UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE

ESCUELA POLITÉCNICA SUPERIOR DE ORIHUELA

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

HYDROSOSTAINABLE ALMONDS: NUTRITIONAL, FUNCTIONAL AND SENSORY QUALITY PARAMETERS AND PRODUCT ACCEPTABILITY IN THE EUROPEAN MARKET

DOCTORAL THESIS

LEONTINA LIPAN

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Bachelor's degree in Food Engineering

Master's in Food Quality Management

Master's in Education

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Thesis for the Degree of Doctor by the

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Thesis presented by Leontina Lipan to qualify for Doctor degree by Universidad Miguel Hernández de Elche

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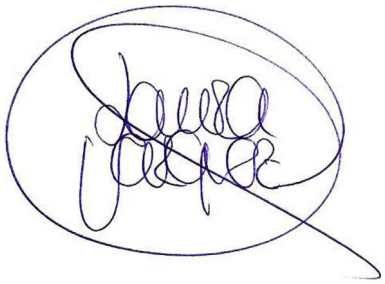

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Dr. Dña. Juana Fernández López, Catedrática de Universidad y Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche (UMH),

CERTIFICA:

Que la Tesis Doctoral titulada "***HydroSOStainable almonds: nutritional, functional and sensory quality parameters and product acceptability in the European market*"** de la que es autora la Máster en Gestión de la Calidad de los Alimentos **Dña. Leontina Lipan**, ha sido realizada bajo la dirección del **Dr. Ángel Antonio Carbonell Barrachina (UMH)** y la codirección de la **Dra. Laura Vázquez Araujo (Basque Culinary Center Innovation)**, actuando como tutora de la misma la Dra. Pilar Legua Murcia (UMH). Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA por tanto, apta para su exposición y defensa pública.

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

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Dra. Dña. Juana Fernández López

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Dedicată părinților mei care m-au învățat să prețuiesc şi să respect roadele pământului.

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QUALITY INDEX OF PUBLICATIONS

Category

This doctoral thesis titled "HydroSOStainable almonds: nutritional, functional and sensory quality parameters and product acceptability in the European market" is classified in a **compendium of publication category** to qualify as Doctor Degree by Universidad Miguel Hernández de Elche. The following publications have been included and their quality, in accordance with the 2019 edition of Journal Citation Reports® (JCR®), are shown:

1. Almond fruit quality can be improved by means of deficit irrigation strategies. 2019. ***Agricultural Water Management***, 217, 236-242. doi: 10.1016/j.agwat.2019.02.041
2. Nutrition quality parameters of almonds as affected by deficit irrigation strategies. 2019. ***Molecules***, 24 (14), 2646. doi: 10.3390/molecules24142646
3. Phytoprostanes and phytofurans–oxidative stress and bioactive compounds–in almonds are affected by deficit irrigation in almond trees. 2020. ***Journal of Agricultural and Food Chemistry***, 68 (27), 7214-7225. doi: 10.1021/acs.jafc.0c02268
4. How does water stress affect the low molecular weight phenolics of hydroSOStainable almonds? 2020. ***Food Chemistry***, 339, 127756. doi: https://doi.org/10.1016/j.foodchem.2020.127756
5. Sensory profile and acceptability of hydroSOStainable almonds. 2019. ***Foods***, 8 (2) 64. doi: 10.3390/foods8020064
6. Long-term correlation between water deficit and quality markers in hydroSOStainable almonds. 2020. ***Agronomy***, 10 (10), doi: https://doi.org/10.3390/agronomy10101470
7. Optimization of roasting conditions in hydroSOStainable almonds using volatile and descriptive sensory profiles and consumer acceptance. 2020. ***Journal of Food Science***, In press. doi: 10.1111/1750-3841.15481

Publication 1			
Lipan, L., Martín-Palomo, M.J., Sánchez-Rodríguez, L., Cano-Lamadrid, M., Sendra, E., Hernández, F., Burló, F., Vázquez-Araújo, L., Andreu, L., Carbonell-Barrachina, Á.A. (2019). Almond fruit quality can be improved by means of deficit irrigation strategies. ***Agricultural Water Management***, 217, 236-242. doi: 10.1016/j.agwat.2019.02.041			
Published	20 May 2019	**ISSN**	0378-3774
Publisher	Elsevier Science BV, PO Box 211, 1000 AE Amsterdam, Netherlands		
Research Domain	Water Resources, Agronomy		
JCR® Category	Water resources		
Quartile 2019	Q1	**Rank**	10/94
Impact Factor	4.021	**5-year Impact Factor**	4.469

Publication 2			
Lipan, L., Moriana, A., López-Lluch, D.B., Cano-Lamadrid, M., Sendra, E., Hernández, F., Vázquez-Araújo, L., Corell, M., Carbonell-Barrachina, Á.A. (2019). Nutrition quality parameters of almonds as affected by deficit irrigation strategies. ***Molecules***, 24 (14), 2646. doi: 10.3390/molecules24142646			
Published	21 July 2019	**ISSN**	1420-3049
Publisher	MDPI AG St. Alban-Angale, 66 Basel, Switzerland 4052		
Research Domain	Chemistry, Multydiscplinar; Biochemistry & Molecular Biology		
JCR® Category	Chemistry, Multidisciplinary		
Quartile 2019	Q2	**Rank**	70/177
Impact Factor	3.267	**5-year Impact Factor**	3.589

Publication 3			
Lipan, L., Collado-González, J., Domínguez-Perles, R., Corell, M., Bultel-Poncé, V., Galano, J.M., Durand, T., Medina, S., Gil-Izquierdo, Á., Carbonell-Barrachina, Á.A. (2020). Phytoprostanes and phytofurans –oxidative stress and bioactive compounds– in almonds are affected by deficit irrigation in almond trees. ***Journal of Agricultural and Food Chemistry***, 68 (27), 7214-7225 doi: https://doi.org/10.1021/acs.jafc.0c02268			
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Research Domain	Food Science & Technology; Agriculture, Multidisciplinary; Chemistry, Applied		
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Quartile 2019	Q1	**Rank**	21/139
Impact Factor	4.192	**5-year Impact Factor**	4.289

Publication 4			
Lipan, L., Collado-González, J., Wojdyło A., Domínguez-Perles, R., Gil-Izquierdo, Á., Corell, M., Moriana, A., Cano-Lamadrid M., Carbonell-Barrachina, Á.A. (2020). How does water stress affect the low molecular weight phenolics of hydroSOStainable almonds? ***Food Chemistry***, 339, 127756. doi:https://doi.org/10.1016/j.foodchem.2020.127756			
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Research Domain	Food Science & Technology; Nutrition & Dietetics; Chemistry, Applied		
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Quartile 2019	Q1	**Rank**	6/139
Impact Factor	6.306	**5-year Impact Factor**	6.219

Publication 5			
Lipan, L., Cano-Lamadrid, M., Corell, M., Sendra, E., Hernández, F., Stan, L., Vodnar, D.C., Vázquez-Araújo, L., Carbonell-Barrachina, Á.A. (2019). Sensory profile and acceptability of hydrosostainable almonds. ***Foods***, 8 (2) 64. doi: 10.3390/foods8020064			
Published	12 February 2019	**ISSN**	0378-3774
Publisher	MDPI AG St. Alban-Angale, 66 Basel, Switzerland 4052		
Research Domain	Food Science & Technology		
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Quartile 2019	Q1	**Rank**	27/139
Impact Factor	4.092	**5-year Impact Factor**	n/a

Publication 6			
Lipan, L., Cano-Lamadrid M., Hernández, F., Sendra, E., Corell, M., Vázquez-Araújo, L., Moriana, A., Carbonell-Barrachina, Á.A. (2020). Long-term correlation between water deficit and quality markers in hydroSOStainable almonds. ***Agronomy***, 10 (10). doi: https://doi.org/10.3390/agronomy10101470			
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Impact Factor	2.259	**5-year Impact Factor**	n/a

Publication 7			
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Research Domain	Food Science & Technology		
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Quartile	Q2	**Rank**	54/139
Impact Factor	2.478	**5-year Impact Factor**	2.693

TABLE OF CONTENT

1. DOCTORAL THESIS STRUCTURE

This Doctoral Thesis has been structured in accordance with the current internal regulation of the Miguel Hernández University of Elche using the option for the Presentation of Doctoral Thesis by Compendium of Publications. Therefore, the structure includes:

- **Abstract/*Resumen***. The most relevant results and conclusions are described in this section (English and Spanish).
- **Introduction.** This part includes the state of the art of almond (*Prunus dulcis*) both in general and growth under deficit irrigation strategies and its nutritional and functional properties.
- **Objectives.** The key objective and specific goals are showed in this part.
- **Material and Methods.** This part comprises methodology, plant material, irrigation treatments, experimental conditions and the analytical methods used to reach the objectives of this research.
- **Publications**. The 7 publications used to develop this Doctoral Thesis are listed below:
  1. Almond fruit quality can be improved by means of deficit irrigation strategies. 2019. *Agricultural Water Management*, 217, 236-242. doi: 10.1016/j.agwat.2019.02.041
  2. Nutrition quality parameters of almonds as affected by deficit irrigation strategies. 2019. *Molecules*, 24 (14), 2646. doi: 10.3390/molecules24142646
  3. Phytoprostanes and phytofurans–oxidative stress and bioactive compounds– in almonds are affected by deficit irrigation in almond trees. 2020. *Journal of Agricultural and Food Chemistry*, 68 (27), 7214-7225. doi: 10.1021/acs.jafc.0c02268
  4. How does water stress affect the low molecular weight phenolics of hydroSOStainable almonds? 2020. *Food Chemistry*, 339, 127756. doi: https://doi.org/10.1016/j.foodchem.2020.127756
  5. Sensory profile and acceptability of hydroSOStainable almonds. 2019. *Foods*, 8 (2) 64. doi: 10.3390/foods8020064
  6. Long-term correlation between water deficit and quality markers in hydroSOStainable almonds. 2020. *Agronomy*, 10 (10) doi: https://doi.org/10.3390/agronomy10101470
  7. Optimization of roasting conditions in hydroSOStainable almonds using volatile and descriptive sensory profiles and consumer acceptance. 2020. *Journal of Food Science*, In press. doi: 10.1111/1750-3841.15481
- **Results and Discussion.** This section gathers the key results of each publication followed by a brief discussion.
- **Conclusions/*Conclusiones*.** This section includes the main conclusions reached with this doctoral thesis and the future research lines (English and Spanish).
- **References.** The present section contains all the literature used to write and justify this Doctoral Thesis following APA 6th edition.

2. ABBREVIATION

AA	Antioxidant Activity
$ABTS^{\bullet+}$	2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AEI	*Agencia Estatal de Investigación*
ANOVA	*Analysis of Variance*
CATA	Check All That Apply
CV	Cultivar
DAD	Diode Array Detector
DI	Deficit Irrigation
DP	Degree of Polymerization
$DPPH^{\bullet}$	2,2-diphenyl-1-picrylhydrazyl
ESI	Electrospray Ionization
ET	Evapotranspiration
FAMEs	Fatty Acids Methyl Esters
FEDER	*Fondo Europeo de Desarrollo Regional*
FID	Flame Ionization Detector
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalents
GC	Gas Chromatography
HCL	Hydrochloric acid
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography
HSD	Honestly Significant Difference
HS-SPME	Headspace Solid Phase Microextraction
JAR	Just About Right
LDL	Low Density Lipoprotein
MUFA	Monounsaturated Fatty Acids
MCI	*Ministerio de Ciencia e Innovación*
MeOH	Methanol
MS	Mass Spectrometry
PAs	Proanthocyanidins
PDA	Photodiode Detector
PhytoFs	Phytofurans
PhytoPs	Phytoprostanes
PUFA	Polyunsaturated Fatty Acids
QTof	Quadrupole and Tandem Time-Of-Flight Mass Spectrometry
RID	Refractive Index Detector
RDI	Regulated Deficit Irrigation
ROS	Reactive Oxygen Species
SFA	Saturated Fatty Acids
SI	Stress Integral
SDI	Sustained Deficit Irrigation
SPE	Solid Phase Extraction
SWP	Stem Water Potential
TPC	Total Phenolic Content
UHPLC	Ultra-High-Performance Liquid Chromatography
UPLC	Ultra-Performance Liquid Chromatography

3. ABSTRACT / *RESUMEN*

Abstract

Water is the most limiting factor in the Mediterranean Basin due to the rainfall shortage and higher rates of annual evapotranspiration (ET). Moreover, climate change is contributing to very frequent and severe drought day by day and agriculture sector needs maximum crop yield which is only feasible if optimum water supply is assured to maintain a normal physiological activity and transport processes. Among the different strategies to maintain the viability of the agricultural sector would be the introduction of drought-tolerant crops; or the implementation of water saving strategies, such as deficit irrigation (DI) that will seek adequate yields, maintaining, or even improving, the quality of the final product.

Therefore, the main aim of this Doctoral Thesis, meant to investigate the effect of different deficit irrigation (DI) strategies on yield and physicochemical, functional, and sensorial parameters of raw and roasted almond (*Prunus dulcis* Mill. *cv.* Vairo), establishing those quality markers required further to identify/certify whether an almond is hydroSOStainable (growth under deficit irrigation strategies) or not; as well as evaluating the sensory profile and international consumers opinion regarding the hydroSOStainable almonds acceptance.

The study was carried out along three seasons (2017-2019), in which, the following irrigation treatments were developed: (i) control treatment (covering 100% of the crop ET), (ii) 2 regulated deficit irrigations (RDI) treatments with a period of water stress during the kernel filling phase (with different stress levels depending on the stem water potential T2=-1.50 MPa and T3=-2.0 MPa) and full irrigated conditions for the rest of the year, and (iii) a sustained deficit irrigation (SDI) in which a lower amount of water was applied during the whole season (T4).

Regarding the effect of DI on agronomical, morphological, and functional parameters of almonds, the results showed that almonds from moderated RDI (T2) were characterized by a redder color, a higher fat, K, glucose and total phenolic content (TPC), together with a greater content of individual phenolic compounds and proanthocyanidins (PAs), degree of polymerization, antioxidant activity, unsaturated fatty acid and total volatile content. Moreover, the TPC was positively correlated with the stress integral (SI), increasing with the water stress in plant. The phytoprostanes (PhytoPs) and phytofurans (PhytoFs) were quantified only in almonds growth under deficit irrigation conditions because in T1 these compounds were below de limit of quantification.

In relation to the sensory profile of almonds and the international consumers acceptance, preference, and willingness to pay it was observed that (i) T2 almonds were characterized by an intense color, and intensity of both sweetness and hardness attributes; and that (ii) T2 almonds were the most liked by both nationalities due to

the sweetness, almond flavor, and crispiness. Consumers are now more aware than ever on the importance of the optimization of water as they were willing to pay a higher price for the hydroSOStainable almonds, which means higher incomes and benefits for farmers.

Additionally, it was observed that water stress accumulated during the studied seasons significantly affected the agronomical and quality characteristics of hydroSOStainable almonds displaying positive relationship with some parameters such as dry weight, color coordinates (*L*a*b**), minerals (K, Fe, and Zn), organic acids (citric acid), sugars (sucrose, fructose and total sugars), antioxidant activity and fatty acids (linoleic, PUFA, SFA, PUFA/MUFA, among others). In contrast, the water stress in almonds showed a negative correlation with yield, water activity, weight, size, some minerals (Ca and Mg), fatty acids (oleic acids, oleic/linoleic ratio, MUFA and PUFA/SFA) and sensory attributes (size, bitterness, astringency, benzaldehyde and woody). In summary, K, Fe, Zn, sucrose, fructose, total sugars, antioxidant activity and the fatty acids can be considered important markers for hydroSOStainable almonds detection.

Moreover, when almonds were processed it was observed that a heat treatment of 170 ºC during 10 min in a convection oven were the optimum roasting conditions from an aromatic, descriptive and affective point of view for the *cv.* Vairo almonds. Besides, deficit irrigation led to sweeter almonds with higher intensity of roasted almond and nutty notes, and with greater content of aroma compounds comparing to the control.

According to the results obtained in this research work, it can be concluded that controlled deficit irrigation strategies can be considered an important tool to reduce the water consumption in almond crop, with significant improvements in fruit bioactive compounds with potential beneficial effect on human health. Moreover, it has been proved that controlling the stress in almond trees can increase both the irrigation water productivity and the farmers profit; by producing environmentally friendly products, better valued by the consumers. In this scenario, government and industry actions might emphasize on providing the right information to the consumers regarding hydroSOStainable products, while agricultural sector might produce these foods helping to combat the water scarcity worldwide.

