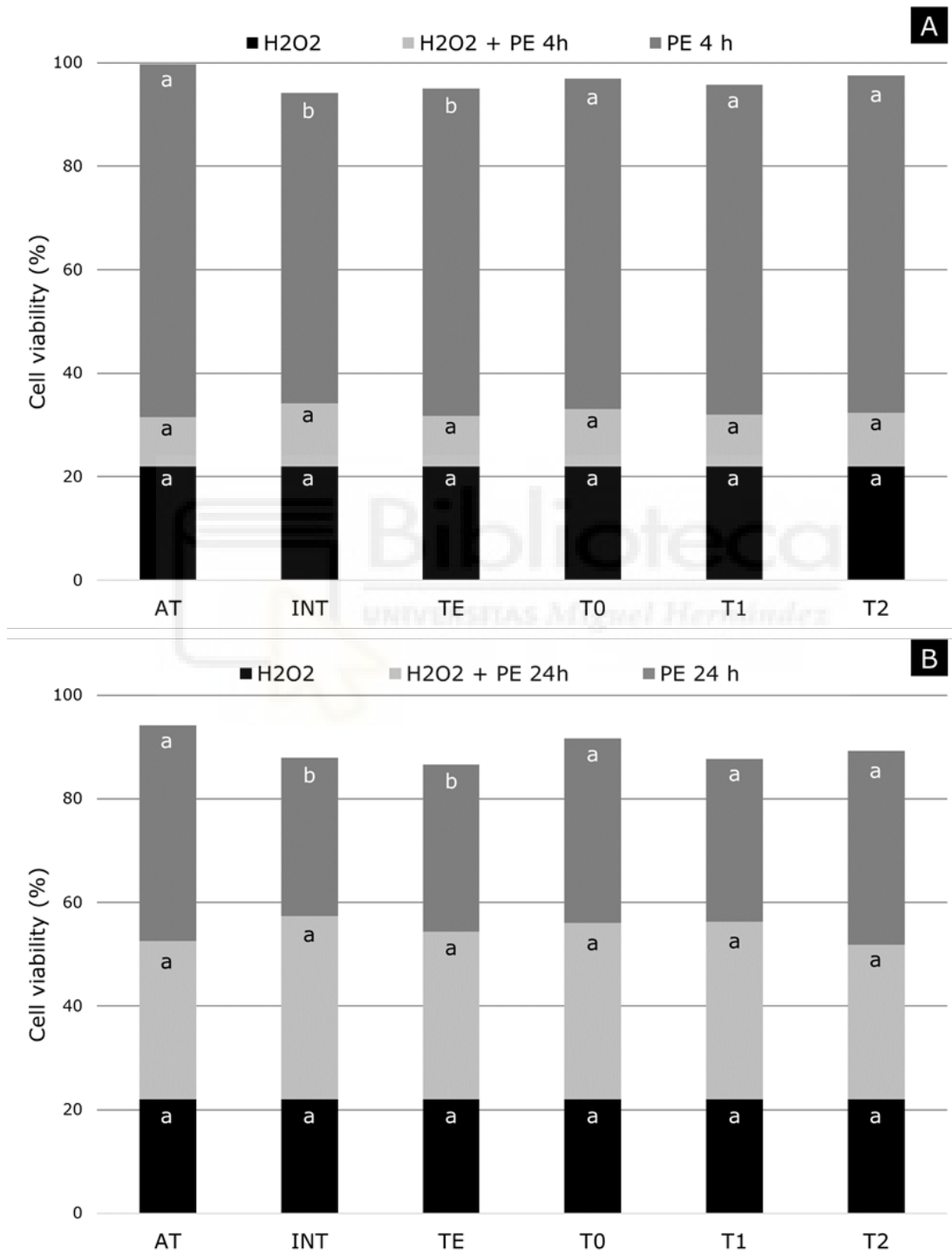


Figure 3. Cellular viability evaluated by MTT method of ARPE-19 cells treated with pistachio extracts (PE), as affected by rootstock and irrigation treatment, at 100 $\mu\text{g mL}^{-1}$ after 4 h (a), and 24 h (b), and then exposed to 10 mM H_2O_2 during 30 min. Different letters within each factor means significant differences ($p \leq 0.05$); Tukey's least significant difference test.





RESULTADOS | 5 Y DISCUSIÓN





Atributos de calidad de diferentes cultivares de pistacho

CAPÍTULO

1

El objetivo de este capítulo fue realizar un estudio completo de las características funcionales, aromáticas y sensoriales de pistachos obtenidos mediante el empleo de ocho cultivares. El propósito del mismo fue proporcionar a los agricultores y a los consumidores toda la información posible para que puedan basar su decisión de plantación o compra, en base a las propiedades funcionales u organolépticas de los frutos.

Los resultados obtenidos en este estudio quedaron recogidos en una publicación denominada "*Functional and sensory properties of pistachio nuts as affected by cultivar*" y está publicada en la revista *Journal of the Science of Food and Agriculture*".

Compuestos fenólicos

Con el fin de estudiar la influencia que el cultivar tiene sobre la composición polifenólica de los pistachos se hizo una determinación de estos mediante cromatografía líquida (LC-PDA-MS-QToF-ESI). Se identificaron y cuantificaron 2 ácidos fenólicos, 9 flavonoles, 1 antocianina y 3 flavan-3-oles (como dímeros de procianidinas y/o (-)-epicatequina y (-)-epicatequina-*O*-hexósido).

El contenido de compuestos fenólicos varió desde los 500 mg 100 g⁻¹ (peso seco) determinados en el cultivar "Sirora" hasta los 6065 mg 100 g⁻¹ (peso seco) determinados en los pistachos obtenidos del cultivar "Larnaka".

Los pistachos son los únicos frutos secos que contienen antocianinas, que son pigmentos que se encuentran en la piel del fruto y son responsables de los colores de muchas verduras y frutas. El compuesto más abundante de esta familia encontrado en las muestras de pistacho (cianidina-3-*O*-galactósido) osciló entre 12,3 y 130,6 mg 100 g⁻¹ (peso seco), siendo los frutos obtenidos de los cultivares "Kastel" y "Avdat" los que tuvieron el contenido de antocianinas más bajo y alto, respectivamente.

Los contenidos encontrados en las muestras bajo estudio de cianidina-3-*O*-galactósido y cianidina-3-*O*-glucósido fueron similares a los encontrados en bibliografía para este tipo de frutos, quedando de manifiesto que las diferencias encontradas se deben, principalmente, al cultivar, el origen geográfico, la etapa de maduración y el procesamiento industrial de los frutos (Ballistreri, Arena, & Fallico, 2009; Bellomo & Fallico, 2007; Seeram *et al.*, 2006; Tomaino *et al.*, 2010).

Procianidinas poliméricas

Se identificaron 3 flavan-3-oles y 4 procianidinas poliméricas en los pistachos bajo estudio. Las concentraciones totales de procianidinas poliméricas oscilaron entre 348 y 5919 mg 100 g⁻¹, siendo la de mayor concentración (-)-epicatequina. Este amplio rango indica que el cultivar tiene una gran influencia en la cantidad de este tipo de compuestos. La concentración más alta de procianidinas poliméricas se encontró en muestras del cultivar "Larnaka" (5919 mg 100 g⁻¹), mientras que las más bajas (de 348 a 506 mg 100 g⁻¹) se encontraron en los cultivares "Aegina", "Avdat" y "Sirora", respectivamente.

Actividad antioxidante

Tras estudiar la actividad antioxidante de las muestras de pistacho mediante el método ABTS⁺, los resultados obtenidos demostraron que los pistachos obtenidos mediante los cultivares "Kerman" (6,21 mmol Trolox 100 g⁻¹) y "Larnaka" (5,88 mmol Trolox 100 g⁻¹) tuvieron los valores más altos de capacidad antioxidante, mientras que los obtenidos por "Mateur" y "Sirora", tuvieron los valores más bajos (0,40 y 0,46 mmol Trolox 100 g⁻¹, respectivamente).