Resumen

El agua es el recurso natural más limitante en los agro-ecosistemas mediterráneos, consecuencia de una climatología caracterizada por la escasez e irregularidad de precipitaciones y unas altas tasas de evapotranspiración. Además, el cambio climático está dificultando aún más si cabe la gestión sostenible de los recursos hídricos generando una mayor incertidumbre y dificultad para mantener la sostenibilidad y competitividad de los sistemas productivos. Dentro de las diferentes estrategias para mantener la viabilidad del sector agrícola estarían la introducción de cultivos tolerantes a la sequía; o la implementación de estrategias de ahorro de agua, tales como el riego deficitario (RD) que permitan alcanzar unos rendimientos adecuados, manteniendo, o incluso mejorando, la calidad del producto final.

Por todo ello, el principal objetivo de esta tesis doctoral fue la evaluación de diferentes estrategias de riego deficitario en el rendimiento y los parámetros fisicoquímicos, funcionales y sensoriales de la almendra (*Prunus dulcis* Mill. *cv.* Vairo) cruda y tostada estableciendo aquellos marcadores de calidad que permitieran identificar/certificar si una almendra es hidroSOStenible (cultivada bajo estrategias de riego deficitario) o no; así como la evaluación del perfil sensorial y de la opinión de los consumidores internacionales con respecto a la aceptación de las almendras hidroSOStenibles.

El estudio se llevó a cabo durante tres campañas consecutivas (2017-2019) en las cuales se desarrollaron los siguientes tratamientos de riego: (i) tratamiento control (que cubre el 100% de la ET del cultivo), (ii) 2 tratamientos de riego deficitario controlado (RDI) con un período de estrés hídrico durante el llenado del grano (con diferentes niveles de estrés dependiendo del potencial hídrico del tallo T2 = -1.5 MPa y T3 = -2.0 MPa) y condiciones de riego completas para el resto del año, y (iii) un riego deficitario sostenido (SDI) en el que se aplicó una menor cantidad de agua durante toda la temporada (T4).

En cuanto a los efectos en los parámetros agronómicos, morfológicos y funcionales de las almendras los resultados mostraron que las almendras obtenidas bajo una estrategia de RDI moderado (T2) se caracterizaron por un color más rojo, mayor contenido de grasa, K, glucosa, contenido de fenoles totales (TPC), un mayor contenido de compuestos fenólicos y proantocianidinas individuales, un mayor grado de polimerización, actividad antioxidante, ácidos grasos insaturados y volátiles. Además, se observó un incremento muy importante en el contenido de fitoprostanos (PhytoPs) y fitofuranos (PhytoFs), siendo cuantificados solo en almendras cultivadas en condiciones de déficit hídrico, ya que en el tratamiento T1 los contenidos se encontraron por debajo del límite de cuantificación.

En cuanto al perfil sensorial de las almendras, la aceptación, preferencia y disponibilidad a pagar por parte de los consumidores internacionales, se observó que las almendras T2 presentaban un color más intenso, mayor intensidad de dulzor y dureza, siendo además este tratamiento el que obtuvo una mayor aceptación debido a su dulzor, sabor a almendras y crujibilidad según los consumidores. Además, estos se mostraron dispuestos a pagar un precio más alto por las almendras hidroSOStenibles, lo que significa mayores ingresos y beneficios para los agricultores.

Por otra parte, se pudo observar que el déficit hídrico acumulado a largo de las campañas de estudio afectaba significativamente a las características agronómicas y de calidad de las almendras hidroSOStenibles que manifestaron una correlación positiva con algunos parámetros tales como: peso seco, coordenadas de color (*L* a* b**), minerales (K, Fe y Zn), ácidos orgánicos (ácido cítrico), azúcares (sacarosa, fructosa y azúcares totales), actividad antioxidante y ácidos grasos (linoleico, PUFA, SFA, PUFA / MUFA, entre otros). Por lo contrario, el estrés hídrico mostró una correlación negativa con el rendimiento, la actividad del agua, el peso, el tamaño, algunos minerales (Ca y Mg), ácidos grasos (ácido oleico, relación oleico/linoleico, MUFA y PUFA / SFA) y atributos sensoriales (tamaño, amargor, astringencia, benzaldehído y amaderado).

Tras el proceso de tostado, se observó que un tratamiento térmico de 170 ºC durante 10 min en un horno de convección eran las condiciones óptimas de tostado desde un punto de vista aromático, descriptivo y afectivo para las almendras de la variedad Vairo. Además, el riego deficitario dio lugar a almendras más dulces con mayor intensidad de notas a almendra tostada, a frutos secos y con un mayor contenido de compuestos aromáticos en comparación con el control.

De acuerdo a los resultados obtenidos en este trabajo de investigación, se puede concluir que las estrategias de riego deficitario pueden considerarse una buena herramienta para reducir el consumo de agua en el cultivo de la almendra, dando lugar a frutos con mejoras significativas en sus compuestos bioactivos. Además, se ha demostrado que controlando el estrés hídrico en los almendros se puede aumentar tanto la productividad del agua como los beneficios para los agricultores al producirse una almendra más sostenible, sabrosa y mejor valorada por los consumidores. Dicho esto, las distintas administraciones y la industria deberían enfatizar sus acciones para proporcionar la información correcta a los consumidores con respecto a los productos hidroSOStenibles, mientras que el sector agrícola debería apostar por la producción de estos alimentos para ayudar a reducir la escasez de agua en el mundo.

4. INTRODUCTION

4.1. Water scarcity and climate change

The global population is yearly increasing being now around 7.8 billion (2020) and is projected to reach 9.5 billion by 2050. As a result, world food demand will surge, which means that population will need three times the basic calories eaten now (Mansvelt, 2011). Unfortunately, water, the basis for our food production, it is a finite resource and currently under heavy restrictions which create a tension among users and an extreme pressure on the environment (FAO, 2017). Different sectors compete with agricultural sector for water use and a growing number of the world regions are reaching alarming levels of water stress. At the same time, climate change also have a significant impact over the water scarcity by affecting rainfall patterns, the variability of precipitations, temperatures and agricultural production (FAO, 2017). This last due to the substantial changes on crop phenological development such as crops cycle shortening, earlier flowering, higher heat and waters stress (Gabaldón-Leal et al., 2017; Lizaso et al., 2018). **Figure 1** illustrates the current water stress levels around the globe, being Spain a country with high-to extremely high levels of water stress (WRI, 2015).

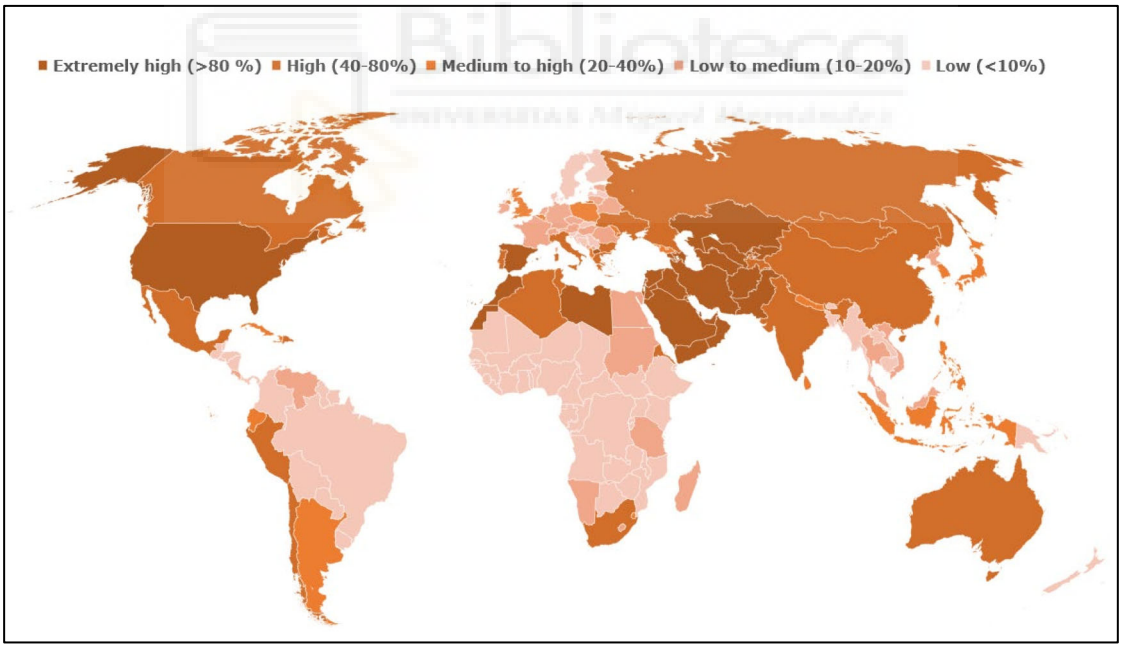


Figure 1. Water stress by country in 2020 (projections are based on a business-as-usual scenario from the data provided by World Resource Institute)

Climate variability and water scarcity affects both social and economic sectors which means that agricultural sector require rapid water management programs able to cope with the threat of the natural resource base sustainability (FAO, 2007).

As shown, Mediterranean regions, particularly Spain, are the most affected areas by water stress, being aridity and low water resources the main characteristics of these areas. It is predicted an increase of 1-fold (from 3.93 in 2020 to 4.22 in 2040) water stress values in Spain by 2040 (WRI, 2015). This change will happen mainly due to the increase in average air temperature (>2-4 ºC), heat waves events, decrease in precipitations (~30%), risks of drought and decrease in crop yields and biodiversity losses (EEA, 2019). The Mediterranean agriculture is characterized by dry-hot summers and low precipitations during the other seasons. Thus, to assure the crop yields and to avoid water deficit in plant, extra water, through irrigation is needed (Goldhamer, Viveros, & Salinas, 2005). However, as the water in this area is reaching worrying limitation, Mediterranean agriculture must adopt a sustainable water management by implementing improved, innovative, and precise irrigation practices to minimize the impact in crop yield and fruit quality.

4.2. Almond crop as a suitable alternative under water scarcity scenarios

As explained before, climate change affects not only the crops yield but also the rest of food chain such as storage, transport conditions and product transformation. This effects being more severe in Mediterranean countries, particularly southern regions in which climate change and water scarcity lead to a gradual deterioration of rural area, reduction productivity of agro-ecosystems, and even land abandonment (IPCC, 2018). For this reason, the Europe Union standard policies incorporated as the main purpose in the new Common Agricultural Policy 2021-2027, the promotion of practices designed to adapt to the climate change situations. Under this circumstances, the use of adapted crops to arid and semiarid environments, the use of tolerant cultivars (*cvs.*) to drought, and agronomic strategies to increase the water use efficiency in agriculture must be seriously considered (Iglesias & Garrote, 2015).

The almond [*Prunus dulcis* a (Mill.) D. A. Webb], is the top produced tree nut, accounting for 31% of the world share, followed by walnut (21%), cashews (17%), pistachios (14%) and hazelnuts (12%) (INC, 2019). Almond is also the main nut crop cultivated in Spain and is it well-known its resistance to drought together with relevant prices in the last years, making almond orchards a good choice to deal with water scarcity issues. The almond belongs to *Rosaceae* family and is one of the oldest cultivated crops in the world. It is currently produced throughout the world, in regions with a Mediterranean, warm, and arid climate.

The world production of in shell almonds (**Figure 2**) is currently dominated by the United States of America (1872500 t), especially California, followed vary far by Spain and Iran with 339033 t and 139029 t, respectively (FAOSTAT, 2018). However, in terms of yield, Australia (1892 kg ha^{-1}) is the second country worldwide, because

is following Californian (4245 kg ha^{-1}) irrigation strategy system which involves the application of full irrigation to achieve the maximum yields (Expósito & Berbel, 2020); while in Spain most of the cultivated area is under rainfed conditions with lower yield values (515 kg ha^{-1}). In Spain, the regions with the largest area of cultivated almond trees is Andalusia (212223 ha), followed by Castilla La-Mancha (133153 ha), Valencian Community (92641 ha), Region of Murcia (79921 ha), Aragon (77572 ha), and Catalonia (23573). However, in terms of the almond production Andalusia (111877 t) still leading the rank, but is followed by Aragon (63235 t), Castilla La-Mancha (53201 t), Valencian Community (40875 t), Region of Murcia (25855 t), and Catalonia (23573 t).

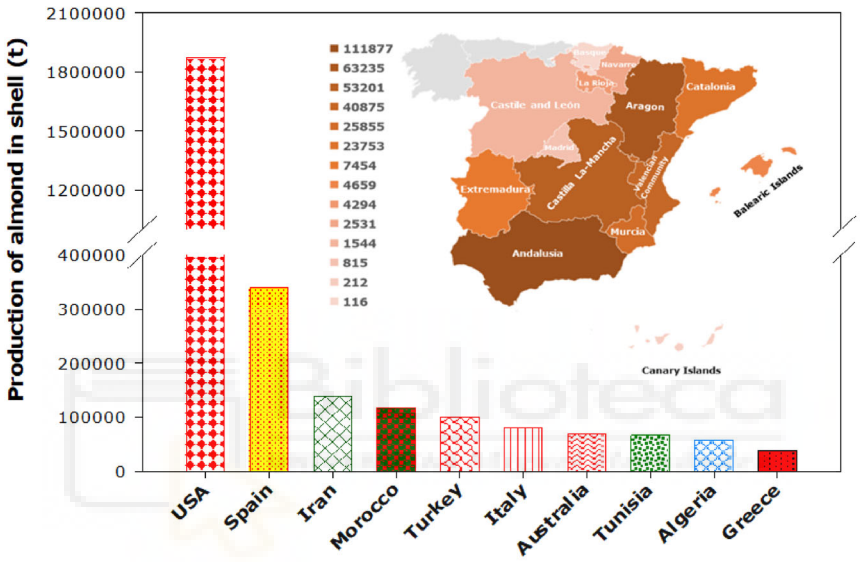


Figure 2. In shell almonds production in tons, data provided by FAOSTAT (2018)

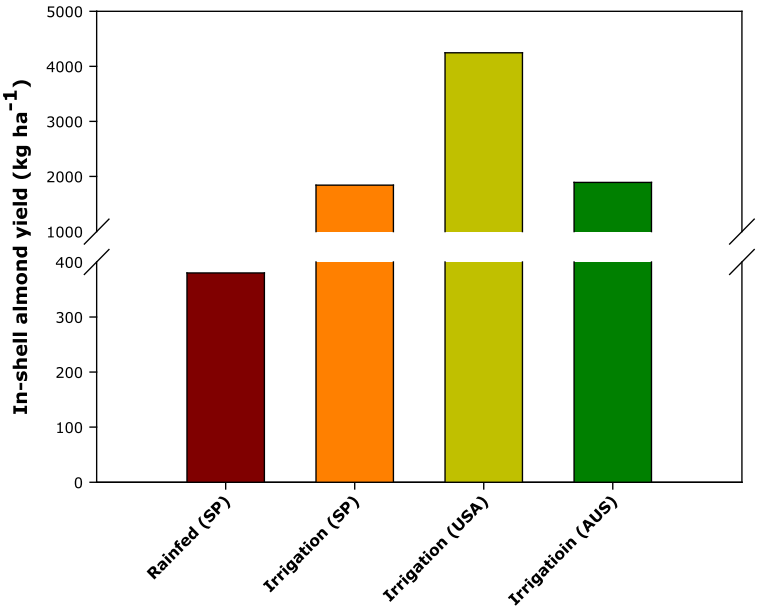


Figure 3. In shell almonds yield, data provided by (FAOSTAT, 2018; MAPA, 2019)

According to Food and Agricultural Organization of the United Nations, the area covered with almonds trees in Spain (657768 ha) was in 2018, 1.5-fold higher than United States of America (USA 441107 ha), but as seen in **Figure 2**, USA produces 4.5-fold more almonds than Spain (FAOSTAT, 2018). This occurs mainly due to water availability, because Spanish almond orchards are mainly located in arid regions where this crop is traditionally associated with marginal areas as South Spain (Arquero, 2013). For instance, in Spain a great area of almond is cultivated under rainfed conditions and in spite of being a drought tolerant specie, water availability is the most limiting factor to reach maximum crop yields (Goldhamer & Fereres, 2017; MAPA, 2019). In **Figure 3**, it can be observed the differences in almond production in rainfed *versus* irrigation conditions in Spain and USA.