Los resultados obtenidos por el método FRAP fueron similares a los descritos anteriormente en el caso del método ABTS⁺, aunque el método FRAP mostró que no solo "Larnaka" (3,61 mmol Trolox 100 g⁻¹) y "Kerman" (3,19 mmol Trolox 100 g⁻¹) presentaron la mayor capacidad antioxidante, sino también muestras de los cultivares "Napolitana" (3,61 mmol Trolox 100 g⁻¹) y "Avdat" (3,19 mmol Trolox 100 g⁻¹) mostraron valores altos. Una vez más, "Sirora" mostró la actividad antioxidante más baja.

Estos resultados demostraron que el contenido total de compuestos fenólicos no era directamente proporcional a la actividad antioxidante, excepto en el caso del cultivar "Sirora", que tenía el contenido más bajo en ambos parámetros. En este sentido, los datos de los ensayos ABTS⁺ y FRAP mostraron que el tipo de compuestos fenólicos (composición) en lugar de su concentración, es el factor responsable de la alta actividad antioxidante. Esta relación puede establecerse, por ejemplo, con la cantidad de ácido gálico que se encuentra en las muestras. Los cultivares "Larnaka" y "Kerman" tenían un alto contenido de este compuesto y, a su vez, eran los que tenían el mejor comportamiento antioxidante en los dos métodos estudiados. Este hallazgo coincidió con otros autores que informaron que las diferencias en las actividades antioxidantes de los extractos de plantas podrían deberse a diferentes

estructuras de extractos de plantas de ácidos fenólicos y compuestos de flavonoides, así como a sus derivados (Rababah, Hettiarachchy, & Horax, 2004; Rajaei, Barzegar, Mobarez, Sahari, & Esfahani, 2010).

Actividad de α -amilasa y α -glucosidasa

Estas enzimas son las responsables de la degradación de los oligosacáridos y disacáridos en monosacáridos, por lo que la inhibición de α -amilasa y α -glucosidasa puede ser una buena estrategia para regular las alteraciones metabólicas relacionadas con algunos tipos de diabetes.

En el caso de ambas enzimas, todas las muestras estudiadas mostraron valores de inhibición muy bajos. Los pistachos obtenidos por medio de los cultivares "Kerman", "Mateur", "Aegina" y "Larnaka" demostraron ser aquellos con un valor más bajo de IC_{50} , es decir, un poder mayor de inhibición mayor. Este comportamiento se observó para las dos enzimas bajo estudio.

Compuestos volátiles

Se aislaron y cuantificaron 13 compuestos volátiles siendo α -pineno y 1-metilpirrol los dos compuestos mayoritarios (entre ambos representaron, aproximadamente, un 94 % de la concentración total).

Los pistachos procedentes de los cultivares "Aegina" y "Napolitana" fueron los que tuvieron una mayor concentración de compuestos volátiles (375 y 370 mg kg^{-1} , respectivamente).

Cabe reseñar que, en la mayoría de las ocasiones, el perfil aromático de un producto no se define por los compuestos mayoritarios que lo componen, sino que en función de los umbrales aromáticos de los mismos y de las sinergias y equilibrio entre concentraciones, compuestos encontrados en muy pequeña concentración puedan ser percibidos con una mayor intensidad. Esta situación, puede ocurrir con compuestos como el limoneno o hexanal, en los que pequeñas variaciones en su concentración pueden percibirse como variaciones en la complejidad del aroma de las muestras.

Análisis sensorial

Para la realización del estudio de análisis sensorial descriptivo llevado a cabo en este estudio, se desarrolló un léxico sensorial formado por 25 atributos

sensoriales, de los cuales 13 fueron relativos a la apariencia de los frutos, 6 referentes al sabor de los mismos y 6 para describir su textura. De todos ellos se apreciaron diferencias significativas en 23.

Los pistachos obtenidos mediante los cultivares "Kerman" y "Mateur" destacaron por la blancura de su cáscara y la uniformidad de los frutos, mientras que los generados mediante los cultivados "Avdat" y "Larnaka" destacaron por la tonalidad púrpura de sus frutos.

Los pistachos procedentes del cultivar "Avdat" fueron los más dulces, los procedentes de "Kerman" tuvieron un mayor sabor a fruto seco (incluido mayor sabor a pistacho) y un mayor postgusto, mientras que los procedentes de "Larnaka" y "Aegina" destacaron por su textura (mayor crujibilidad y dureza).

CONCLUSIONES

Los pistachos procedentes del cultivar "Larnaka" tuvieron un mayor contenido de polifenoles y procianidinas poliméricas. Las muestras de los cultivares "Kerman" y "Larnaka" tuvieron los valores más altos de actividad antioxidante, así como un mayor efecto inhibitorio de la α -amilasa y la α -glucosidasa. Con respecto al sabor, "Kerman" fue el cultivar con la intensidad de sabor más alta, que podría estar relacionada, entre otros factores, con una mayor concentración de limoneno. Las muestras "Kerman" y "Mateur" destacaron por su blancura de la piel, mientras que "Avdat" y "Larnaka" tuvieron el color de piel más intenso (púrpura). Por último, "Aegina" y "Larnaka" tuvieron mayores intensidades de dureza y textura crujiente. En consecuencia, no es fácil hacer una recomendación final sobre qué cultivar es el mejor, y esta recomendación debe hacerse de acuerdo con objetivos específicos; por ejemplo, si se requieren pistachos con alta funcionalidad o propiedades saludables, "Larnaka" y "Kerman" son las mejores opciones, mientras que si la elección debe estar basada en unas mejores características sensoriales "Kerman" es el más adecuado.



Concepto de hidrosostenibilidad

CAPÍTULO

2

El objetivo principal de este capítulo fue determinar qué aspectos impulsan la intención de compra de los consumidores a la hora de adquirir productos sostenibles. Esta información, es de vital importancia a la hora de establecer estrategias de marketing que permitan una puesta en valor de los pistachos obtenidos mediante estrategias de riego deficitario controlado (pistachos hidroSOS). Los resultados obtenidos quedaron reflejados en una publicación titulada "*Opinion of Spanish consumers on hydrosustainable pistachios*", publicada en la revista "*Journal of Food Science*".



Grupos focales

Las frutas y verduras cultivadas bajo estrategias de riego deficitario controlado (RDI, por sus siglas en inglés) se denominan productos hidroSOStenibles o hidroSOS. Para conocer la opinión que los consumidores tienen acerca de este tipo de estrategias, y cuáles eran sus enfoques a la hora de vender o comprar este tipo de productos, se llevaron a cabo estrategias de grupos focales. Todos los términos surgidos durante el desarrollo de estas se agruparon en 4 categorías: agricultura, salud, atributos sensoriales y precio; aunque esta última, como veremos más adelante se trató de manera individualizada.

Inicialmente, todos los términos surgidos a lo largo de las sesiones de grupos focales se enumeraron dentro de una categoría; a continuación, se eliminaron los términos redundantes y se combinaron conceptos similares o complementarios en un solo término. De esta forma, se obtuvieron 3 términos incluidos en la categoría "agricultura", 5 en la categoría "salud" y otros 3 en la categoría "propiedades sensoriales". El hecho de que en la categoría relacionada con atributos saludables surgiesen más términos que en las otras dos, es la atención que actualmente tienen los consumidores por el consumo de productos funcionales.

Uno de los conceptos clave tratados a lo largo de los grupos focales fue la necesidad de destacar los productos hidroSOS del resto de los productos del mercado; para lo cual, mencionaron como necesario incluir información relativa al concepto en el paquete o envoltorio de venta. Tras conocer esta información se desarrolló el logo de producto hidroSOStenible o hidroSOS. Por otra parte, surgió la necesidad de implementar un índice que fuera capaz de clasificar los productos en base al ahorro hídrico o buen manejo hídrico tenido a lo largo de su cultivo.

Análisis conjunto

Tras llevar a cabo un análisis conjunto de las combinaciones surgidas con los términos englobados en las tres categorías (45 combinaciones entre las categorías agricultura, salud y sensorial) se obtuvieron las 6 mejores combinaciones (en base a la media y la mediana de las mismas). La categoría más importante para los consumidores fue aquella que hacía referencia a términos relacionados con la salud (43,8 %), seguida por los otros dos factores (agricultura y sensorial) con un 28 % cada uno de ellos.