Recently, the almond cultivated surface has significantly increased both in USA and Spain (**Figure 4**), especially in irrigated areas previously occupied by other crops (CAPDR, 2016). This occurs mainly due to the relevant increases in prices, between 6.0 and 8.5 € kg^{-1} during 2014-2016, and stabilized at ~6 € kg^{-1} farmer price after 2016 up to now (Junta de Andalucía, 2019)

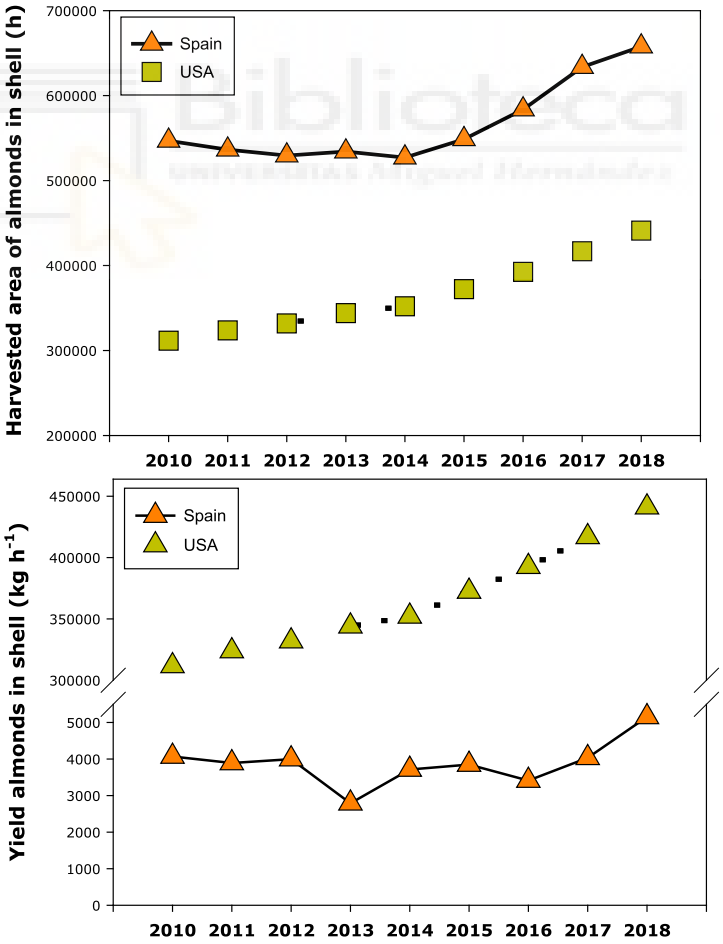


Figure 4. Almonds harvested area and yield in Spain and USA, data provided by FAOSTAT (2018)

As observed, the cultivated surface augmented yearly in Spain, nevertheless the crop yield only in the last years started to increase, which explains that this crop has been progressively introduced under irrigation, following agronomic practices previously designed by countries with maximum crop yields. Considering the water consumption growth, climate change, and water restrictions already existed in southern Spain, it is necessary to consider urgently viable water management strategies to do an efficient use of water in Mediterranean areas.

4.3. Deficit irrigation strategies in almond crop

Deficit irrigation strategies are agronomic practices, highly recommended in trees, which help to achieve sustainable and competitive almond yields under water limitation circumstances without committing the fruit quality (Fereres & Soriano, 2007; Gutiérrez-Gordillo, Durán-Zuazo, & García-Tejero, 2019). Deficit irrigation strategies (DI) refers to an agricultural practice in which the irrigation water is applied below the crop evapotranspiration (ET=the sum of humidity loss through soil evaporation and plant transpiration). Regulated deficit irrigation (RDI) was developed in 1970s and is a strategy in which the water is reduced or even eliminated in a certain period of the growing cycle in which the plant is less sensitive to the water stress (Du, Kang, Zhang, & Davies, 2015; Egea et al., 2013). Beside increasing the water productivity, this strategy, also raise the profitability for growers because RDI is also used to control the excessive vegetative growth, helping in this way to reduce costs on pruning and to guide the nutrients through fruit growing with impact on yield and quality (Doll, 2014). Sustained deficit irrigation (SDI) consists of applying a less and uniform amount of water (below crop ET) during the whole season, rather than focusing only in one period like in RDI (Goldhamer et al., 2005). In this way, a progressive stress in plant is created throughout the season by not refilling the root zone completely while irrigation (Lipan et al., 2018).

The optimum water requirements on almond ranges between 9,000-13,500 m^3 ha^{-1}, depending on different agronomical factors (cultivar, rootstock, canopy size, tree spacing and location) (Goldhamer & Girona, 2012; López-López et al., 2018). Recently, it was reported that the optimum water requirements in almonds growth under climatic conditions of the Guadalquivir river (Seville, Spain) are ~8000 m^3 ha^{-1} (García-Tejero, Gutiérrez Gordillo, Souza, Cuadros-Tavira, & Durán Zuazo, 2019; Manuel López-López et al., 2018). Considering the optimum water requirements in almonds and the water allocation in southern Spain (~3500 m^3 ha^{-1}), the introduction of almond tree as an alternative crop would only be reasonable if irrigation productivity can be improved under water scarcity scenarios by means of DI strategies, minimizing the crop yield and without affecting the fruit quality.

Saying this, to achieve success by using DI, important factors such as: irrigation strategy and crop phenology must be considered. The almond phenology consists of 3 very differentiate stages (**Figure 5**):

- Stage 0-dormant Bloom.
- Stage I-fruit growth and vegetative development.
- Stage II-kernel filling with dry matter accumulation and pre-harvest.
- Stage III-post-harvest in which reserves accumulation and buds differentiation occurs before leaf-fall (Doll, 2014).

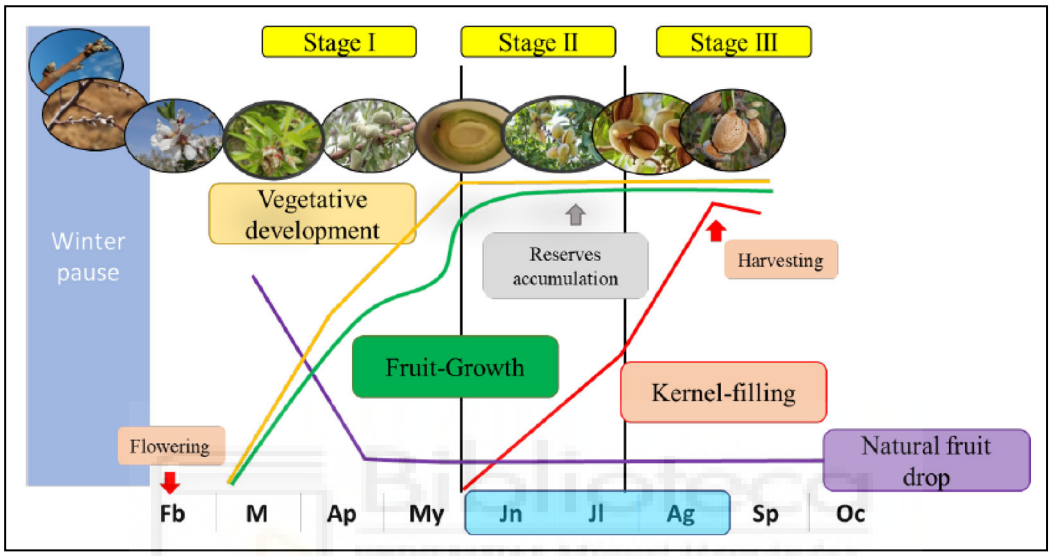


Figure 5. Almond tree growth and development (Gutiérrez-Gordillo et al., 2020)

In the almond crop, the less sensitive phenological period to water stress is the stage II, which coincide with the kernel filling and with the summer months when the evaporative demand reach the highest levels (Doll, 2014; Egea et al., 2013; Goldhamer et al., 2005).

As observed, in the Mediterranean countries the almond development starts in February with bloom and vegetative development and finish in August-September with the harvest period (Goldhamer & Girona, 2012). These periods are highly dependent on the cultivar (*cv.*), for instance the earliest blooming *cv.* is Desmayo Llargueta which bloom at the beginning of February, followed by *cvs.* Marcona, Marta and Nonpareil (7 days later), and *cvs.* Guara, Lauranne, Vairo and Glorieta (more than 20 days later than *cv.* Llargueta). On the other hand, harvesting time also change within each cultivar, in this case, *cvs.* Guara and Marta are harvested at the beginning of August (3rd and 6th August), *cvs.* Vairo and Lauranne mid-August (13th), while *cvs.* Llargueta and Marcona at the end of August (28th) and beginning of September (5th). The flowering and vegetative growth is significantly influenced by the stored reserves from the previous seasons during pre-harvest and post-harvest

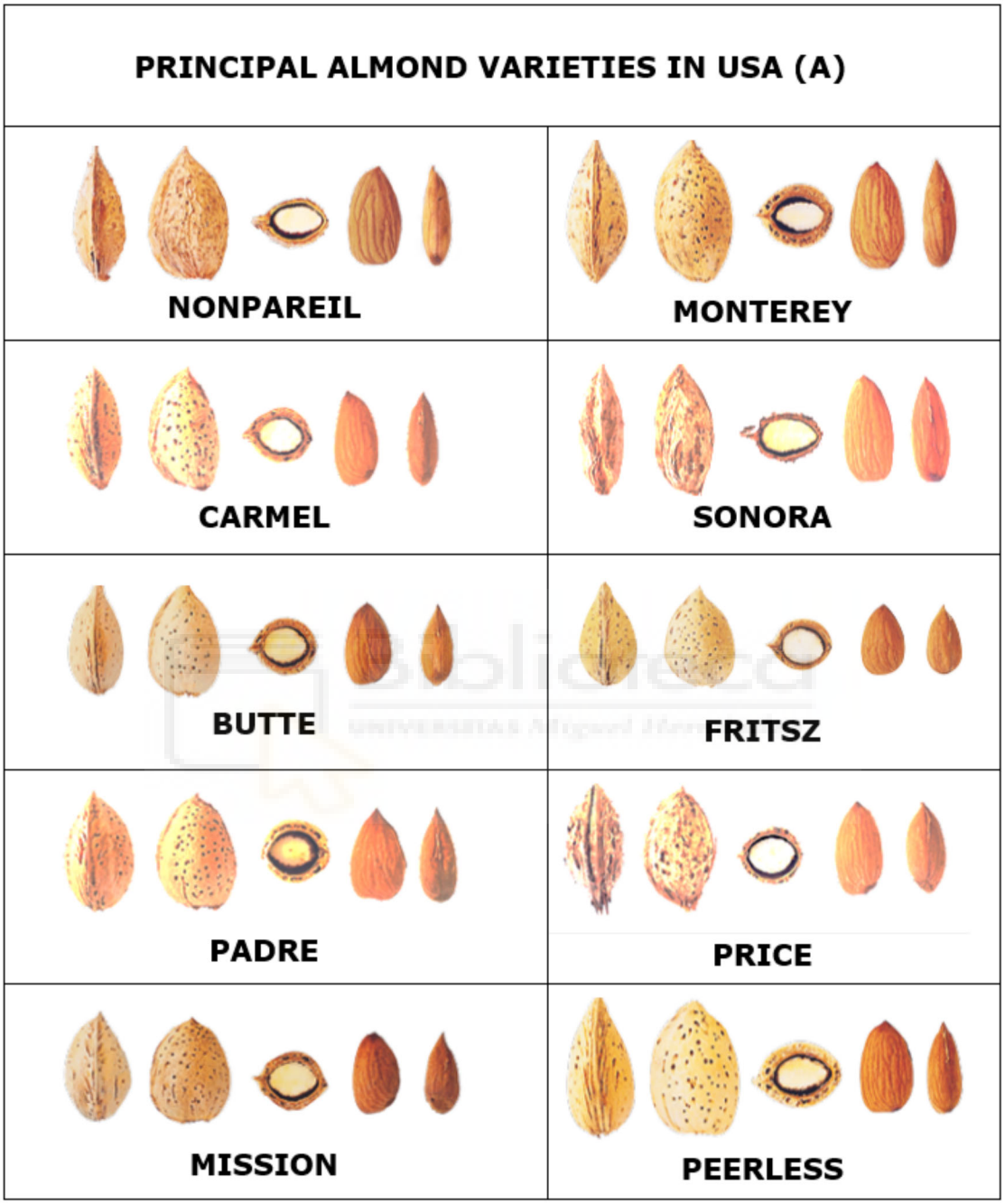
because in this moment the accumulation of carbohydrates occurs and directly affect the yield in the next year (Esparza, DeJong, & Weinbaum, 2001; Esparza, DeJong, Weinbaum, & Klein, 2001).

Many authors have already studied the RDI strategy in different stages of almond growing cycle and depending on the phenological phase the RDI can have a negative or positive effect on yield and fruit quality. For instance, (i) applying RDI during **Stage I**, might lead to small fruits, fruits abortion and poor canopy development which will negatively affect the photosynthetic capacity; (ii) similarly, reducing water irrigation through RDI in **Stage III** will affect the fruit set and vegetative growing in Stage I of the following season due to a lower accumulation of carbohydrates, and consequently will affect the final crop yield (Goldhamer & Viveros, 2000; Romero, Botia, & Garcia, 2004); (iii) finally, imposing the RDI during the kernel filling phase or **Stage II** a great adaptation of almond to water stress was reported (García-Tejero et al., 2019; Goldhamer et al., 2005; Gutiérrez-Gordillo et al., 2019; Romero et al., 2004). Thus, flowering and fruit set is highly dependent on the previous season, while the fruit growth is more dependent on the water and nutrients from the current season (Esparza, DeJong, & Weinbaum, 2001; Goldhamer & Girona, 2012).

4.4. Almond cultivars

As showed before, USA and mainly California is the leader in almond production followed by Spain which is the first almond producer in Europe. California accounts ~30 almond varieties and 10 of them represents more than 70% of total production, being Nonpareil the cultivar with the widest range of uses among marketing classification (Almond Board of California, 2020). Regarding Spanish almonds, the most widely cultivated varieties are: Marcona, Desmayo Largueta and Comuna (a mix of different varieties) which accounts for 45% of the Spanish current production in rainfed conditions, while under irrigation, Marcona has a minimal implantation, gaining importance modern varieties such as Ferragnes, Ferraduel and Garrigues. Usually Spanish varieties are more appreciated by Spanish industry than the American cultivars, particularly *cv.* Marcona, which is best paid due to its appreciable sensory properties and low production rate (Vázquez-Araújo, Enguix, Verdú, García-García, & Carbonell-Barrachina, 2008). For instance, *turrón* (a traditional Spanish confectionery) industry, only use Spanish cultivars (Marcona, Largueta and Comuna) for the *turrón* with Protected Geographical Indication, while the American varieties can only be used in those non-protected ones (Vázquez-Araújo et al., 2008). Although the Spanish varieties are grown in the vicinity of *turrón* area, their prices are significantly higher than that of the American varieties, mainly due to the sensory

characteristics such as *turrón* flavor and texture, and lower production. **Figure 6** lists the most important cultivars in these 2 countries.



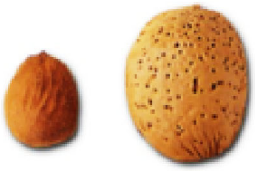
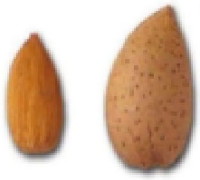
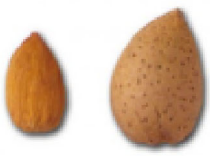
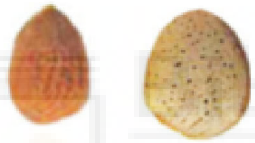
PRINCIPAL ALMOND VARIETIES IN SPAIN (B)			
MARCONA		DESMAYO LARGUETA	
"COMUNA"			
VAIRO		GUARA	
SOLETA		LAURANNE	
BELONA		MARTA	
RAMILETTE		CONSTANTÍ	
FERRADUEL		TARRACO	
FERRAGNÈS		MARINADA	

Figure 6. American (A) and Spanish (B) main varieties (Almond Board of California, 2020; IRTA, 2017; Viveros Caliplant, 2020)

4.5. Almond anatomy and its nutritional, functional, and sensorial traits

The almond is a drupe fruit (**Figure 7**), which means that is formed both by fleshy (mesocarp) and stony (endocarp or shell) layers that surround the kernel which is the only edible part (Grundy, Lapsley, & Ellis, 2016). Depending on the variety, the shell can be very hard (*cvs*. Vairo, Guara, Marta), needing tools to break it, semi hard (Lauranne); or so soft (*cv*. Nonpareil) that it breaks with the pressure of the fingers. The kernel, or the edible part, is protected by a reddish-colored integument (skin) that, although it is edible and contains most of the polyphenols, is often eliminated during the manufacturing process of almonds.

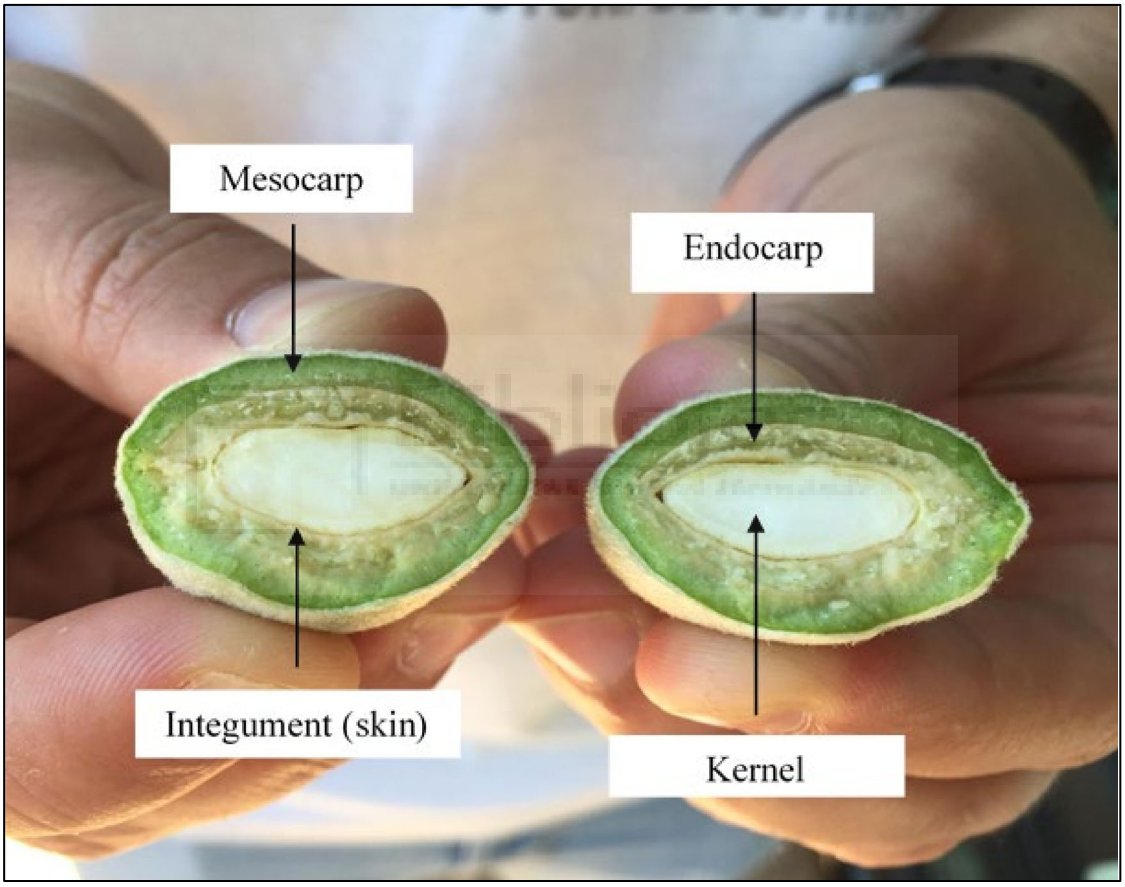


Figure 7. Almond structure in Stage II-Kernel filling phase (~15 May in *cv*. Vairo, Sevilla, Spain)

Almond is the most consumed tree nut in the world accounting for 30% of the total tree nut estimated consumption, being Europe the biggest consuming region for nuts in general and USA, India, Germany and Spain the main consumers of almonds (INC, 2019). As observed, Spain is the 4th country in almond consumption ranking worldwide (~67000 t) with an increase of 1.9% for the almond consumption at the end of 2018 (INC, 2019; MAPA, 2018). Almond kernels are extensively consumed in the Mediterranean diet either as a snack or as ingredient for confectionery (mainly

turrón) and bakery due to their high nutrition value and distinctive flavor (Xiao et al., 2014). The estimated average intake was around 0.26 kg/person/year while the average cost was 3.07 €/person/year (MAPA, 2018).

Almonds are nuts associated with healthy snacks due to their chemical composition (**Table 1**) which includes: protein with a high digestibility coefficient, monounsaturated fatty acids, dietary fiber (in insoluble: soluble ratio of 4: 1), vitamin E, riboflavin and important minerals (K, Mg, P, Mn, Cu, Fe, and Zn). The chemical composition depends mainly on the cultivar and post-harvest handling (Gama, Wallace, Trueman, & Bai, 2018). For example, the fat content in commercial and local almond varieties from different countries (Egypt, India, Iran, Italy, Portugal, Spain, Tunisia and Turkey) ranged between 44 and 61% (Yada, Lapsley, & Huang, 2011). Studies on local almond varieties but non-commercial also showed different percentages on lipid content these ranging from 25-61% in Turkey, 43-63% in Afghanistan, 40-67% in Spain and 49-66% in the USA (California). Regarding the lipid profile, the five main fatty acids in decreasing order are: oleic (62-80%), linoleic (22-26%), palmitic, stearic and palmitoleic. The main free amino acid of almonds is asparagine with a content of 20-50% of the total of amino acids. Finally, the carbohydrates found in almonds are sugars and polysaccharides with and without starch (cellulose and hemicellulose), with a great variability depending on the cultivar. The latter are not digestible and therefore do not provide energy to the body. However, their physiological effect is beneficial for the human organism since together with oligosaccharides, pectins, gums, waxes and lignin form the "dietary fiber", essential for a healthy diet (Yada et al., 2011).

Table 1. Chemical composition of raw almond in different cultivars (Barreca et al., 2020; King et al., 2019; Lipan et al., 2020; Lipan et al., 2019; Summo et al., 2018)

	Moisture	Fat	Protein	Carbohydrates	Ash	MUFA	PUFA	SFA
				%				
Largueta	5.68	50.6	19.7	20.9	3.20	63.8	26.2	10.2
Marcona	6.10	52.7	21.2	22.3	3.41	69.8	21.3	8.97
Ferragnès	6.50	50.1	18.4	22.4	2.86	69.2	21.6	9.20
Guara	5.30	47.3	20.5	23.9	3.02	67.7	22.0	10.2
Texas	6.45	48.8	20.1	21.7	2.92	68.4	22.8	8.80
				g 100 g^{-1}				
Vairo	3.80	48.8	24.7	-	2.93	23.6	8.12	4.00
Marta	-	-	-	-	3.26	24.3	6.35	2.96
Guara	-	-	-	-	3.36	22.0	5.69	2.99
Lauranne	-	-	-	-	3.37	21.5	6.70	2.95
Nonpareil	4.00	47.6	20.9	23.7	2.80	30.7	10.6	3.50
Carmel	4.10	48.2	20.6	22.6	2.80	28.9	13.0	3.60
Bute	4.70	50.0	20.7	-	2.80	29.4	13.9	4.10
Sonora	4.10	50.2	22.4	-	3.00	31.4	12.4	3.90

Moreover, the almond skin contains 70-100% of the total phenols however, most of the time is eliminated from the almond manufacturing process (Monagas, Garrido, Lebrón-Aguilar, Bartolome, & Gómez-Cordovés, 2007). Both non-flavonoids

and flavonoids has been identified in almonds, the former including vanillic, and *p*-hydroxybenzoic acids and the latter including compounds belonging to flavanols being proanthocyanidins the most abundant [(+)catechin, (-)-epicatechin, procyanidins], flavonols (3-O-glucosides, -galactosides, and -rutinosides of quercetin, kaempferol, and isorhamnetin and its corresponding aglycones), dihydroflavonols (morin and dihydrokaempferol) and flavanones (naringenin-7-*O*-glucoside, eriodictyol-7-*O*-glucoside, and eriodictyol-7-*O*-galactoside, and their corresponding aglycones) (Milbury, Chen, Dolnikowski, & Blumberg, 2006; Monagas et al., 2007; Sang et al., 2002).

Finally, almond also contain phytoprostanes (PhytoP) and phytofurans (PhytoF) which are compounds derived through non-enzymatic pathway from α-linolenic acid and by the enhanced formation of reactive oxygen species (ROS) (Imbusch & Mueller, 2000). The PhytoP and PhytoF production is increased by ROS, because although in normal growing conditions plants are continuously generating ROS, under conditions of biotic and abiotic stress this process significantly increase (Cruz De Carvalho, 2008; Thoma et al., 2003; Zoeller et al., 2012).

A daily consumption of almonds can help to maintain a healthy lifestyle essential to prevent chronic diseases. Thus, the increase in almond consumption may be associated to their nutrients and phytochemicals which are strongly related to cardiovascular benefits (Alasalvar & Bolling, 2015). The epidemiological studies and clinical trials carried out so far have reported positive effects regarding the consumption of almonds against cardiovascular diseases, obesity, hypertension, diabetes which might be related to important bioactive compounds that almonds contain: oleic acid, linoleic, linolenic, polyphenols, proanthocyanidins, phytoprostanes, phytofurans, vitamin E, sterols, etc. (Gulati, Misra, & Pandey, 2017; Jenkins et al., 2002). For example, it was observed that a daily consumption of almonds improved the concentration of α-tocopherol in plasma and decreased the cholesterol levels (Jambazian, Haddad, Rajaram, Tanzman, & Sabaté, 2005). Other studies showed that the antioxidants in almonds help to prevent oxidative stress and therefore the diseases that occur with it (Mandalari et al., 2011). Polyphenols (flavonoids and proanthocyanidins), phytoprostanes and phytofurans are compounds with anti-oxidative and anti-inflammatory effect in human body which help to protect the cells against damage produced under oxidative stress conditions and have a key role in the neuronal function regulation (Barden et al., 2009; Jenkins et al., 2002; Minghetti et al., 2014). Additionally, the consumption of almonds helped to reduce plasma triglycerides and LDL cholesterol (7 g / day of almonds reduces the concentration of LDL cholesterol by 1%). Moreover, helped to increase the resistance of LDL cholesterol to oxidation, and to increase the concentration of HDL cholesterol

in humans (Jenkins et al., 2002). Other studies showed that frequent consumption of almonds reduces the risk of cardiovascular diseases, and the cardioprotective effect was associated to a lower atherogenic profile of plasma and to the reduction in glycemic excursion (Cesarettin Alasalvar & Shahidi, 2008). Finally, it was observed that people who consume nuts regularly have a lower waist circumference and an improved metabolic profile (Barreca et al., 2020).

All these scientific studies led the Food and Drug Administration of the US to publish in 2003 a qualified health claim associated to nuts consumption (**almonds**, Brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pecans, pine nuts, pistachio nuts, and walnuts), declaring 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*" (FDA, 2017). For instance, by eating 42.5 g of almonds account for approximately 246 kcal; 9 g of protein [18% of the daily value (DV)]; 21 g of fat from which 13 g monounsaturated fatty acids (MUFAs) 92% of the DV, 5 g polyunsaturated fatty acids (PUFAs) and 2 g of saturated fatty acids (SFA), 9 g of carbohydrates and 5 g of fiber (20% of the DV) (Lee-Bravatti et al., 2019; Liu et al., 2017).

4.6. Roasting process as an alternative to raw almond consumption

Almonds are widely consumed in the Mediterranean area in different food applications. In Spain, they are eaten as fresh, but the most common way of consuming as a snack is in the form of roasted almonds. Besides, they represent the major ingredient in confectionary products such as "turrón" (a traditional Spanish sweet made from almonds and honey). The roasting process is the key unit operation in the processing of almonds and other nuts, with a long tradition operated since humans used fire to cook (Perren et al., 2013). This is a thermal process in which different changes occurs to the product, such as (i) typical roast flavor notes; (ii) roast color with a reduction in lightness, increase in darkness and yellowish brown hue; and (iii) texture changes from hard or rubbery to typical crisp texture of roasted almonds. Roasting process always involves dehydration and this moisture removal lead to the desired color, flavor, and texture. For this reason, roasting is highly dependent on the initial moisture content and size which usually change with farming, variety, season, and storage conditions (Vázquez-Araújo et al., 2009). Drier almonds will reach their optimal quality before those with a higher moisture content, which means that depending on the moisture content and chemical composition the almonds might be roasted separately to avoid over roasting or raw product properties. If the roasting time or temperature are not correct, the almonds would remain raw inside, not generating the aromatic compounds typical of a roasted nut (pyrazines, furans, and pyrroles) and with an inappropriate texture (soft and slightly

crunchy). However, an excess of time or temperature can lead to burn notes and disproportionate dark color with undesirable toxic compounds and less attractive for consumers (Verdú et al., 2007). Moreover, roasting at elevated temperatures may decrease beneficial lipids, phytosterols, tocopherols, riboflavin, thiamin, and lysine leading to negative changes on the nutritional properties of nuts. On the other hand, almond is the only nut in which beside reducing sugars, also contains the amino acid asparagine which are the precursors for the acrylamide formation, compound which degrade at high temperatures (Perren et al., 2013). Consequently, the roasting process might be optimized for each nut, variety, and growing conditions.

Overall, this Doctoral thesis is part of a research line developed since 2013 by Food Quality and Safety Research Group (Universidad Miguel Hernández de Elche) which deals with hydroSOStainable concept, specially created to reduce the irrigation water consumption in different crops such as: olives, pomegranate, pistachios and now almonds. The hydroSOStainable products, refers to products cultivated under controlled deficit irrigation strategies by creating a stress in plant which will rebound in the fruit quality by increasing its bioactive compounds.

This Doctoral Thesis is funded by the Spanish Government [*Ministerio de Ciencia e Innovacion* (MCI), *Agencia Estatal de Investigacion* (AEI)], through a coordinated research project (hydroSOS) including the Universidad Miguel Hernández de Elche (AGL2016-75794-C4-1-R, *Productos hidroSOStenibles: identificación de debilidades y fortalezas, optimización del procesado, creación de marca propia, y estudio de su aceptación en el mercado europeo*, hydroSOS foods) and the Universidad de Sevilla (AGL2016-75794-C4-4-R); these projects have been also funded by *Fondo Europeo de Desarrollo Regional* (FEDER) "*Una manera de hacer Europa*", (MCI/AEI/FEDER, UE). From this project, the present Doctoral Thesis investigate the effect of RDI and SDI strategies on almond *cv*. Vairo. For this purpose, yield, morphological, nutritional, functional, and sensory parameters have been analyzed during 3 seasons to evaluate the water stress effect on the yield and in raw and roasted fruit quality.

5. AIM AND OBJECTIVES

The overall aim of this Doctoral Thesis was to study the effect of different deficit irrigation strategies on yield and physicochemical, functional, and sensorial characteristics of raw and roasted almonds (*cv.* Vairo) with the purpose of reducing the irrigation water consumption, increasing the kernel quality and identifying essential hydroSOStainable markers on almond crop.

To reach the main purpose, the following specific objectives were established (**Figure 8**):

- ***Objective 1.*** To determine the effect of deficit irrigation strategies on agronomic, morphological, and functional parameters after one season of water stress.
- ***Objective 3.*** To determine the descriptive sensory profile of hydroSOStainable almonds and the international consumers' acceptance, preference, and willingness to pay.
- ***Objective 2.*** To correlate water stress response with quality parameters after 3 years of experiments (2017, 2018, 2019) and to identify those parameters that behave in the same way throughout the trials. These results are essential to establish the future hydroSOStainable markers needed for hydroSOStainable certification.
- ***Objective 4.*** To determine the best roasting conditions for hydroSOStainable almonds in terms of physicochemical parameters, descriptive sensory profile, and consumers acceptance and to check the effect of deficit irrigation strategy on roasted almond quality.