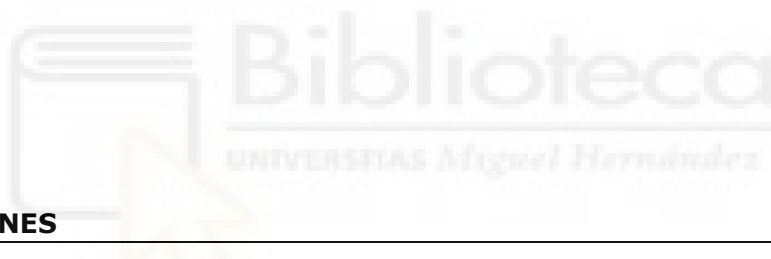
La elección "producto de España" fue la característica agrícola más apreciada. Este resultado de la encuesta coincidió bastante bien con los comentarios obtenidos durante el trabajo de los grupos focales, en los que un alto porcentaje de participantes mencionó que los "alimentos locales" eran atractivos para ellos. Está demostrado que, para los consumidores, la comida local no se percibe como costosa, al contrario de la comida orgánica, y esto lleva a opiniones más positivas hacia este tipo de productos. Además, los consumidores declararon que estaban dispuestos a pagar dinero extra por productos claramente etiquetados como locales porque quieren apoyar a los agricultores y la economía de proximidad.

Dentro de la categoría de "salud", el hecho de que los pistachos sean una fuente de energía no impulsó la voluntad de los consumidores para comprar pistachos, quizás debido a las preocupaciones de los consumidores con respecto a los productos grasos con altos niveles de energía. En el lado opuesto, los conceptos "rico en antioxidantes" y "perfil de ácidos grasos saludables" fueron valorados positivamente por los consumidores españoles. Por lo tanto, estas dos declaraciones de propiedades saludables pueden ser útiles cuando se promueven los pistachos en España y en la Unión Europea.

Finalmente, el factor más importante relacionado con las propiedades sensoriales de los pistachos fue su crujibilidad, y el concepto "divertido de comer". Por otra parte, el concepto de "combinación tostada y salada" no era demasiado atractivo para los consumidores, tal vez por incluir la palabra "salado" que parece no muy popular en este momento (actualmente, los consumidores desean reducir su consumo de sal y se están tomando medidas administrativamente para que esto ocurra). En base a estos resultados, es importante que durante el procesamiento de los pistachos se garantice que estén lo más crujientes posible y que tengan un contenido de sal reducido.

Intención de compra

La disposición a pagar por los pistachos se vio afectada tanto por la información de riego (convencional versus RDI) como por la ubicación geográfica (Galicia o Valencia). Los consumidores españoles estaban dispuestos a pagar un precio significativamente más alto ($p < 0,01$) por los pistachos hydroSOS en comparación con las muestras convencionales. De hecho, estaban dispuestos a pagar 1,72 € por bolsa de 0,125 kg, lo que equivale a un precio final de 13,76 € kg⁻¹ por los pistachos hydroSOS, en comparación con 12,72 € kg⁻¹ por los convencionales. Atendiendo a las diferencias geográficas, los consumidores de Galicia estaban dispuestos a pagar un precio significativamente más alto ($p < 0,05$) por los pistachos que los de la región de Valencia, exactamente 13,60 en comparación con 12,88 € kg⁻¹. La hipótesis inicial de este estudio fue que los consumidores de Valencia deberían estar dispuestos a pagar precios más altos porque el agua es más escasa allí que en Galicia; Sin embargo, la situación real era exactamente la opuesta. Este hallazgo experimental podría deberse al hecho de que todos los consumidores en España son conscientes de la importancia del agua y de la reducción drástica de la disponibilidad de agua de riego.



CONCLUSIONES

Tras el análisis mediante grupos focales, análisis conjunto e intención de compra llevados a cabo en este estudio, se demostró que es necesario diferenciar/resaltar los productos hydroSOS del resto que no lo son, que los consumidores de frutos secos se fijan en los atributos saludables de los mismos a la hora de comprarlos, que en el caso particular de los pistachos lo que más valoran es la crujibilidad de estos, y que estarían dispuestos a pagar hasta un 10 % por aquellos pistachos obtenidos mediante técnicas que ahorren agua durante su cultivo.



Calidad física, química y sensorial de pistachos hidroSOS

CAPÍTULO

3

El objetivo principal de este capítulo fue determinar la influencia que el riego deficitario controlado, así como el empleo de tres portainjertos tenían sobre la calidad física, química y sensorial de los pistachos. Los resultados obtenidos quedaron reflejados en dos publicaciones, la primera de ellas titulada "*Influence of regulated deficit irrigation and rootstock on the functional, nutritional and sensory quality of pistachio nuts*" publicada en la revista "*Scientia Horticulturae*", y la segunda, titulada "*Volatile, sensory and functional properties of hydroSOS pistachios*" publicada en la revista "*Foods*". Los resultados de ambas publicaciones se corresponden a dos años sucesivos del cultivo.

Análisis morfológico

Los pistachos obtenidos del portainjerto *P. integerrima* tuvieron un peso y tamaño mayor en el primero de los años, mientras que los procedentes del portainjerto *P. terebinthus* fueron los más pesados y grandes en el segundo año. En ambos años, los pistachos de *P. terebinthus* fueron los que un mayor número de pistachos abiertos presentaron y en ninguno de estos años se detectó que el empleo de los portainjertos bajo estudio tuviese incidencia significativa sobre el color de los frutos obtenidos.

En el caso del estudio de la influencia del riego sobre la morfología de los frutos, los pistachos obtenidos mediante RDI tuvieron un mayor peso que los pistachos regados completamente (control, 100 % ETo). No hubo diferencias significativas en el número de pistachos abiertos o cerrados ni en el color de las muestras. Sí se apreciaron diferencias significativas en el tamaño, puesto que los pistachos obtenidos bajo un RDC 2 (estrés severo) tuvieron ligeramente menor tamaño que los pistachos control.

Ácidos grasos

Se identificaron 9 ácidos grasos en los pistachos, de los que 4 fueron ácidos grasos saturados, 3 monoinsaturados y 2 poliinsaturados. El ácido graso mayoritario encontrado fue el ácido oleico (~52 %), seguido del ácido linolénico (~31 %).

El empleo de RDI T1 durante la fase II del cultivo de pistacho incrementó ligeramente el contenido de ácido oleico durante el primer año de estudio, mientras que el segundo año no hubo diferencias significativas entre los tratamientos de riego estudiados. En el caso del estudio de los distintos portainjertos, en el primero de los años bajo estudio, los pistachos obtenidos mediante el empleo de *P. terebinthus*

fueron los que tuvieron un mayor contenido de ácido oleico, mientras que los obtenidos mediante *P. atlantica* fueron los que tuvieron una menor concentración. En el segundo año de estudio no se observaron diferencias significativas entre los portainjertos estudiados.

Minerales

Del estudio de la composición mineral de los pistachos obtenidos mediante diferentes portainjertos, se desprende que aquellos cultivados bajo *P. integerrima* tuvieron menores concentraciones de Mg, Cu, Mn, Fe y Zn; aunque no se observaron diferencias significativas en el caso del potasio, mineral más abundante en este fruto seco y al cual deben buena parte de sus alegaciones nutricionales.

En el caso de los pistachos obtenidos bajo diferentes riegos, los del tratamiento T2 presentaron mayores contenidos de Na, Ca y Cu, y menor concentración de K y Mn que los pistachos control; en los obtenidos bajo el tratamiento RDI T1 no se observaron diferencias significativas en el contenido de K ni Na, y sí en el caso del Ca (al igual que en el otro tratamiento de restricción hídrica, el Ca se vio ligeramente incrementado). La disminución en el agua de riego podría causar, entre otras cosas, una acumulación de los contenidos de Ca y Na disponibles en el sistema suelo-planta al reducir las pérdidas por lixiviación, lo que lleva a una menor absorción y acumulación de K y Mn. Esto podría justificarse por el efecto antagónico que Ca y Na pueden causar en el resto de los minerales.

Azúcares y ácidos orgánicos

Tres azúcares (maltitol, rafinosa y sacarosa) y tres ácidos orgánicos (fumárico, oxálico y shikimico) se identificaron y cuantificaron en las muestras de pistacho bajo estudio.

Las muestras obtenidas bajo el tratamiento de riego T1 tuvieron menor concentración de ácido fumárico ($0,287 \text{ g L}^{-1}$) que las muestras control ($T0 = 0,315 \text{ g L}^{-1}$), mientras que los pistachos T2 ($0,287 \text{ g L}^{-1}$) quedaron estadísticamente relacionadas con los otros dos tratamientos. En el resto de los ácidos y azúcares orgánicos no se encontraron diferencias estadísticas significativas entre los tratamientos.

En el caso de los pistachos obtenidos mediante distintos portainjertos, los obtenidos mediante *P. integerrima* presentaron menor concentración de sacarosa

(19,770 g L⁻¹) que *P. terebinthus* y *P. atlantica* (22,505 y 24,977 g L⁻¹, respectivamente). En el caso de los ácidos orgánicos, *P. integerrima* fue el portainjerto que produjo mayores concentraciones de los 3 ácidos estudiados, mientras que *P. terebinthus* tuvo menos cantidad de ácidos oxálico y fumárico.

Compuestos volátiles

En el análisis de la composición volátil de los pistachos se identificaron 31 compuestos, siendo α -pineno (~35 %), limoneno (~14 %) y β -mirceno (~11 %) los tres compuestos mayoritarios.

Los pistachos obtenidos mediante el tratamiento de riego T1 no tuvieron diferencias estadísticamente significativas en su composición volátil con respecto a los pistachos del tratamiento control (T0). Por otro lado, los pistachos obtenidos bajo RDI T2 tuvieron menores cantidades de α -pineno, dodecano y tridecano, y un alto contenido de β -mirceno y limoneno (en comparación con la muestra control).

En el caso de los diferentes portainjertos, *P. integerrima* tuvo el mayor contenido de α -pineno (principal compuesto volátil); *P. atlantica* obtuvo frutos con alto contenido de β -mirceno, dodecano y tridecano; y *P. terebinthus* condujo a pistachos con un mayor contenido de limoneno.

Según los resultados obtenidos, los principales compuestos volátiles encontrados en el perfil volátil de los pistachos α -pineno, limoneno y β -mirceno (compuestos sensoriales relacionados con descriptores de madera, cítricos y frutales, respectivamente), pueden usarse como una herramienta de control para estudiar la calidad del aroma de este fruto seco.

Actividad antioxidante

La aplicación de un riego deficitario controlado leve, o tratamiento RDI T1, no tuvo ninguna incidencia significativa en la actividad antioxidante de las muestras de pistachos, con respecto a la muestra control (T0). En cambio, cuando la restricción hídrica aplicada fue severa (T2), la actividad antioxidante de los pistachos sí se vio disminuida. Esto ocurrió en los métodos DPPH y FRAP, sin embargo, la determinación de la actividad antioxidante mediante el método ABTS no arrojó ninguna diferencia significativa entre las muestras.

Este resultado indica que, bajo un alto estrés hídrico se produce una regulación estomática importante y el CO₂ producido se utiliza para el mantenimiento del

metabolismo primario; mientras que, bajo un estrés hídrico leve o suave, el CO₂ se redistribuye a la formación de metabolitos secundarios (compuestos de naturaleza antioxidante).

En el caso de los pistachos obtenidos mediante distintos portainjertos, en el primero de los años estudiados no se obtuvieron diferencias significativas entre las tres muestras, mientras que, en el segundo de los años, los pistachos obtenidos con el portainjerto *P. integerrima* fueron los que tuvieron valores de actividad antioxidante más altos (en los tres métodos estudiados).



Polifenoles totales

El mismo comportamiento que el descrito en el apartado de actividad antioxidante, se observó tras analizar la cantidad de polifenoles totales presentes en las muestras de pistachos obtenidos bajo portainjertos: el primero de los años no se observaron diferencias mientras que, en el segundo, *P. integerrima* obtuvo valores más altos.

En el caso del riego, en este caso, los pistachos obtenidos mediante RDI T1 fueron los que tuvieron un mayor valor de polifenoles totales, reforzando la hipótesis descrita previamente en la que se preveía un incremento de metabolitos secundarios como mecanismo de defensa ante un estrés leve.



Análisis sensorial descriptivo

De los 15 atributos sensoriales estudiados en el análisis descriptivo de las muestras de pistachos obtenidos bajo diferentes riegos, se encontraron diferencias significativas en 5 de ellos: color, tamaño, sabor a pistacho, postgusto y crujibilidad. El tratamiento RDI T1 no presentó ninguna diferencia significativa frente a las muestras control en ninguno de estos atributos; sí las presentó el tratamiento T2, obteniendo menores intensidades en los atributos color, tamaño, sabor a pistacho y postgusto, y teniendo una mayor crujibilidad. Esta situación pudo darse debido a la aparición de fibrosidad en el fruto debido al estrés al que estuvo sometido.

En el caso del estudio de los diferentes portainjertos se observaron diferencias significativas en 10 de los 15 atributos estudiados. Los pistachos de *P. atlantica* fueron los de mayor tamaño; los de *P. integerrima* destacaron por tener un mejor perfil de sabor; y los de *P. terebinthus* tuvieron una mejor textura (duros, crujientes, poco friables y adhesivos).

Análisis sensorial afectivo

En el estudio de análisis sensorial afectivo participaron unos 200 consumidores españoles, mexicanos y polacos, de los que el 35 % tenía entre 18 y 25 años, el 32 % tenía entre 26 y 35 años, el 15 % entre 36 y 45 años, el 17 % entre 46 y 55 años, y el 2 % restante, tenía más de 55 años.

Para esta determinación solo se analizó la influencia que riego deficitario controlado tenía sobre la calidad de los pistachos, dejando de lado el efecto del portainjerto. Esto fue debido a que únicamente se tuvo oportunidad de llevar a cabo una sesión de análisis con los consumidores en cada uno de los países y hubo que minimizar el número de muestras para no saturar a los panelistas.

Los tratamientos de riego tuvieron incidencia significativa en 3 de los trece atributos sensoriales bajo análisis: sabor a pistacho (ID), aceitosidad y la satisfacción general. Los pistachos obtenidos bajo RDI T1 obtuvieron mayores intensidades de sabor a pistacho y satisfacción general que las muestras control y T2. En el caso del atributo aceitosidad, el tratamiento T1 obtuvo las mismas intensidades que el tratamiento control, mientras que T2 tuvo una intensidad menor.

Cuando forzamos a los consumidores a elegir (entre las tres muestras estudiadas) cuál era su muestra favorita, los pistachos obtenidos bajo RDI T1 fueron los que más gustaron en cada uno de los países (en el caso de Polonia, no se observaron diferencias estadísticas entre T1 y T0). Los consumidores mencionaron que las principales razones para seleccionar la muestra preferida fueron el sabor a pistacho (~ 83 %), la textura crujiente (~ 65 %), el regusto (~ 45 %) y la dureza (~ 30 %). Por otro lado, la muestra que menos gustó a los consumidores en cada uno de los países fue T2. Se observaron los mismos resultados cuando se les preguntó a los consumidores sobre su disposición a pagar las muestras en estudio.

CONCLUSIONES

Los resultados obtenidos en este estudio demostraron que aplicar riego deficitario controlado leve durante el cultivo de pistacho (T1) condujo a obtener pistachos con las mismas propiedades morfológicas, contenido total de polifenoles, actividad antioxidante, composición volátil y propiedades sensoriales que los pistachos obtenidos bajo tratamientos de riego completo (T0). Además, T1 condujo a pistachos con un mejor perfil de ácidos grasos y fue la muestra favorita de los consumidores internacionales. Por el contrario, cuando la reducción hídrica fue severa

(T2), los pistachos tuvieron menos actividad antioxidante, menos contenido de polifenoles y fueron los menos preferidos para los consumidores.