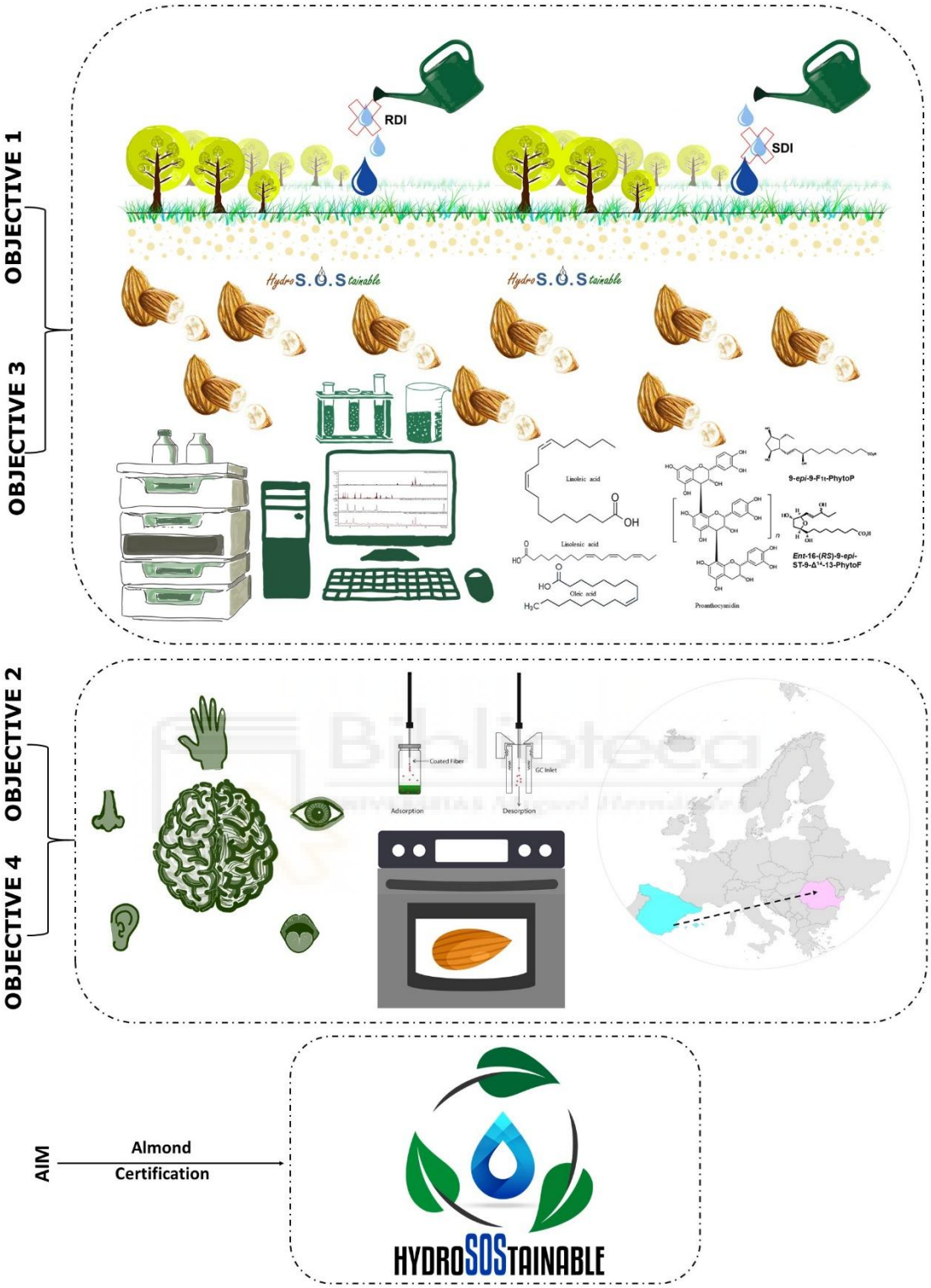
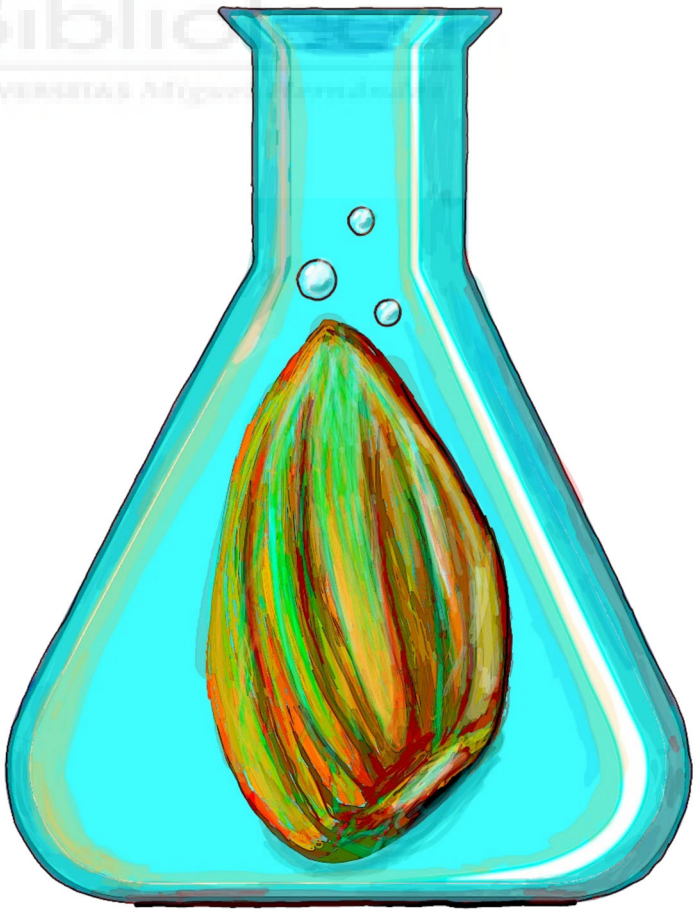


Figure 8. Graphical visualization of the Doctoral Thesis objectives

6. MATERIALS AND METHODS



This section includes a brief description of the experimental design, irrigation treatments, morphological, nutritional, functional, and sensory methods used for the characterization of the studied almonds, as well as the roasting process. Additional details on the methodology and all the materials used can be found in the published manuscripts that compose this thesis.

6.1. Plant material, growing conditions, and experimental design

The experiment was carried out during 2017, 2018 and 2019 in a commercial farm "La Florida", located in Dos Hermanas (Seville, Spain). The climate is Mediterranean with an average annual temperature of 17.5 ºC; during winter 11.7 ºC and during summer 26.1 ºC. Almonds were 7 years old at the beginning of the experiment and belonged to *cv.* Vairo. The orchard (**Figure 9**) has 2 almond cultivars Guara and Vairo; the tree spacing was 6 m × 8 m. The experimental plots consist of 4 lines of 4 trees and the measurements were carried out in the central trees of *cv.* Vairo. The irrigation was done using a drip irrigation line (3.8 L h^{-1}) with 0.4 m between drippers. The applied water was monitored with a flowmeter installed on each plot.

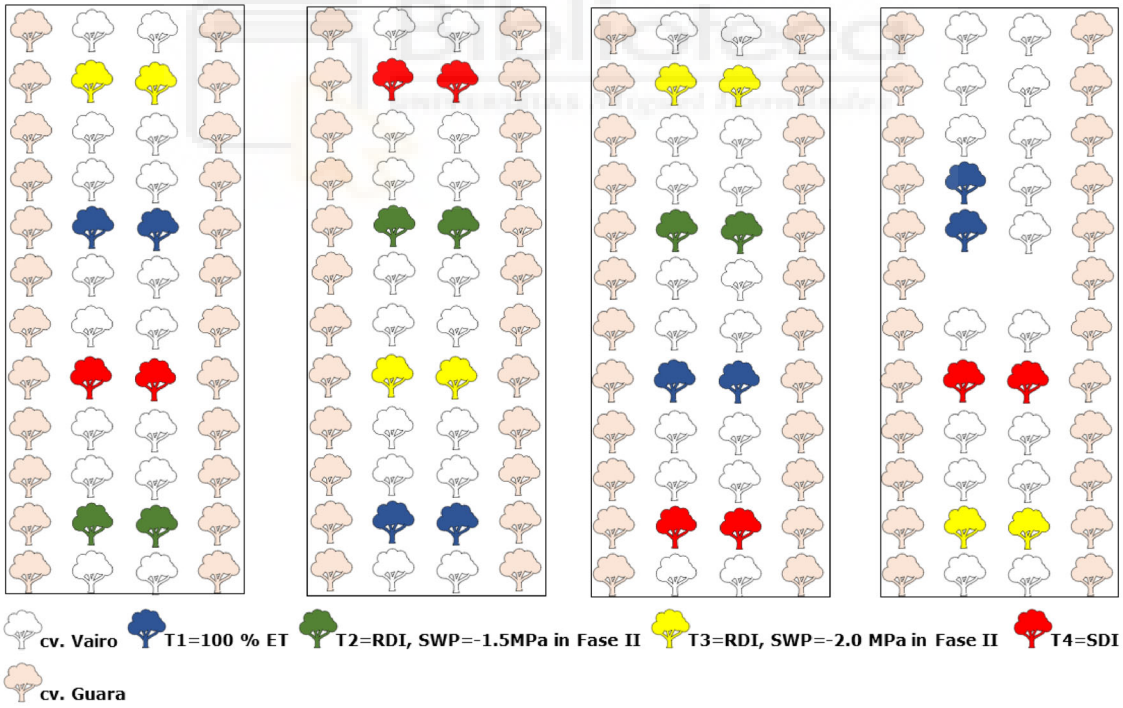


Figure 9. Representation of the experimental plots in the commercial farm (SWP=midday stem water potential)

The irrigation treatments were scheduled based on the threshold values of the midday (12 h solar time) stem water potential (SWP) obtained by using a pressure

chamber (PMS Instrument Company, Albany, OR, USA). The following irrigation treatments were evaluated:

- **T1** = **full irrigation**, to assure the crop needs. Water needs were estimated with crop evapotranspiration (ET) approach according to Steduto, Hsiao, Fereres, and Raes (2012) using reduction coefficients (Kr) around 0.6. Moreover, the water status was calculated using midday stem water potential and compared to McCutchan and A Shackel (1992) baseline. If water status was below the expected one, irrigation was increased in 150% ET.
- **T2** = **moderate regulated deficit irrigation** (RDI) the water stress was imposed during the stage II or kernel filling period; almond trees were irrigated when SWP was below -1.5 MPa, and for the rest of the time, trees were irrigated to keep a SWP as the baseline proposed by McCutchan and A Shackel (1992). That shows the optimum midday stem water potential in relation with vapor pressure deficit (VPD) (1):

  $$SWP = (-0.41) \times (-0.12\, VPD)\ (1)$$

  where: SWP = optimum midday stem water potential (MPa) and VPD = vapor pressure deficit (KPa)
- **T3** = **severe RDI**; the same as T2, but during stage II the trees were irrigated when SWP was below -2.0 MPa.
- **T4** = **sustained deficit irrigation** (SDI); the same as T3 but tree water status was not considered. Irrigation was applied in a constant daily rate around 1-2 mm per day.

The threshold values of midday stem water potential (SWP) were measured weekly (**Figure 10**) with the pressure chamber (PMS Instrument Company, Albany, OR, USA). These values were used for the irrigation schedule by evaluating the stress level in plant with the methodology proposed by Myers (1988) according to the following expression (2):

$$SI = |\sum(\psi\text{stem} - (-0.2)) \times \text{n}\ \ (2)$$

where *SI* was the stress integral, ψstem is the average midday stem water potential for any interval and *n* is the number of days in the interval.

The harvest was done with a self-propelled trunk shaker, with collector at mid-August. The 4 repetitions per treatment were separately collected and sun-dried until getting a moisture content below 5%.

Figure 10. Images from "La Florida" farm during the agronomic measurements

6.2. Roasting of almonds

Almonds from T1 (optimum water requirements) and T2 (moderate RDI) with similar moisture content and size were chose for testing the roasting process. The experiment was carried out in a hot-air circulation drying oven Distform My Chef (Lleida, Spain); batches of 200 g of almonds were roasted at three temperatures (150, 170 and 190 ºC) and constant time of 10 min. These conditions were established according to literature (Lukac et al., 2007; Vázquez-Araújo et al., 2008; Lin et al., 2016) and preliminary experiments.

6.3. Morphological and physical characterization

For the almond weight, size (length, width, thickness), instrumental color and texture, 100 almonds per treatment (25 × 4 repetitions) were measured (**Figure 11**). The weight and size were measured for in-shell almonds and kernels using a scale with a 0.1 mg precision (Mettler Toledo model AG204, Barcelona, Spain) and a digital caliper (Mitutoyo 500-197-20, Kawasaki, Japan) respectively. The CIE*L*a*b** color coordinates were measured with a Minolta Colorimeter CR-300 (Minolta, Osaka, Japan) while the almond texture [fracturability (mm), hardness (N), work done to shear (Ns), average force (N) and number of fractures (peaks count)] were determined using a texture analyzer (Stable Micro Systems, model TA-XT2i, Godalming, UK).

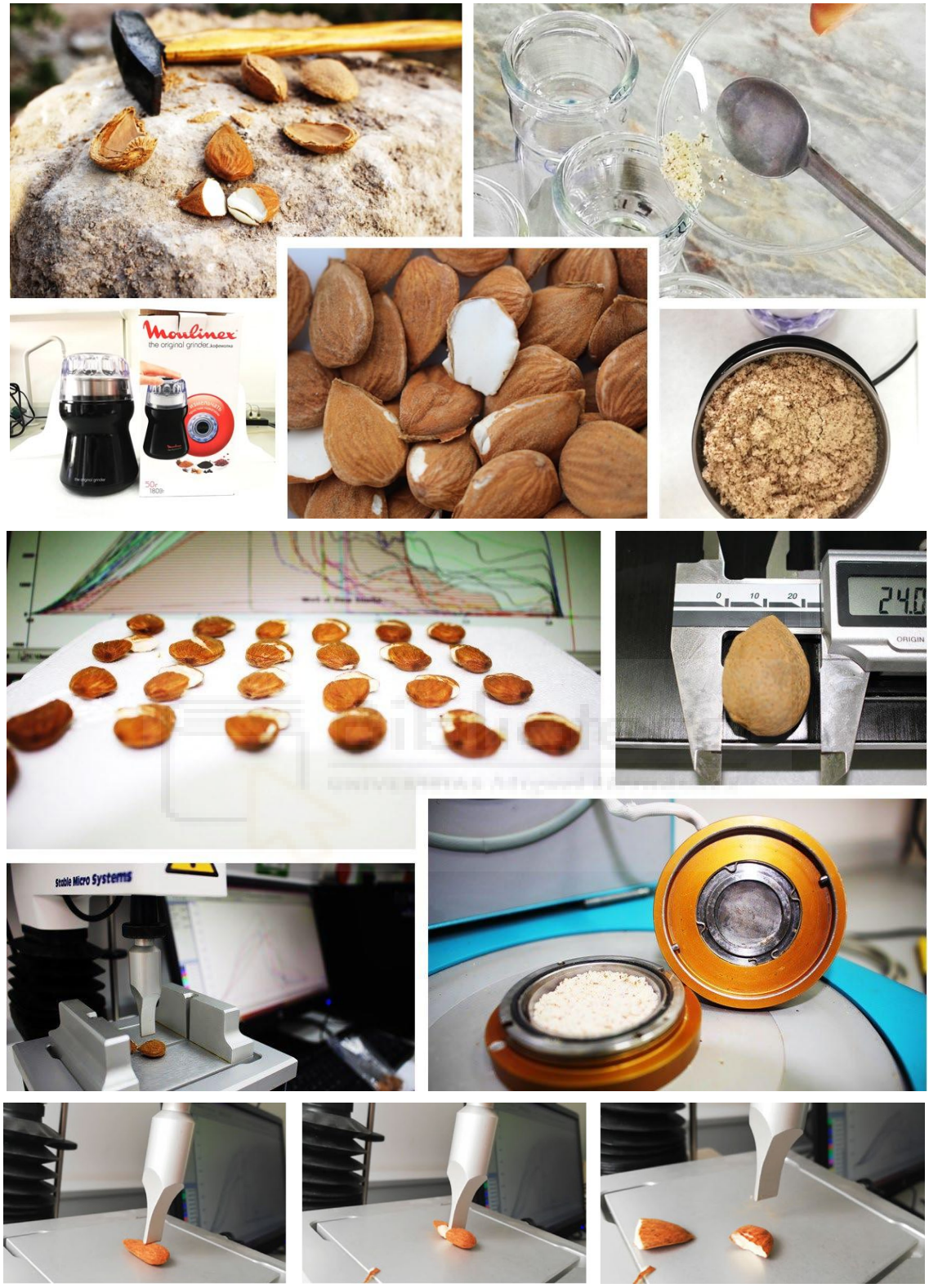


Figure 11. Shelling, morphological, and physical analyses

For the dry weight determination, 2 g of almonds were grinded and dried to constant mass in a stove at 60 ºC and the water activity of grinded almonds was measured with an A_w-meter (Novasina aw-Sprint TH500; Pfaffikon, Zurich, Switzerland). All analyses were run in quadruplicate.