En el caso de los pistachos obtenidos bajo diferentes portainjertos, *P. integerrima* dio lugar a pistachos con alto peso, menor contenido de sacarosa, mejores propiedades funcionales y una mejor textura que *P. atlantica* y *P. terebinthus*.





Calidad funcional de pistachos hidroSOS

CAPÍTULO

4

El objetivo de este capítulo fue evaluar el perfil de los compuestos bioactivos (identificación y cuantificación de polifenoles, procianidinas poliméricas, contenido de triterpenoides y α -amilasa) de los pistachos obtenidos mediante el empleo de los portainjertos *P. atlantica*, *P. integerrima* y *P. terebinthus*, así como por el efecto del riego deficitario controlado. Los resultados obtenidos tras la realización de estas determinaciones se reflejaron en una publicación titulada "*Phenolic and triterpenoid composition and inhibition of α -amylase of pistachio kernels (*Pistacia vera* L.) as affected by rootstock and irrigation treatment*" publicada en la revista "*Food Chemistry*".

Análisis cualitativo de polifenoles

Se identificaron 17 compuestos en el extracto metanólico de las muestras de pistacho: 8 flavonoles, 4 flavan-3-oles, 2 antocianinas, 2 ácidos fenólicos y 1 flavanona. Los compuestos fenólicos se identificaron de acuerdo con sus tiempos de retención, masas moleculares, patrones de fragmentación, espectros característicos y referencias bibliográficas.

El grupo de polifenoles predominante fue flavan-3-oles, siendo el compuesto principal identificado como (+)-catequina, con un ion (M-H)⁻ a m/z 289.0727 y fragmentos de MS/MS a m/z 245, 205 y 179. También se identificó (-)-epicatequina con el mismo (M-H)⁻ ion que la anterior, pero diferente tiempo de retención. Además de estos compuestos, se identificaron dos dímeros de procianidinas con m/z 577. En el caso de flavonoles, hubo dos tipos de derivados, con un fragmento a m/z 285 y 301, característicos de kaempferol y quercetina, respectivamente. Además, se encontraron dos antocianinas con un fragmento a m/z 287, cianidin-3-O-galactósido y cianidin-3-O-glucósido (picos a diferentes tiempos de retención). Finalmente, hubo una flavanona, eriodictyol-3-O-glucósido ([MH]⁻ a m/z 449), y un desoxidihexósido, con un patrón de fragmentación característico (M-H)⁻ a m/z 309.

Análisis cuantitativo de polifenoles

El compuesto fenólico encontrado en mayor concentración en las muestras de pistacho fue (+)-catequina con valores de 97 y 143 mg (100 g)⁻¹, seguido de (-)-epicatequina con valores de 25,5 a 42,6 mg (100 g)⁻¹.

El empleo de los diferentes portainjertos tuvo influencia en el contenido de ácido cafeico y keampferol-O-dihexósido, teniendo las muestras procedentes del portainjerto *P. terebinthus* los contenidos más altos 9,18 y 1,91 mg (100 g)⁻¹,

respectivamente. Para el resto de los compuestos fenólicos, el empleo de los portainjertos no tuvo ninguna incidencia significativa en la composición fenólica de los frutos obtenidos.

En el caso de los pistachos obtenidos mediante distintos tratamientos de riego deficitario controlado, se observó una disminución lineal en el contenido de (+)-catequina con respecto al estrés hídrico aplicado; los pistachos T0 tuvieron la concentración más alta [143 mg (100 g)⁻¹], seguidos de los obtenidos mediante el tratamiento T1 [119 mg (100 g)⁻¹], y en última instancia por los procedentes del tratamiento T2 [97,6 mg (100 g)⁻¹]. Sin embargo, los pistachos obtenidos mediante un riego deficitario controlado leve (T1) tuvieron una mayor concentración de keampferol-3-*O*-glucósido y quercetina-*O*-galoil-hexósido.

La concentración de procianidinas poliméricas (PP) en las muestras de pistacho varió de 323 a 443 mg (100 g)⁻¹ y se encontraron diferencias estadísticamente significativas para ambos factores en estudio, portainjertos y tratamiento de riego. Los pistachos obtenidos con portainjertos de *P. terebinthus* y *P. atlantica* [443 y 415 mg (100 g)⁻¹, respectivamente] tuvieron un mayor contenido de PP que los obtenidos con el portainjerto de *P. integerrima* [323 mg (100 g)⁻¹]. En el caso de los pistachos obtenidos mediante diferentes tratamientos de riego, la aplicación de los tratamientos de RDI causó un aumento significativo del contenido de PP, aumentando de 352 mg (100 g)⁻¹ en las muestras control hasta 427 y 404 mg (100 g)⁻¹ en muestras T1 y T2, respectivamente. Este incremento, provocado por el estrés hídrico, podría estar relacionado con el hecho de que el déficit hídrico puede conducir a un aumento en los niveles de fenilalanina libre (un precursor en la síntesis de PP).

Si analizamos los resultados desde el punto de vista de la concentración total de compuestos fenólicos, *P. integerrima* fue el portainjerto que condujo a una menor concentración de compuestos, mientras que la aplicación de un riego deficitario controlado leve (T1) incrementó la concentración de estos compuestos (~10 % más).



Cuantificación de triterpenoides

Se determinaron 3 triterpenoides en los pistachos obtenidos mediante diferentes portainjertos y riegos: ácido betulínico, ácido oleanólico y ácido ursólico. El contenido total promedio fue de ~644 µg g⁻¹, y no se vio afectado por ninguno de los factores bajo estudio. El contenido (promedio) individual para cada uno de los triterpenoides estudiados fue de 98, 60 y 486 µg g⁻¹, para el ácido betulínico, oleanólico y ursólico, respectivamente.

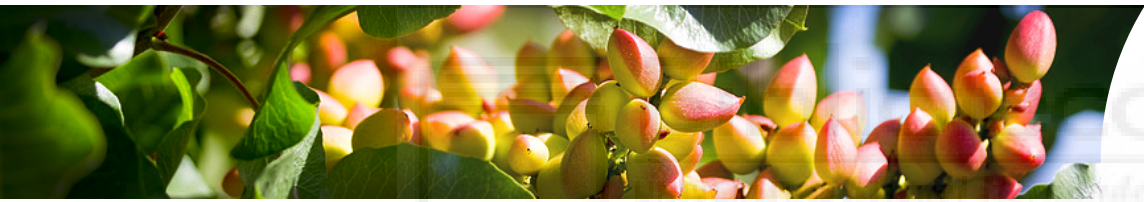
El empleo de los diferentes portainjertos únicamente tuvo incidencia significativa en la concentración de ácido betulínico, siendo *P. terebinthus* y *P. atlantica* las muestras que presentaron una mayor concentración. En el caso de los diferentes riegos, no se observaron diferencias significativas en la concentración de ninguno de los tres triterpenoides estudiados.

α -Amilasa

Del estudio del potencial que tienen los pistachos para inhibir la enzima α -amilasa se demostró que el empleo de los portainjertos *P. atlantica*, *P. integerrima* y *P. terebinthus* no tuvo ninguna incidencia (resultados expresados como mg ml⁻¹ necesarios para inhibir el 50 % de la concentración de enzima, IC₅₀). En el caso de los pistachos obtenidos mediante distintos riegos sí se apreciaron diferencias significativas. Los frutos procedentes de pistachos regados mediante riego deficitario controlado tuvieron valores más altos de inhibición que la muestra control. Es decir, los resultados mostraron que la aplicación de tratamientos de RDI no eliminó la actividad inhibidora de la α -amilasa en el pistacho. Esta actividad podría estar relacionada con su mayor contenido fenólico, ya que no se ha demostrado que los triterpenoides tengan ningún efecto significativo sobre esta enzima; Además, este efecto podría estar relacionado con la capacidad antioxidante de los compuestos fenólicos.

CONCLUSIONES

Tras comparar los resultados obtenidos sobre la calidad funcional de los pistachos obtenidos mediante el empleo de 3 portainjertos (*P. atlantica*, *P. integerrima* y *P. terebinthus*), se demostró que el uso de *P. terebinthus* y *P. atlantica* generó pistachos con una mayor calidad funcional (mayor contenido de ácido betulínico y polifenoles totales), en comparación con los frutos obtenidos mediante el portainjerto *P. integerrima*. En el caso del riego, tras la aplicación de un tratamiento de riego deficitario controlado leve (T1) se obtuvieron pistachos con un mejor perfil funcional (mayor contenido de polifenoles y procianidinas poliméricas), que en el caso de las muestras regadas hasta cubrir sus necesidades.



Propiedades antimutagénicas y citoprotectivas

CAPÍTULO

5

El objetivo de este capítulo fue estudiar el efecto que el riego deficitario controlado y el empleo de 3 portainjertos tienen sobre las propiedades antioxidantes, antimutagénicas y citoprotectoras de los pistachos. Los resultados derivados de esta investigación se publicaron en la revista "Molecules" en un artículo titulado "Antioxidant, antimutagenic and cytoprotective properties of hydrosol pistachio nuts".

Antioxidantes

En el caso que ahora nos ocupa, se realizó una determinación de los compuestos con capacidad antioxidante presentes en los extractos obtenidos (fracción metanólica) de los pistachos.

Los pistachos obtenidos tras el empleo del portainjerto *P. terebinthus* durante el cultivo fueron los que presentaron una mayor actividad antioxidante tras someter las muestras a los métodos ABTS, FRAP y DPPH. Por el contrario, a nivel general, *P. atlantica* fue quien obtuvo valores más bajos de actividad antioxidante y *P. integerrima* valores intermedios. Estos resultados están en concordancia con los obtenidos previamente (capítulo anterior) en los que se demostró que *P. terebinthus* conducía a pistachos con un mayor contenido polifenólico y mayor contenido de ácido betulínico (triterpenoide).

En el caso de los pistachos obtenidos mediante diferentes tratamientos de riego, se observó que la actividad antioxidante se mantuvo o incluso se mejoró (como en el caso del método DPPH) cuando se restringió el agua durante el cultivo (tratamientos RDI T1 y T2).

Antimutagenicidad

El análisis de mutagenicidad se realizó siguiendo el test de Ames, empleando *Salmonella typhimurium* TA100 en presencia de azida de sodio como mutágeno. No se observaron diferencias significativas entre los pistachos obtenidos mediante diferentes portainjertos ni entre los obtenidos mediante diferentes estrategias de riego.

Los extractos de pistacho no fueron tóxicos ni mutagénicos para las bacterias (a las concentraciones de 20, 2 y 0,2 mg mL⁻¹) y el crecimiento bacteriano fue normal. Esto indica que los compuestos fenólicos presentes en los extractos no tienen actividad contra la azida de sodio.

Citotoxicidad

En este apartado hacemos resumen del efecto que el RDI y el empleo de portainjertos tuvieron sobre la actividad citotóxica en cuatro líneas celulares cancerosas y una no cancerosa. Como control positivo se empleó cisplatino, un agente de control citotóxico, que es un fármaco genotóxico que se usa en tratamientos de quimioterapia.

En ninguna de las líneas cancerosas estudiadas se encontraron diferencias significativas asociadas a cualquiera de los factores bajo estudio (riego y portainjerto). Los extractos de pistacho estudiados demostraron tener el mismo efecto sobre las líneas cancerosas que una dosis de cisplatino que $50 \mu\text{g mL}^{-1}$.

En la línea celular normal, ARPE-19, no se encontraron diferencias entre las muestras que se obtuvieron mediante diferentes tratamientos de riego, pero sí entre las obtenidas por diferentes portainjertos, siendo *P. atlantica* la que garantizó una mayor viabilidad celular ($\sim 7\%$ más de viabilidad). Una cuestión importante a señalar es que ninguno de los extractos de pistacho redujo la viabilidad celular de ARPE-19 por debajo del 85% ; por lo tanto, no pueden considerarse como citotóxicos en la concentración estudiada.

Estos resultados demostraron que los compuestos presentes en los extractos de pistacho no tuvieron ningún efecto tóxico en las células normales (ARPE-19). Por el contrario, podrían afectar significativamente a las cuatro líneas de cáncer estudiadas, siendo la línea celular A549 la más resistente y HeLa la más sensible. Esta actividad citotóxica frente líneas celulares cancerosas puede deberse al contenido de triterpenoides presentes en los frutos de pistacho.

Protección frente al daño oxidativo

Para evaluar el efecto de protección que los compuestos presentes en los extractos de pistacho tuvieron frente al daño oxidativo se incubaron células normales de retina (ARPE-19) con los extractos, durante 4 y 24 h, y después se sometió a las células a peróxido de hidrógeno a una concentración de 10 mM .

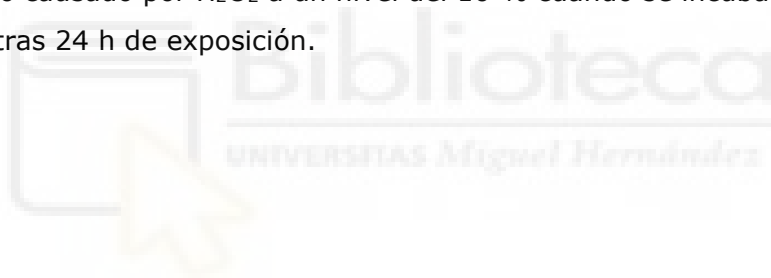
No se observaron diferencias estadísticamente significativas entre los pistachos obtenidos mediante diferentes estrategias de riego ni entre los cultivados bajo distintos portainjertos.

Tras incubar las células normales con los extractos de pistacho durante 4 h se consiguió reducir en un 10% el daño oxidativo provocado por el H_2O_2 . Cuando se

incubaron durante 24 h, el daño oxidativo se redujo en un 35 %. El incremento del tiempo de exposición, probablemente, aumentó la cantidad de compuestos del pistacho en el interior de la célula, por lo que se logró una mayor protección contra el estrés oxidativo causado por los radicales peróxido (formados a partir del H₂O₂ por las reacciones de Fenton o Haber-Weiss).

CONCLUSIONES

Los resultados experimentales demostraron que el uso del portainjerto de *P. terebinthus* produjo pistachos con mayor actividad antioxidante, mientras que la aplicación de estrategias RDI mantuvo o mejoró la capacidad antioxidante de los frutos. Ni el uso de diferentes portainjertos ni la aplicación de RDI durante el cultivo de pistacho tuvieron un impacto significativo en el potencial antimutagénico y la actividad citoprotectora de los pistachos. De las cuatro líneas celulares de cáncer estudiadas, A549 fue la más resistente y HeLa fue la más sensible a los extractos de pistacho. Los extractos de pistacho protegieron con éxito las células ARPE-19 del daño oxidativo causado por H₂O₂ a un nivel del 10 % cuando se incubaron durante 4 h, y al 35 % tras 24 h de exposición.



6

CONCLUSIONES

CONCLUSIONS



CONCLUSIONES

De entre los ocho cultivares de pistacho estudiados, Kerman destacó por su funcionalidad y sus propiedades sensoriales, especialmente por una mayor intensidad de sabor a pistacho.

Los consumidores de frutos secos valoran positivamente los productos saludables, con buenas propiedades sensoriales y aquellos obtenidos respetando el medio ambiente, estando dispuestos a pagar hasta un 10 % más por un producto que cumpla dichas condiciones.

La aplicación de un tratamiento de riego deficitario moderado (T1, durante la fase fenológica II del cultivo del pistacho, el potencial hídrico del tallo se mantuvo en -1.5 MPa), produce pistachos con igual morfología, mejores atributos funcionales (entre los que destacan un mayor contenido de polifenoles), mejor calidad química (gracias principalmente a un mejor perfil de compuestos volátiles) y mejor aceptación sensorial que los pistachos no estresados hídricamente; todo ello con un ahorro de más de un 30 % de agua y, en consecuencia, con una menor repercusión medioambiental y mayores beneficios económicos.