6.4. Proximate analysis

Protein, fat, ash, and carbohydrate content were also measured (**Figure 12**) using Kjeldhal method for protein determination, Soxhlet equipment for fat content and a muffle furnace (Hobersal, Barcelona, Spain) model 12 PR/300 series 8B for the ash content. Finally, total carbohydrates content was determined by subtracting the ash, protein, and fat percentages from 100%. Analyses were run in quadruplicate and results were expressed as g kg^{-1} dry weight (dw).

Figure 12. Proximate analyses determination

6.5. Minerals

Minerals content was determined (**Figure 13**) by digesting 0.5 g of grinded (Moulinex grinder, AR110830) almond for 10 s with 8 mL of concentrated HNO_3 and 2 mL H_2O_2 (30%) with a START D Medium Microwave Digestion (SK-10). Then, Unicam Solaar 969; atomic absorption-emission spectrometer (Unicam Ltd., Cambridge, UK) was used for minerals measurements. Calcium, Mg, Fe, Cu, Mn and Zn was determined by atomic absorption while K was measured using atomic emission (Lipan et al., 2019). Analyses were run in quadruplicate and results were expressed as g kg^{-1} dw.

Figure 13. Digestion and minerals determination through atomic absorption and emission spectrometer

6.6. Organic acids and sugars

The organic acids and sugars were extracted as previously described by Lipan et al. (2019) by homogenizing (Ultra Turrax T18 Basic) 1 g of grinded almond with 5 mL phosphate buffer 50 mM (pH = 7.8) followed by filtration and injection. The identification and quantification were carried out in a high-performance liquid chromatography Hewlett Packard (Wilmington DE) series 1100 (HPLC). Sugars were detected through refractive index detector (RID) while the organic acid absorbance was read at 210 nm with a diode-array detector (DAD). Analyses were run in quadruplicate and results were expressed as g kg^{-1} dw.

6.7. Antioxidant activity and total phenolic content

The antioxidant activity (AA) and total phenolic content (TPC) were measured in three different matrixes (**Figure 14**): whole kernel, kernel skin and de-skinned kernel. The skin was removed by (i) immersing the kernels in boiling water (100 ºC) for 2 min or by (ii) submerging the kernels in water at room temperature for 20 min followed by manual skin removal. All almond parts (whole kernels, skins, and de-skinned kernels) were dried during 24 h at room temperature and, then, finely ground. For the extraction 0.5 g of sample were sonicated with 10 mL of extractant [MeOH/water (80:20, v/v) + 1% HCl at 20 ºC] for 15 min and stored for 24 h at 4 ºC. The extract was sonicated again under the same conditions, followed by centrifugation and supernatant separation.

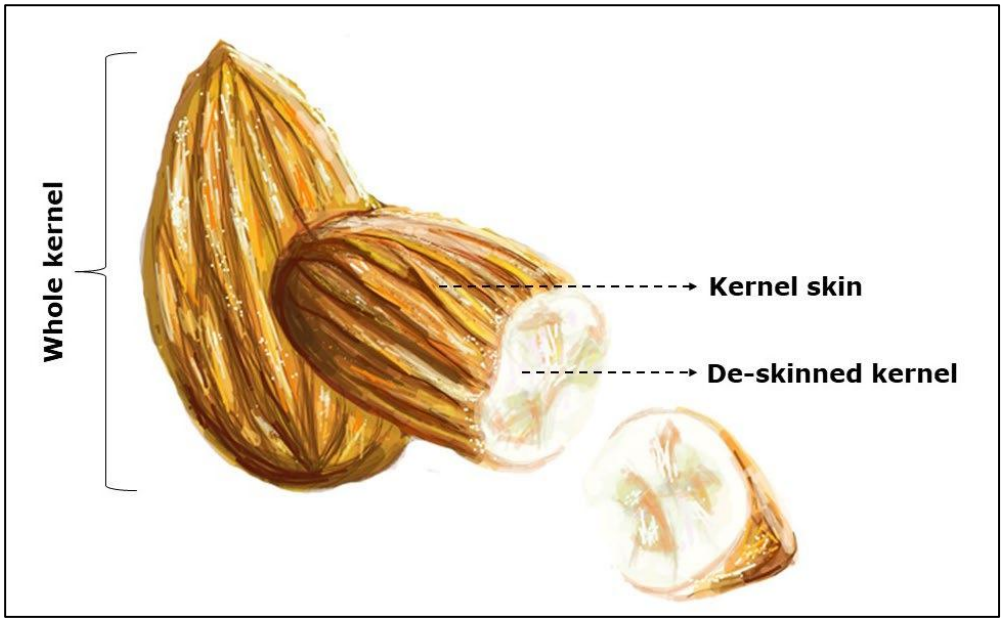


Figure 14. Kernel parts used for the AA and TPC measurements

The radical scavenging activity of the obtained extracts was measured using 3 methods due to the different action mechanism of each one: (i) $ABTS^{\bullet+}$ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], (ii) $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) and (iii) FRAP (ferric reducing antioxidant power) (Benzie & Strain, 1999; Brand-Williams, Cuvelier, & Berset, 1995; Re et al., 1999). All measurements were done in a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK). The quantification was performed based on the calibration curve of Trolox prepared in a concentration ranging from 0.50 to 5.00 mmol Trolox L^{-1}, and curves showed good linearity (R^2>0.99) and results were expressed in mmol Trolox kg^{-1}.

For the TPC, the same extract prepared for AA analyses was measured with Folin-Ciocâlteu colorimetric method in the same equipment, at 765 nm. Quantification was based on the gallic acid calibration curve and the results were expressed as gallic acid equivalents (GAE) g^{-1}.

6.8. Extraction, identification, and quantification of individual phenolic compounds

The individuals polyphenols identification and quantification was performed as previously described by Noguera-Artiaga, Pérez-López, Burgos-Hernández, Wojdyło, and Carbonell-Barrachina (2018). For this, 1.0 g of whole/deskinned kernels or 0.5 g of skin were mixed with 5 mL of aqueous methanol (30:70, v/v) and 1% of ascorbic acid (v/w). The mixture was stirred, sonicated (ultrasonic bath JP Selecta S.A, model 3000512, Barcelona, Spain), and kept in darkness, at room temperature, for 24 h. The suspension was, then, centrifuged, and the supernatant was filtered and was

directly injected into an ultra-performance liquid chromatography equipment (UPLC). The compound identification was done using ultra-performance liquid chromatography (UPLC) coupled with a photodiode detector (PDA; Waters, Milford, MA, USA) quadrupole and tandem time-of-flight mass spectrometry (QTof) (Waters, Manchester, UK), equipped with an electrospray ionization (ESI) source.

The phenolic compounds were recorded at 280 nm (flavan-3-ols), 320 nm (phenolic acids), and 360 nm (flavonols and flavanones) and the quantification was done by injecting calibration curves of standards according to Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016). All analyses were performed in quadruplicate and results were expressed as mg $(100\ g)^{-1}$ dw of almonds kernel.

6.9. Extraction and determination of proanthocyanidins

Proanthocyanidins were analysed only in whole kernels following the protocol previously described by Gironés-Vilaplana et al. (2014). Thus, 100 mg of ground kernels were mixed with 1 mL of methanol/water (70:30, *v/v*) and acidified with 1% of formic acid. The solution was vortexed, sonicated and were kept overnight at 4 ºC. The mixture was sonicated again, centrifuged (model EBA 21, Hettich Zentrifugen), filtered and directly injected into an Agilent HPLC 1100 series model equipped with a photodiode array (PDA) detector and a mass detector in series (Agilent Technologies, Waldbronn, Germany). Proanthocyanidins were identified and quantified by using their UV spectra recorded at 280 nm, the molecular mass and daughter ions acquired in the negative mode on the mass spectrometer. All analyses were performed in quadruplicate and results were expressed as mg 100 g^{-1} dw of almonds kernel.

6.10. Analysis of polymeric procyanidins through phloroglucinol method

The phloroglucinolysis of almonds samples were done as Wojdyło et al. (2016). Whole, deskinned, and skin kernel powder (50 mg) were stirred with a solution of 0.3 M HCl in MeOH containing phloroglucinol (75 g L^{-1}) and ascorbic acid (10 g L^{-1}) and cooled during 5 min. To stop the reaction, 0.6 mL of aqueous sodium acetate (0.2 mol L^{-1}) were added, followed by 5 min cooling and centrifugation. Chromatographic separation was run on a BEH Shield RP C18 (2.1 mm × 50 mm; 1.7 m) with a precolumn (Waters Corp.) at 15 °C. The liquid chromatograph used for analysis of polymeric proanthocyanidins was a Waters (Milford, USA) system equipped with DAD and scanning fluorescence detectors. Fluorescence was recorded at the excitation wavelength of 278 nm and emission wavelength of 360 nm, while Procyanidin B_2 was used as a reference compound. The identification of each proanthocyanidin was done based on their retention time and UV-*vis* spectra while

the degree of polymerisation (DP) was calculated from the molar ratio of all flavanol units to the terminal units (−)-epicatechin and (+)-catechin (Wojdyło, Oszmiański, Teleszko, & Sokół-Łetowska, 2013). Analyses were done in quadruplicate and results were expressed as mg 100 g^{-1} dw of almonds kernel.

6.11. Fatty acids

The fatty acids methyl esters (FAMEs) were methylated according to Lipan et al. (2019) and analyzed as Tuberoso, Kowalczyk, Sarritzu, & Cabras (2007). The extraction was done with 40 mg of grinded almond, dichloromethane (Cl_2CH_2) and methanolic NaOH for 10 min at 90 ºC. Later BF_3 methanolic was added followed by 30 min rest in dark for reaction. Finally, the FAMEs were extracted from the mixture with hexane. The FAMEs separation was carried out in Shimadzu GC17A gas chromatography coupled with a flame ionization detector and a DB-23 capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) J&W Scientific, Agilent Technologies. The identification of methylated FAMEs peaks was done by comparing the retention times of the FAMEs Supelco MIX-37 standards. Results were expressed quantitatively as g kg^{-1} concentration using methyl nonadecanoate as internal standard.

6.12. Phytoprostane and phytofurane analysis

Ground almond (~1 g) was suspended in 2.5 mL of a solution containing MeOH:BHA (1 g L^{-1}) (99.9:0.1, v:v). Then, the mixture was stirred, sonicated, centrifuged and filtered through a pre-activated Sep-Pack C_{18} cartridge (Waters, Milford, MA) (Collado-González et al., 2015; Domínguez-Perles et al., 2018). The phytoprostanes (PhytoPs) and phytofurans (PhytoFs) were isolated by using a dilution followed by a solid-phase extraction (SPE). This emulsion was passed through a pre-activated Strata X-AW cartridge (**Figure 15**). The compounds were eluted with MeOH and dried using SpeedVac concentrator (Savant SPD121P, Thermo Scientific, MA, USA). The dried extracts were reconstituted with milliQ water/acetic acid and methanol:acetic acid and filtered through PVDF filters (Millipore, MA, USA) and 20 µL of each sample were analyzed in triplicate. The chromatographic separation of the almond PhytoPs and PhytoFs was carried out as previously described (Collado-González et al., 2015; Domínguez-Perles et al., 2018) by using UHPLC coupled to a 6460 triple quadrupole-MS/MS (Agilent Technologies, Waldbronn, Germany), with a BEH C_{18} analytical column (2.1 × 50 mm, 1.7 µm) (Waters, Milford, MA). The quantification of PhytoPs and PhytoFs detected in almonds performed according to standard curves freshly prepared using authentic standards. Both PhytoPs and

PhytoFs were synthesized according to the previous reports (Cuyamendous et al., 2015; El Fangour et al., 2004; El Fangour, Guy, Vidal, Rossi, & Durand, 2005; Pinot et al., 2008). The results were expressed in ng 100 g^{-1}.

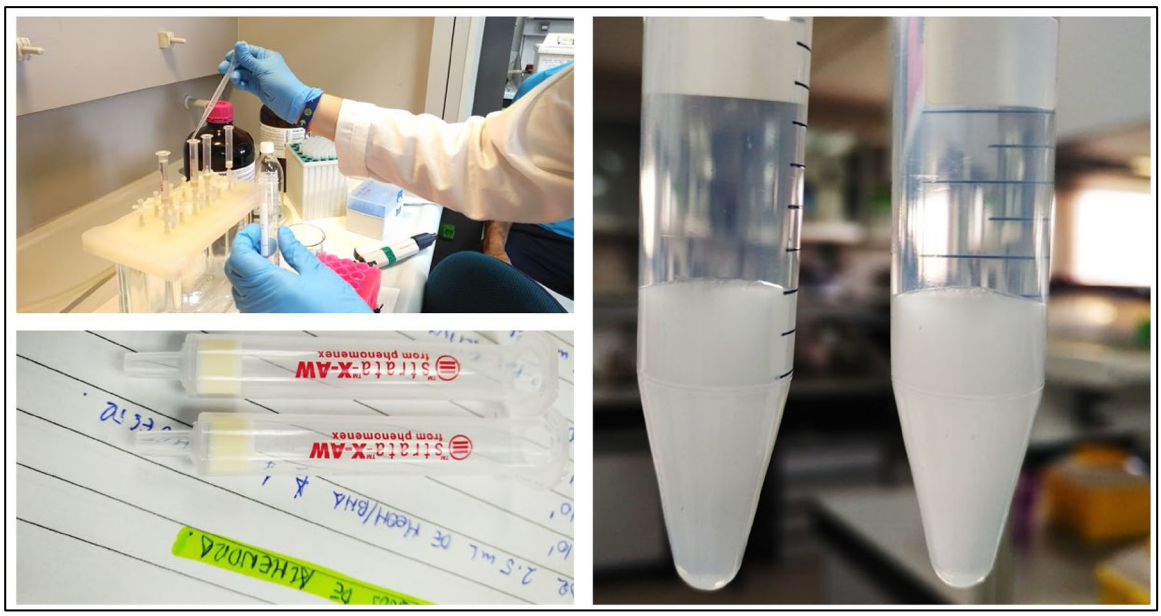


Figure 15. Extraction of the almond phytoprostanes and phytofurans

6.13. Volatile compounds

Volatile compounds were extracted using headspace solid phase microextraction (HS-SPME) and a 50/30 µm DVB/CAR/PDMS fiber special chosen for its high capacity to trap volatile compounds from fruits and nuts (**Figure 16**). For extraction, 2 g of ground raw almond and β-ionone (100 mg L^{-1}) internal standard were added to a hermetic vial which was placed in a water bath with controlled temperature to assure 40 ºC in the vial (to simulate the mouth temperature when chewing almonds). Once the temperature was reached and was constant, the fiber was introduced in the headspace of the vial for 50 min and later desorbed for 3 min in the injector port of a gas chromatograph Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) coupled with mass spectrometer (MS) detector Shimadzu GC-MS QP-5050A used for the volatile compounds identification. For the roasted almonds, only 1 g of ground sample together with 500 µL of 12.5% aqueous NaCl and 2.5 µL of 2-acethylthiazole (1000 mg L^{-1}) as internal standard were used for the volatiles extraction for 35 min. The semi-quantification of the volatile compounds was done based on the use of the internal standard (β-ionone and 2-acethylthiazole) while the volatile compounds identification was done by using 3 methods: (a) retention indexes, (b) GC-MS retention times of authentic chemicals, and (c) mass spectra (authentic chemicals and NIST05 spectral library collection) (NIST, 2018).