La aplicación de un tratamiento severo de riego deficitario controlado (T2, durante la fase fenológica II del cultivo del pistacho, el potencial hídrico del tallo se mantuvo en -2.0 MPa) tiene incidencia negativa en algunas propiedades claves en la calidad del pistacho, como el tamaño, la actividad antioxidante y las propiedades sensoriales.

La aplicación de tratamientos de riego deficitario controlado no merma las propiedades antimutagénicas y citotóxicas que los compuestos presentes en el pistacho tienen frente a células cancerígenas. Por otra parte, el riego deficitario controlado mantiene la capacidad de inhibición de α -amilasa característica de este fruto.

Entre los portainjertos estudiados, *P. terebinthus* es el que produce frutos de mayor calidad: frutos más grandes, de mayor peso, mejor textura, con mejor perfil de ácidos grasos, mejor capacidad antioxidante y mayor contenido total de polifenoles.

Con base en los resultados aquí descritos, la combinación surgida del empleo del portainjerto *P. terebinthus* y la aplicación de un riego deficitario controlado moderado (T1), aplicados al cultivar Kerman, es la mejor opción para obtener frutos con una mejor funcionalidad y calidad sensorial.

CONCLUSIONS

Among the eight pistachio cultivars studied, Kerman stood up for its functionality and sensory properties, especially for a higher intensity of pistachio flavor.

Nut consumers valued healthy products positively, with good sensory properties and those obtained respecting the environment, and were willing to pay up to 10% more for a product that meets these conditions.

The application of a moderate regulated deficit irrigation treatment (T1, during the phenological phase II of pistachio cultivation, stem water potential remained at -1.5 MPa), produces pistachios with equal morphology, better functional attributes (among which a higher content of polyphenols), better chemical quality (thanks mainly to a better profile of volatile compounds) and better sensory acceptance than pistachios not hydric stressed; all this, with a saving of more than 30% of water and, consequently, with a lower environmental impact and greater economic benefits.

The application of severe regulated deficit irrigation treatment (T2, during the phenological phase II of pistachio cultivation, the stem water potential remained at -2.0 MPa) has a negative impact on some key properties in pistachio quality, such as size, antioxidant activity and sensory properties.

The application of regulated deficit irrigation treatments does not reduce the antimutagenic and cytotoxic properties that the compounds present in pistachio have against cancer cells. On the other hand, regulated deficit irrigation maintains the ability to inhibit α -amylase, characteristic property of this nut.

Among the rootstocks studied, *P. terebinthus* is the one that produces fruit with a higher quality: larger and heavier nuts, with better texture, with a better fatty acid profile, better antioxidant capacity and higher total polyphenol content.




Based on the results obtained, the combination arising from the use of the *P. terebinthus* rootstock and the application of a moderate regulated deficit irrigation (T1), applied to Kerman cultivar, is the best option to obtain fruits with better functionality and sensory quality.

INVESTIGACIONES FUTURAS

7



En base a los resultados obtenidos durante el desarrollo de esta Tesis Doctoral, surgen nuevos escenarios que pueden ser estudiados con el fin de profundizar más en el efecto que el riego deficitario controlado tiene sobre la calidad de los frutos. Estos son algunos que pueden ser de especial interés:

-  Aplicar tratamientos de riego deficitario controlado manteniendo los niveles de estrés hídrico por debajo de -1.5 MPa durante un tiempo más prolongado, para estudiar si es posible reducir aún más, si cabe, el aporte hídrico manteniendo la calidad de los frutos obtenidos.
-  Fraccionar los extractos de pistacho con el fin de aislar los compuestos presentes y poder caracterizar más certeramente los compuestos responsables de sus propiedades citotóxicas y antioxidantes.
-  Implementar tratamientos de riego deficitario controlado en otros cultivos leñosos, con el fin reducir el aporte hídrico destinado a la agricultura y, al mismo tiempo, mejorar la calidad de los frutos obtenidos.



∞ | REFERENCIAS

- A -

- Acar, I., Kafkas, S., Kapchina-Toteva, V., & Ercisli, S. (2017). Effect of rootstock on fat content and fatty acid composition of immature pistachio kernels. *Comptes rendus de l'Académie bulgare des Sciences*, 70(7), 1049-1056.
- Acquaviva, R., Sorrenti, V., Santangelo, R., Cardile, V., Tomasello, B., Malfa, G., Vanella, L., Amodeo, A., Genovese, C., Mastrojeni, S., Pugliese, M., Ragusa, M., & Di Giacomo, C. (2016). Effects of an extract of *Celtis aetnensis* (Tornab.) Strobl twigs on human colon cancer cell cultures. *Oncology Reports*, 36(4), 2298-2304. doi: 10.3892/or.2016.5035
- Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). *Crop evapotranspiration – guidelines for computing crop water requirements*. FAO Irrigation and drainage paper 56. Rome.
- Arpaci, S., Atli, H. S., Tekin, H., & Burak, M. (2006). Studies on spring frost resistance of some pistachio (*Pistacia vera*) cultivars. *Acta Horticulturae*, 726, 391-395.

- B -

- Ballistreri, G., Arena, E., & Fallico, B. (2009). Influence of ripeness and drying process on the polyphenols and tocopherols of *Pistacia vera* L. *Molecules*, 14(11), 4358-4369. doi: 10.3390/molecules14114358
- BEDCA (2019). Base de datos española de composición de alimentos. Consultada en diciembre 2019. <https://www.bedca.net/>
- Behboudian, M. H., & Mills, T. M. (1997). Deficit irrigation in deciduous orchards. In J. Janick (Ed.), *Horticultural Reviews* (pp. 105-131).
- Bellomo, M., & Fallico, B. (2007). Anthocyanins, chlorophylls and xanthophylls in pistachio nuts (*Pistacia vera*) of different geographic origin. *Journal of Food Composition and Analysis*, 20(3-4), 352-359. doi: 10.1016/j.jfca.2006.04.002
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. doi: 10.1006/abio.1996.0292
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25-30. doi: 10.1016/s0023-6438(95)80008-5
- Campana, B. M. R., & Ochoa, M. J. (2008). Propagación vegetativa o agámica de especies frutales. In G. O. Sozzi (Ed.), *Árboles frutales. Ecofisiología, cultivo y aprovechamiento* (pp. 133-197). Buenos Aires, Argentina: Facultad de Agronomía.

- C -

- Carbonell-Barrachina, A. A., Memmi, H., Noguera-Artiaga, L., del Carmen Gijón-López, M., Ciapa, R., & Pérez-López, D. (2015). Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *Journal of the Science of Food and Agriculture*, 95(14), 2866-2873. doi: 10.1002/jsfa.7027
- Carbonell-Barrachina, A. A., Memmi, H., Noguera-Artiaga, L., Gijon-Lopez, M. C., Ciapa, R., & Perez-Lopez, D. (2015). Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *Journal of the Science of Food and Agriculture*, 95(14), 2866-2873. doi: 10.1002/jsfa.7027
- Couceiro, J. F., Guerrero, J., & Gijón, M. C. (2010). Cultivo ecológico de especies alternativas leñosas en secano (El pistachero). *Agricultura Ecológica en Secano*. Madrid: Ediciones Mundi-Prensa.
- Couceiro, J. F., Guerrero, J., Gijón, M. C., Pérez-López, D., Moriana, A., & Rodriguez, M. (2013). *El Cultivo del Pistacho*. Madrid: Ediciones Mundi-Prensa.
- Cragg, G. M., & Newman, D. J. (2005). Plants as a source of anti-cancer agents. *Journal of Ethnopharmacology*, 100(1-2), 72-79. doi: 10.1016/j.jep.2005.05.011

- D -

- Dreher, M. L. (2012). Pistachio nuts: Composition and potential health benefits. *Nutrition Reviews*, 70(4), 234-240. doi: 10.1111/j.1753-4887.2011.00467.x

- F -

- FAOSTAT. (2019). *Datos sobre alimentación y cultura*. Roma, Italia: Organización de las Naciones Unidas para la Alimentación y la Agricultura.
- Fereres, E., & Goldhamer, D. (1990). Deciduous fruit and nut trees. *Agronomy (USA)*.
- Ferguson, L. (2005). *Pistachio production manual* (4th ed.). CA (USA): Fruit and Nut Research and Information Center, University of California.

- G -

- Gijón, M. C. (2013). *Relaciones hídricas y manejo del riego en pistachero (Pistacia vera L.)*. Universidad de Córdoba, Córdoba.
- Gijón, M. C., Gimenez, C., Perez-López, D., Guerrero, J., Couceiro, J. F., & Moriana, A. (2010). Rootstock influences the response of pistachio (*Pistacia vera* L. cv. Kerman) to water stress and rehydration. *Scientia Horticulturae*, 125(4), 666-671. doi: 10.1016/j.scienta.2010.05.026
- Gijón, M. C., Gimenez, C., Perez-López, D., Guerrero, J., Couceiro, J. F., & Moriana, A. (2011). Water relations of pistachio (*Pistacia vera* L.) as affected by

phenological stages and water regimes. *Scientia Horticulturae*, 128(4), 415-422. doi: 10.1016/j.scienta.2011.02.004

Gijón, M. C., Gimenez, C., Pérez-López, D., Guerrero, J., Couceiro, J. F., & Moriana, A. (2011). Water relations of pistachio (*Pistacia vera* L.) as affected by phenological stages and water regimes. *Scientia Horticulturae*, 128(4), 415-422. doi: 10.1016/j.scienta.2011.02.004

Goldhamer, D. A. (1995). Irrigation management. In L. Ferguson (Ed.), *Pistachio Production Manual* (pp. 71-81). University of California, Davis, CA (USA): Fruit and Nut Research and Information Center.

Goldhamer, D. A. (1995). Irrigation management. In L. Ferguson (Ed.), *Fruit and nut research and information center* (pp. 71-81). California, USA: University of California.

Goldhamer, D. A., Viveros, M., & Salinas, M. (2006). Regulated deficit irrigation in almonds: Effects of variations in applied water and stress timing on yield and yield components. *Irrigation Science*, 24(2), 101-114. doi: 10.1007/s00271-005-0014-8

Guerrero, J., Gijón, M. C., Moriana, A., & Couceiro, J. F. (2006). Pistachio cultivation in Castilla La Mancha: twenty years later. *Nucis*, 13, 41-45.

- H -

Hernández, F., Noguera-Artiaga, L., Burló, F., Wojdyło, A., Carbonell-Barrachina, A. A., & Legua, P. (2016). Physico-chemical, nutritional, and volatile composition and sensory profile of Spanish jujube (*Ziziphus jujuba* Mill.) fruits. *Journal of the Science of Food and Agriculture*, 96(8), 2682-2691. doi: 10.1002/jsfa.7386

- K -

Khazir, J., Mir, B. A., Pilcher, L., & Riley, D. L. (2014). Role of plants in anticancer drug discovery. *Phytochemistry Letters*, 7(1), 173-181. doi: 10.1016/j.phytol.2013.11.010

- M -

Mandalari, G., Bisignano, C., Filocamo, A., Chessa, S., Sarò, M., Torre, G., Faulks, R. M., & Dugo, P. (2013). Bioaccessibility of pistachio polyphenols, xanthophylls, and tocopherols during simulated human digestion. *Nutrition*, 29(1), 338-344. doi: 10.1016/j.nut.2012.08.004

- Maron, D. M., & Ames, B. N. (1983). Revised methods for the *Salmonella* mutagenicity test. *Mutation Research/Environmental Mutagenesis and Related Subjects*, 113(3), 173-215. doi: 10.1016/0165-1161(83)90010-9
- Memmi, H., Couceiro, J. F., Gijón, C., & Pérez-López, D. (2016). Impacts of water stress, environment and rootstock on the diurnal behaviour of stem water potential and leaf conductance in pistachio (*Pistacia vera* L.). *Spanish Journal of Agricultural Research*, 14(2). doi: 10.5424/sjar/2016142-8207
- Memmi, H., Gijón, M. C., Couceiro, J. F., & Pérez-López, D. (2016). Water stress thresholds for regulated deficit irrigation in pistachio trees: Rootstock influence and effects on yield quality. *Agricultural Water Management*, 164, 58-72. doi: 10.1016/j.agwat.2015.08.006
- Moriana, A., Memmi, H., Centeno, A., Martín-Palomo, M. J., Corell, M., Torrecillas, A., & Pérez-López, D. (2018). Influence of rootstock on pistachio (*Pistacia vera* L. cv Kerman) water relations. *Agricultural Water Management*, 202, 263-270. doi: 10.1016/j.agwat.2017.12.026

- N -

- Namvar, F., Mohamed, S., Fard, S. G., Behravan, J., Mustapha, N. M., Alitheen, N. B. M., & Othman, F. (2012). Polyphenol-rich seaweed (*Eucheuma cottonii*) extract suppresses breast tumour via hormone modulation and apoptosis induction. *Food Chemistry*, 130(2), 376-382. doi: 10.1016/j.foodchem.2011.07.054

- P -

- Portalfruticola. (2017). Agrotecnia: compendio de injertos frutales. Cómo hacerlos. Tipos, época y compatibilidades por especie. Consultado en diciembre de 2019. <https://www.portalfruticola.com/noticias/2017/07/05/compendio-de-injertos-frutales-como-hacerlos-tipos-epoca-y-compatibilidades-por-especie/>

- R -

- Rababah, T. M., Hettiarachchy, N. S., & Horax, R. (2004). Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. *Journal of Agricultural and Food Chemistry*, 52(16), 5183-5186. doi: 10.1021/jf049645z
- Rajaei, A., Barzegar, M., Mobarez, A. M., Sahari, M. A., & Esfahani, Z. H. (2010). Antioxidant, anti-microbial and antimutagenicity activities of pistachio

(*Pistachia vera*) green hull extract. *Food and Chemical Toxicology*, 48(1), 107-112. doi: 10.1016/j.fct.2009.09.023

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10), 1231-1237. doi: 10.1016/s0891-5849(98)00315-3

- S -

Seeram, N. P., Zhang, Y., Henning, S. M., Lee, R., Niu, Y., Lin, G., & Heber, D. (2006). Pistachio skin phenolics are destroyed by bleaching resulting in reduced antioxidative capacities. *Journal of Agricultural and Food Chemistry*, 54(19), 7036-7040. doi: 10.1021/jf0614948

- T -

Tomaino, A., Martorana, M., Arcoraci, T., Monteleone, D., Giovinazzo, C., & Saija, A. (2010). Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie*, 92(9), 1115-1122. doi: 10.1016/j.biochi.2010.03.027

- V -

Valentini, G. (2003). *La injertación en frutales*. Buenos Aires, Argentina: Instituto nacional de tecnología agropecuaria.

- W -

Wojdyło, A., Carbonell-Barrachina, Á. A., Legua, P., & Hernández, F. (2016). Phenolic composition, ascorbic acid content, and antioxidant capacity of Spanish jujube (*Ziziphus jujube* Mill.) fruits. *Food Chemistry*, 201, 307-314. doi: 10.1016/j.foodchem.2016.01.090

Wojdyło, A., Nowicka, P., Carbonell-Barrachina, Á. A., & Hernández, F. (2016). Phenolic compounds, antioxidant and antidiabetic activity of different cultivars of *Ficus carica* L. fruits. *Journal of Functional Foods*, 25, 421-432. doi: 10.1016/j.jff.2016.06.015

Wojdyło, A., Oszmiański, J., & Bielicki, P. (2013). Polyphenolic Composition, Antioxidant Activity, and Polyphenol Oxidase (PPO) Activity of Quince (*Cydonia oblonga* Miller) Varieties. *Journal of Agricultural and Food Chemistry*, 61(11), 2762-2772. doi: 10.1021/jf304969b

World Health Organization. (2018). Cancer, key facts. Consultada en diciembre de 2019. <https://www.who.int/news-room/fact-sheets/detail/cancer>