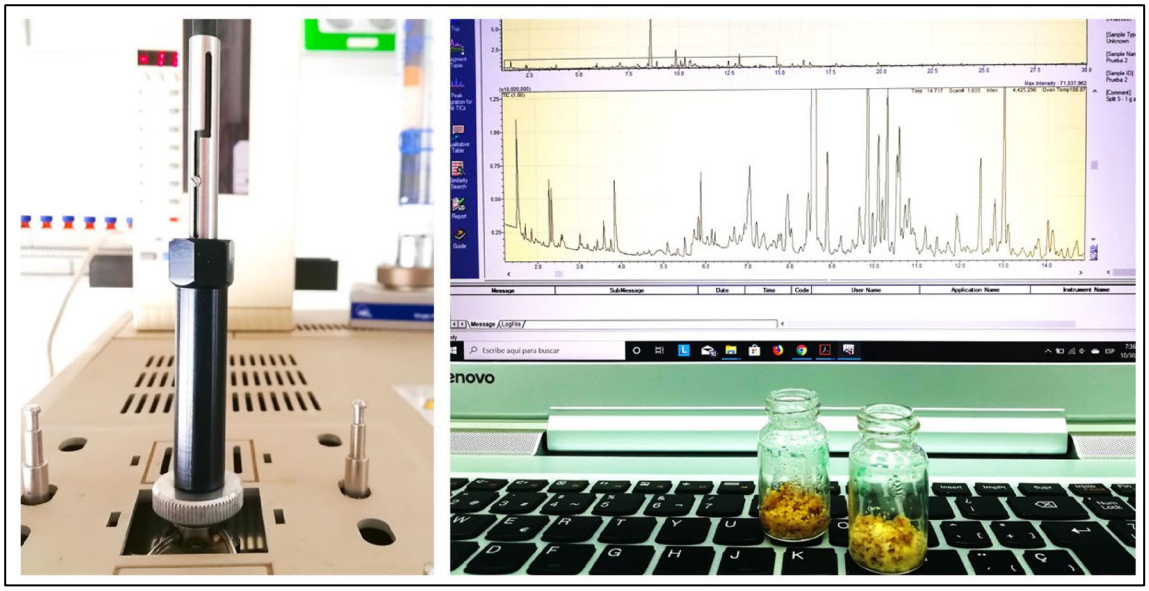


Figure 16. Extraction and identification of the volatile compounds

6.1. Descriptive sensory analysis

Ten trained panelists from the Food Quality and Safety Group (Miguel Hernández University of Elche, Orihuela, Alicante, Spain) were used for descriptive analysis. The panel had a vast experience in tasting almond and *turrón* (traditional Spanish dessert made basically of toasted almonds and honey); 4 orientation session were done previous to the almonds tasting, where the panelists chose reference products (**Figure 17**) for each attribute and decided the final list of descriptors. After the orientation sessions, each panelist received 4 samples corresponding to the different irrigation treatments and 3 evaluations per sample were done. In the case of the roasting almonds, panelists received 6 coded samples corresponding to 2 irrigation treatments × 3 roasting temperatures and were tasted in the same conditions as previously described. A 0 to 10 numerical scale was used by the panelists to quantify the intensity of the almond attributes, where 0 represented none and 10 extremely strong with a 0.5 increment.

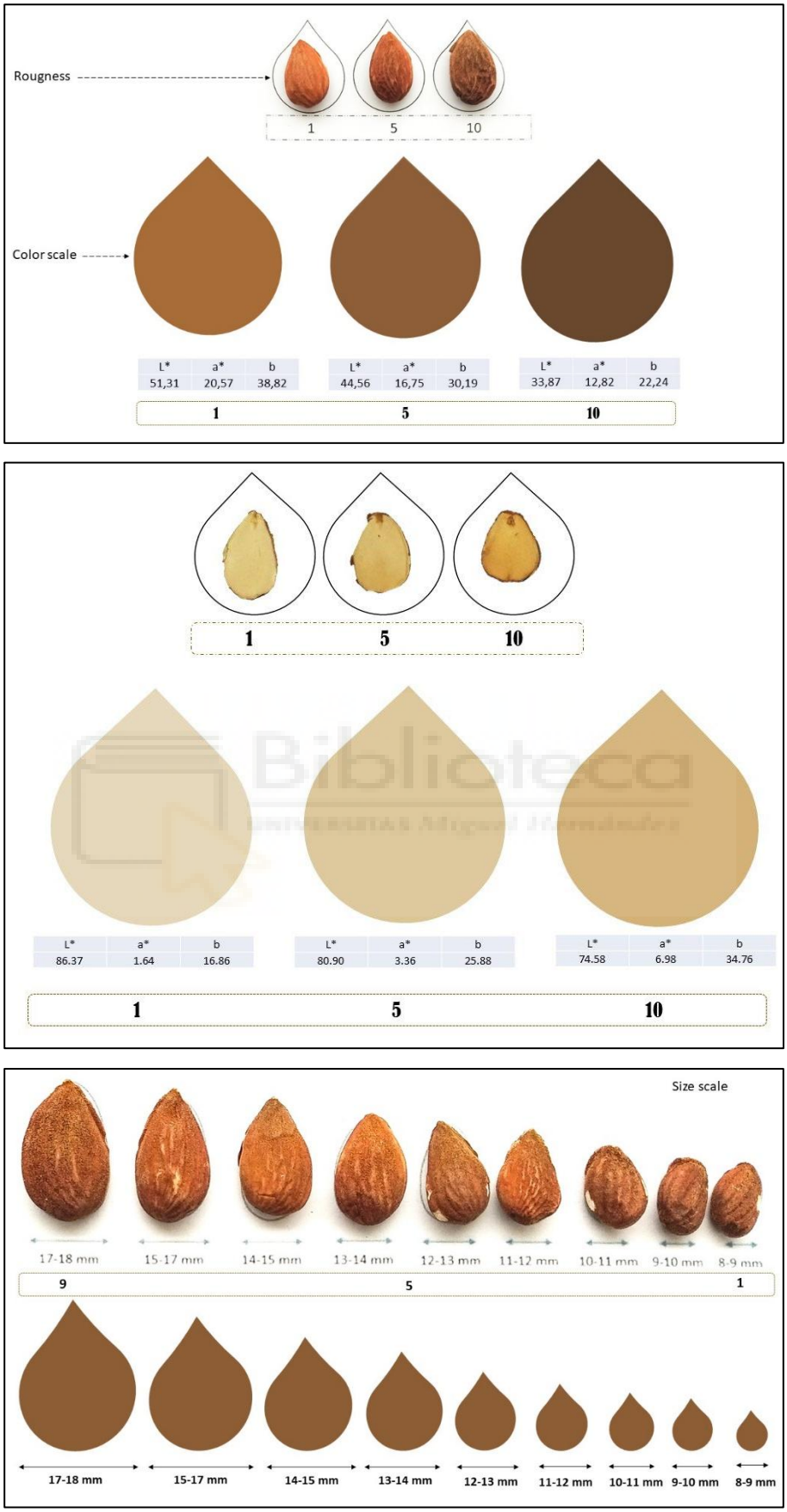


Figure 17. Example of scales used by trained panel as references to evaluate the almond appearance (roughness, outside color, inside color, and size)

6.2. Affective sensory analysis

Affective sensory analysis (**Figure 18**) was done with 100 recruited consumers from Spain and other 100 from Romania who were asked about the willingness to taste almonds and the frequency of nuts consumption. Only consumers who declared no allergy to nuts and with a frequency of consumption for at least once per week were recruited. Demographic questions regarding gender, age, nuts consumption frequency, allergies, intolerances, or diet restriction were also included in the questionnaire. Spanish to Romanian back-translation procedure was conducted to avoid major misunderstandings during the evaluation. Consumers were provided with 4 samples randomly served in cups with 3 digit codes and asked for global satisfaction degree using a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely) and attributes intensity using Just About Right (JAR) questions. Moreover, preference and some sociodemographic items were also asked by using *Check All That Apply* (CATA) question type. The affective tests were also carried out in special tasting rooms with individual booths and according to a randomized block design. For the optimization of the roasting process 100 consumers from Spain were recruited and a study was conducted using the conditions previously described.

Figure 18. Captures recorded during the affective tests

6.3. Consumer willingness to pay for hydroSOStainable almonds

The willingness to pay test was carried out with consumers from Spain and Romania to check their willingness to pay for the hydroSOStainable almonds depending on different countries. For this, consumers received a price for the conventional almonds (2.60 €/200 g) and 4 options to choose the price were willing

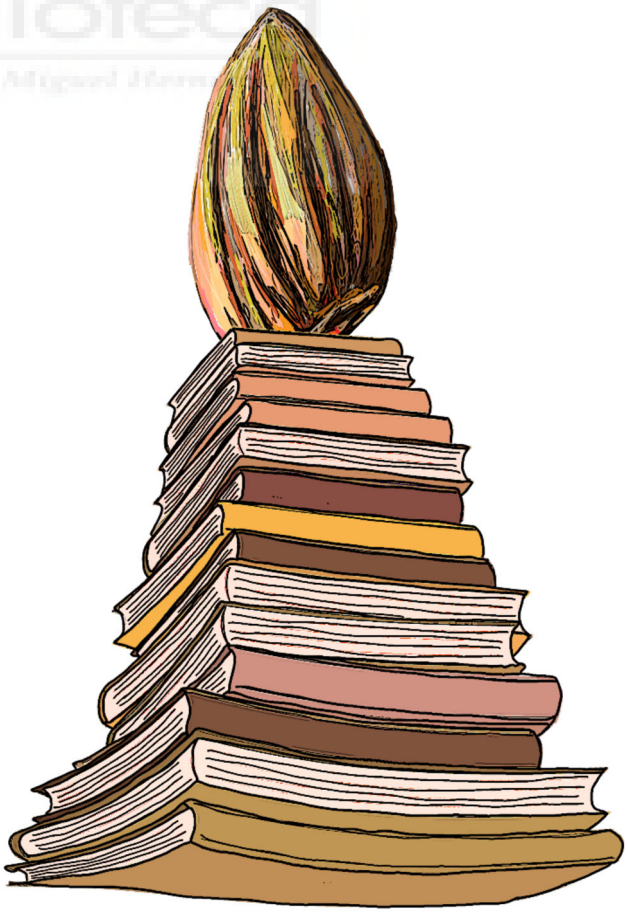
to pay for the hydroSOStainable almond (same price, 0.5 € more, 1.0 € more or more than 1 €).

6.4. Statistical analysis

The present results are mean values of, at least, 3 repetitions. One, two or three-ways analysis of variance (ANOVA) and then Tukey's HSD (honestly significant difference) multiple range test was used to determine significant differences among irrigation treatments, the affective sensory data (factor 1: irrigation treatment, and factor 2: country), and the panel consistency in the descriptive sensory analysis data (factor 1: irrigation treatment; factor 2: session; and, factor 3: panelist), respectively. Statistically significant differences were considered when $p<0.05$ according to the following levels of significance: $p<0.05$=*, $p<0.01$=**, and $p<0.001$=***. Pearson's correlation was carried out with the same program in which data were subjected to correlation tests in order to check the relationship among variables. Additionally, principal component analysis (PCA regression map) was run to rapidly imagine and examine the relationship between instrumental parameters and sensory descriptors. Partial least-square regression (PLS) analysis was also used to determine the drivers of liking by assessing the relationship of the volatile composition and the descriptive analysis with the overall liking data. Finally, Penalty analysis was performed to supply information about the possible improvement of samples, and for these analyses, JAR sensory data was used. Mean drops (penalties) *versus* the percentage of the consumers (providing each response in the mean drop plot) were graphically represented.

Statistical analysis was performed using XLSTAT Premium 2016 (Addinsoft Inc, New York) while figure representation was done with Statgraphics Plus (Version 3.1, Statistical Graphics Corp., Rockville, MA, USA).

7. PUBLICATIONS



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Almond fruit quality can be improved by means of deficit irrigation strategies

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ABSTRACT

Water scarcity is considered one of the biggest global risks worldwide, not only because affects every continent but mostly because it can have dramatic impact in a long term. Deficit irrigation strategies can help in coping with this water scarcity and optimizing the water efficiency. Both regulated deficit irrigation (RDI) in moderate and severe levels and sustained deficit irrigation (SDI) were applied in almond nut crop within this study and quality parameters of obtained fruits were analyzed. Almost all morphological and physicochemical parameters were not affected by the water stress. However, statistically significant differences among treatments were observed for the fat content, the highest value being reached by moderate RDI treatment. Besides, differences were also found for total organic acids content, calcium, potassium, manganese and for six fatty acids (myristic, palmitic, margaric, *cis*-heptadecenoic, *cis*-vaccenic, and arachidic acids) content. According to experimental findings, it can be concluded that irrigation strategies do not affect almond fruit quality; being possible to increase the final quality of nuts, when moderate RDI is applied.

1. Introduction

Fresh water availability and its scarcity are serious issues worldwide and drives inevitably to accept that Spanish agriculture same as other Mediterranean regions must consider to implement different strategies in order to achieve a higher resilience in terms of water scarcity (García Tejero and Duran Zuazo, 2018). Moreover, around 70% of all fresh water withdrawals are consumed by agriculture sector and used for the food production which more than 40% comes from irrigated fields (Du et al., 2015). The Mediterranean agriculture must be involved in projects on how to manage the water productivity, because of being a great example for arid and semiarid farming fields (Egea et al., 2013). Spain is a country with high levels of water stress, particularly in the southern side, due to the low rainfall and the high evaporative claim during the almond growing season. Despite the relative tolerance of almond to water stress there are plenty of authors agreeing that the irrigation is essential to produce high quality almonds and to improve the yield (Goldhamer et al., 2005).

On the other hand, many researches have recommended deficit irrigation strategies (DI) to increase water productivity of many crops. In this regard, using DI strategies, a new generation of hydro-sustainable food products originates: *hydroSOS* (Cano-Lamadrid et al., 2015; Carbonell-Barrachina et al., 2015; Lipan et al., 2018; Noguera-Artiaga et al., 2016; Sanchez-Rodriguez et al., 2018). The *hydroSOS* products are those obtained from plants submitted to a water stress and are characterized by a higher amount of bioactive compounds, among other properties. Although, there is research in different fruits such as olives or pistachios, there is a lack of studies evaluating the effects of water stress on almond nut quality.

The aim of this work was to determine the impact of different

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irrigation strategies on almond yield and quality; suggesting the best way of optimizing the water resources in the almond crop of the Mediterranean area.

2. Materials and methods

2.1. Plant material, growing conditions and experimental design

The trial was carried out during 2017 season in a commercial orchard belonged to the farm "La Florida", located in Dos Hermanas (Seville, Spain). Mediterranean climate with average temperature of 17.5 °C, average maximum and minimum temperatures of 24.5 and 11.5 °C. Almonds were 7 years-old and belonged to Vairo genotype. Tree spacing followed an 8 m × 6 m square pattern, with tree soil cover in January 2017: 25.2% T1, 27.0% T2, 23.3% T3 and 21.82% T4. Trees were irrigated using a drip irrigation line (3.8 L h^{-1}), with drippers separated 0.4 m. Applied water was calculated using a flowmeter on each plot, 4 in each treatment. Four irrigation treatments were evaluated:

- **T1**: A full irrigated control treatment which received, total irrigation requirements during the irrigation period.
- **T2 (RDI):** A moderate regulated deficit irrigation treatment, in which the water stress was imposed during kernel filling stage. In this treatment, irrigation scheduling was based in measurements of midday stem water potential (SWP) and maximum daily shrinkage (MDS). During kernel filling, this treatment was irrigated when SWP was below -1.5 MPa or MDS signal was higher than 1.75. Out of this period, trees were irrigated by using the baseline suggested for McCutchan and Shackel (1992) or MDS signal equal to 1.
- **T3 (RDI)**: A severe regulated deficit irrigation treatment, in which water stresswas imposed during the same period that the previous one but with a maximum seasonal water applied of 100 mm. Thus, under this strategy, trees were irrigated when SWP was below −2 MPa or MDS signal was higher than 2.75. In the other periods, irrigation was conducted as previously described in the T2 treatment.
- **T4 (SDI):** A sustained deficit irrigation with a maximum water applied of 100 mm during the irrigation period.

Stem water potential (ψ_{stem}) was measured using a pressure chamber (PMS Instrument Company, USA), being calculated the water stress integral (SI) following the methodology proposed by Myers (1988) according to the following expression (1):

$$SI = |\sum (\psi_{stem} - (-0.2)) \times n \quad (1)$$

where *SI* was the stress integral, ψ_{stem} is the average minimum stem water potential for any interval and *n* is the days numbers in the interval.

At the end of season (August 7, 2017), monitored trees were harvested (28 weeks after blossom) with a self-propelled trunk shaker with collector, monitoring each tree separately and around 6 kg of in-shell almonds were sent to Miguel Hernández University for quality analysis. Previously, each sample set extended horizontally until getting a moisture content below 5%.

2.2. Physico-chemical analysis

2.2.1. Kernel ratio

The ratio between the mass of in-shell almonds and kernel was calculated from 12 kg of whole fruit per treatment.

2.2.2. Weight and size

All physicochemical analysis were performed in raw almonds and for the morphological analysis 100 almonds per treatment (25 samples × 4 trees/treatment) were randomly selected and analyzed by measuring the weight and size (length, width, thickness) of both in-shell almond and kernel using a digital caliper (Mitutoyo 500-197-20, Kawasaki, Japan) and a scale (Mettler Toledo model AG204, Barcelona, Spain) respectively.

2.2.3. Instrumental color

Color measurements were done in 100 kernels per treatment and were run using a Minolta Colorimeter CR-300 (Minolta, Osaka, Japan). This spectrophotometer used a D_{65} illuminant and a 10° observer as references. The color was provided as CIE$L^*a^*b^*$ coordinates defining the color in a three-dimensional space. The color was expressed in three numerical values which includes L^* for the lightness ($L^* = 0$ black; $L^* = 100$ white), a^* for the green-red (a^* = red; -a^* = green) while b^* for the blue-yellow components (b^* = yellow; -b^* = blue).

2.2.4. Instrumental texture analysis

Almond texture was determined using a texture analyzer (Stable Micro Systems, model TA-XT2i, Godalming, UK) with a 30 kg load cell and a probe (Volodkevich Bite Jaw HDP/VB): trigger was set at 15 g, test speed was 1 mm s^{-1} over a specified distance of 3 mm. One hundred almonds per treatment were used to determine texture attributes. The obtained parameters were: fracturability (mm), hardness (N), work done to shear (Ns), average force (N) and number of fractures (peaks count).

2.2.5. Dry weight

For the dry weight determination, 2 g of almonds of each sample were grinded and dried to constant mass in a stove at 60 °C (AOAC, 1995a).

2.2.6. Water activity

Water activity of grinded almonds was measured with an a_w-meter (Novasina aw-Sprint TH500; Pfaffikon, Zurich, Switzerland). Four replications per treatment were conducted.

2.2.7. Proximate analysis

Protein, fat, ash and carbohydrate content was carried out in triplicate. Nitrogen of 1 g of grinded almond was determined with the Kjeldhal method and later converted to protein by multiplying a factor of 5.7 (AOAC, 1995b). For fat content determination, 2 g of fine ground almond was subjected to extraction with diethyl ether for 1.5 h in a Soxhlet equipment. Moreover, 0.5 g of grinded almond and introduced in a muffle furnace (Hobersal, Barcelona, Spain), model 12 PR/300 series 8B, set at 650 °C for 6 h were used to determine the ash content (AOAC, 1995a). Finally, total carbohydrates content was determined by subtracting the ash, protein and fat percentages from 100% (Jinapong et al., 2008).

2.2.8. Organic acids and sugars

Organic acids and sugars were done as described by Lipan et al. (2018) using an HPLC equipment with some modification. The extraction consists of homogenization of 1 g of sample with 5 mL of phosphate buffer followed by filtration and injection. On the one hand, sugars were detected by using the refractive index detector (RID) and organic acid absorbance was measured at 210 nm using a diode-array detector (DAD).

2.2.9. Mineral content determination

Minerals content was determined by digesting 0.5 g of sample with 8 mL of concentrated HNO_3 and 2 mL H_2O_2 (30%) using a START D Medium Microwave Digestion (SK-10). Determination of macro-nutrients (Ca, Mg, and K) and micro-nutrients (Fe, Cu, Mn and Zn) in the previously mineralized samples was performed using a Unicam Solaar 969 atomic absorption-emission spectrometer (Unicam Ltd., Cambridge, UK). More specific Ca, Mg, Fe, Cu, Mn and Zn was

determined by atomic absorption while potassium and sodium were the elements measured using atomic emission (Cano-Lamadrid et al., 2018).

2.2.10. Fatty acids analysis

The fatty acids methyl esters (FAMEs) were prepared using *in-situ* methylation, with some modification (Lipan et al., 2018) and analyzed according to Tuberoso et al. (2007). Grinded almond (40 mg) was saponified with 100 µL of dichloromethane (Cl_2CH_2) and 1 mL of sodium methoxide solution and refluxed for 10 min at 90 °C. Later 1 mL of BF_3 methanolic was added followed by 30 min rest in dark for reaction. Finally, the FAMEs were extracted from the mixture using 1.5 mL hexane. The FAMEs were separated in a Shimadzu GC17 A gas chromatography coupled with a flame ionization detector and a DB-23 capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) J&W Scientific, Agilent Technologies. The carrier gas (Helium) flow rate was 1.1 mL min^{-1} and 35 mL min^{-1} at the make-up point, the injector temperature was 240 °C and the detector 260 °C. The injection volume was 0.8 µL (split ratio 1:20). The temperature program was as follows: initial temperature 100 °C held for 1 min, temperature gradient of 3 °C min^{-1} until 220 °C, followed by a gradient of 5 °C min^{-1} until 245 °C and keeping 245 °C during 1 min. The identification of methylated fatty acids (FAME) peaks was carried out by comparing the retention times of the FAME Supelco MIX-37 standards. Results were expressed quantitatively as g kg^{-1} concentration using methyl nonadecanoate as internal standard.

2.3. Statistical analysis

One-way analysis of variance (ANOVA) was conducted for the statistical analysis of data, and, then, the data was subjected to Tukey's multiple range test. Statistically significant differences were considered when $p < 0.05$ and were performed using XLSTAT Premium 2016 (Addinsoft, New York, USA) and Statgraphics Plus (Version 3.1, Statistical Graphics Corp., Rockville, MA, USA).

3. Results and discussion

3.1. Irrigation

Table 1, shows the amount of water received for each treatment. Significant differences among control irrigation (T1) and deficit treatments were found. Minimum values of SWP of each treatment presented statistically significant differences with T2 and T3 samples having the lowest values and T4 similar to the control. Stress integral showed significant differences among the control (T1), RDI (T2, T3) and SDI (T4). In all deficit treatments, the level of water stress was severe; however, no significant differences were found among treatments in yield (T1:2.2 t ha^{-1}; T2: 1.9 t ha^{-1}; T3: 1.9 t ha^{-1}; T4: 1.9 t ha^{-1}). With stress levels achieving a reduction of 10–20 % the targeted levels for T3 and T4 trees; these levels of stress may influence the yield of the next harvest, because the post-harvest period is especially sensitive to the deficit (García Tejero et al., 2018).

Table 1
Applied water (AW, mm), Minimum stem water potential (min ψ_{stem} MPa) and water stress integral (SI, MPa x day) in each regulated deficit irrigation treatment during kernel filling in 2017.

Irrigation treatments	AW	Min ψ_{stem}	SI
ANOVA test[†]			
2017	***	**	***
Tukey's Multiple Range Test[‡]			
2017			
T1	433 ± 26 a	−1.55 a	54.2 c
T2	148 ± 24 b	−2.03 b	91.7 ab
T3	103 ± 3.0 b	−2.08 b	94.9 a
T4	114 ± 13 b	−1.83 ab	74.7 b

[†] NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.

[‡] Values (means of 4 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

3.2. Effect of deficit irrigation on the morphological and physico-chemical parameters

The different deficit irrigation strategies evaluated in the present study did not have a significant effect on most of the almond quality parameters, only a^* color coordinate. This finding agreed with other studies on deficit irrigation not only in almonds but also with pistachio and olives, in which many quality parameters were not affected by deficit irrigation strategies if applied at moderate levels (Cano-Lamadrid et al., 2015; Carbonell-Barrachina et al., 2015; Egea et al., 2009; Sanchez-Rodriguez et al., 2018; Zhu et al., 2015).

As said above just the a^* coordinate (the green–red) and the *Hue* angle were significantly different for treatments when analyzing morphology, instrumental color, and texture (Table 2). The a^* color coordinate gives information about whether a sample is redder, when positive values are obtained, or greener for the negative ones. Table 2 indicates that almonds from the T2 treatment were more reddish when compared to the other treatments under study, especially those from the control treatment, T1. *Hue* coordinate was lower in T2 treatment indicating an intensification on the typical brown color of the almond skin (Martínez-Esplá et al., 2017).

For the texture parameters, no significant differences were observed among the applied treatments. This finding agreed with results on almonds by Cornacchia et al. (2010) in almond trees growing under RDI and partial root-zone drying (PRD) but also in (i) orange trees in the study by Stagno et al. (2015) and (ii) apples under deficit irrigation and sustainable strategies in the study by Du et al. (2015). On the contrary, harder texture was found in studies about pistachio and softest texture in olives under severe water stress conditions (Cano-Lamadrid et al., 2015; Carbonell-Barrachina et al., 2015). Both color and texture are important parameters which the best described the raw almond quality (Contador et al., 2015). Textural properties, specially crunchiness, are of utmost importance for the consumer acceptability (Cheely et al., 2018).

Kernel ratio was not affected by the irrigation treatments, neither the dry weight of the samples (Table 3). This finding agreed with Stewart et al. (2011) who concluded that both crop consumptive water use and irrigation could be decreased without having a significant effect on the almond yield. On the contrary, Egea et al. (2013) showed, in their study about long term deficit irrigation in almond trees, that water stress had a negative impact on the kernel yield during 2002 and 2005, but no differences were observed during 2006 (last year of study with moderate strategies). Regarding kernel dry weight, Egea et al. (2009) concluded that water stress applied at severe levels had a negative impact. Not reducing the almond yield with the deficit irrigation under study signify important gains for almond growers which can produce the same amount of almonds reducing the water consumption.

The average value of moisture content was around 3.9% (39 g kg^{-1}) and no statistically significant differences among treatments were observed. This finding agreed with Cornacchia et al. (2010), who observed no differences on moisture content among treatments. Moisture content is the total quantity of water contained in a product; because the almond is a low-moisture food, the industry standards for the raw almonds range between 3–6 % moisture content. These values are considered as the optimal ones for minimum biological reactions and essential to preserve the almond quality increasing the shelf life (Huang, 2014).

The optimal a_w values for almonds, stored in cool and dry conditions, range from 0.3 to 0.6 (Gama et al., 2018; Huang, 2014). All 4

Table 2
Morphology, instrumental color and instrumental texture of raw almonds as affected by deficit irrigation treatments.

Irrigation Treatment	Weight (g)		Size (mm)						Kernel Color coordinates				Kernel Cutting Force			
	Whole	Kernel	Shell	Whole Length	Kernel Length	Whole Width	Kernel Width	Whole Thickness	Kernel Thickness	L^*	a^*	b^*	Hue	Fracturability (mm)	Hardness (N)	Work to shear (Ns)	Average force (N)	Number of fracture
ANOVA test[†]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS	NS	NS	NS
Tukey's Multiple Range Test[‡]																		
T1	4.96	1.49	3.47	36.9	26.9	26.1	17.0	16.6	8.36	44.6	16.7 b	30.2	60.9 ab	1.78	73.8	65.7	36.5	8.93
T2	4.85	1.46	3.39	36.6	26.5	26.1	16.9	16.3	8.35	44.9	17.4 a	30.8	60.5 b	1.77	73.9	66.8	37.4	9.33
T3	4.96	1.46	3.50	36.9	26.6	26.4	16.8	16.3	8.13	44.8	17.2 ab	30.7	60.7 ab	1.79	72.8	64.4	35.7	9.09
T4	5.06	1.51	3.56	36.0	26.9	26.5	16.9	16.5	8.44	45.2	17.3 ab	31.2	61.0 a	1.82	72.2	65.9	35.7	9.26

[†] NS = not significant at $p < 0.05$; *. **. and ***. significant at $p < 0.05$. 0.01. and 0.001, respectively.
[‡] Values (means of 100 replications) followed by the same letter within the same column and factor were not significantly different ($p < 0.05$), according to Tukey's.

Table 3
Effect of deficit irrigation treatments on yield.

Irrigation Treatment	Kernel Ratio (g kg $^{-1}$)	Dry Weight	Water Activity (aw)	Kernel units (kg)	Defects kernel units (kg)
ANOVA test[†]	NS	NS	**	NS	NS
Tukey's Multiple Range Test[‡]					
T1	322	962	0.57 b	690	40.0
T2	307	957	0.59 a	720	50.0
T3	309	963	0.59 a	700	80.0
T4	309	960	0.60 a	710	80.0

[†] NS = not significant at $p < 0.05$; *. **. and ***. significant at $p < 0.05$. 0.01. and 0.001, respectively.
[‡] Values (means of 16 replications) followed by the same letter within the same column and factor were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

Table 4
Effect of deficit irrigation treatments on physicochemical parameters of raw almonds (dry weight = dw).

Irrigation Treatment	Protein (g kg $^{-1}$)	Fat	Ash	Carbohydrates	Organic acids	Sugars
ANOVA test[†]	NS	***	NS	***	**	NS
Tukey's Multiple Range Test[‡]						
T1	247	488 c	29.3	275 b	11.1 b	45.3
T2	248	523 a	28.7	245 d	11.2 b	46.1
T3	247	495 b	29.7	266 c	13.5 ab	43.9
T4	248	460 c	30.6	303 a	15.3 a	42.4

[†] NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
[‡] Values (mean of 8 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

treatments under study reached values between these limits, but, T2, T3 and T4 showed significantly higher a_w.

Chemical composition of the almonds is presented in Table 4. Neither protein nor ash content were statistically significant different among treatments. This result was similar to those reported by Egea et al. (2009) but did not agree with those reported by Sanchez-Bel et al. (2008), who observed higher protein content for non-irrigated almond farming when compared to the drip-irrigated ones. Almonds from T2 showed significantly higher fat content and the opposite phenomenon was observed for T4. Previous studies proved an increase in the lipid content of almonds and pistachio under moderate RDI (Carbonell-Barrachina et al., 2015; Egea et al., 2009). Zhu et al. (2015) stated that almond fat content was constant under moderate DI, and it did not increase when an excess of irrigation water was applied and started to decrease under severe water stress conditions. Both Nanos et al. (2002) and (Sanchez-Bel et al., 2008) showed a constant fat content when irrigated with non-irrigated samples were compared. Many authors have observed a negative correlation between protein and fat content, when studying almonds growing under water stress: a higher fat content was associated with a lower protein content. However, this phenomenon was not observed in the current study (Egea et al., 2009).

Regarding the carbohydrates, the highest mean value was observed for the T4 samples and the opposite for almonds T2. The almond carbohydrates include polysaccharides frequently linked to dietary fiber and a relatively low amount of soluble sugars, which are responsible for the sweet taste to the almond.

Total sugar content was not influenced by the treatments studied (Table 4) and agreed with other studies about the same topic (Egea et al., 2009; Nanos et al., 2002). Statistically significant differences

were observed in total organic acids content among treatments, with T4 recording the highest values of all treatments (Table 4). This finding disagreed with the results obtained by other authors, who observed no significant differences in organic acids among treatments (Egea et al., 2009; Sanchez-Bel et al., 2008).

From a commercial and industrial point of view the chemical composition of almonds it is very important and depends on the almond production destiny. Food industry, such as almond oil production or *turron* (typical Spanish desert) might be interested in almonds with high fat content (Rabadán et al., 2017; Verdú et al., 2007). From a nutritional perspective, many studies suggest that the almond lipid fraction might be the principal factor to influence alteration in insulin sensitivity and satiety (Mori et al., 2011). Almond protein content for instance can supply the recommended daily intake of protein for children (23.0–36.0 g) and is also a good source for adults (44–56 g of protein recommended daily intake) (Sobowale, 2015). The present study suggested that deficit irrigation did not reduce the protein content with a mean value of 247.5 g kg^{-1}. Eating ~ 30 g of almonds (~ 20 almonds Vairo cultivar) would represents ~ 17% of the total protein daily recommended intake for adults.

3.3. Mineral content

Both the soil and also the irrigation water have an influence in the mineral content of almonds. The main almond minerals include Ca, Cu, Fe, Zn, K, P, Se, and Na (Saura et al., 1988; Yada et al., 2011). The elements studied in the current experiment were Ca, Mg, K (macro-nutrients) and Fe, Cu, Mn and Zn (micro-nutrients). The contents of Mg, Fe, Cu and Zn were not statistically significantly affected by irrigation treatments (Table 5); in fact, the main minerals affected were Ca and K, with T3 treatment leading to the lowest Ca content while T2 led to the highest content of K. All treatments reached a high content of K, which can lead to the conclusion that almonds might be a good source of this element (mean values of all treatments 7.2 g kg^{-1}).

Alimohammadi et al. (2012), in their research about the effect of deficit irrigation in different phenological stages of almond fruit growth and development, found no significant differences for both leaf and fruit mineral contents. Similar results were obtained by Carbonell-Barrachina et al. (2015), who stated that mineral content of pistachio nuts was only slightly affected by the irrigation treatments. They found only two elements (Cu and Zn) which increased with water stress. Other studies in different fruits, such as grapes, olive, tomatoes and apple, also concluded that deficit irrigation strategies can be used without a negative impact on mineral nutrition both in leaves and fruit (Nakajima et al., 2004).

The only element which showed a decrease in deficit irrigation strategies in leaves of olive and grapes was K but no significant differences were obtained in fruits. In the present study, K increased for

Table 5
Minerals content of raw almonds as affected by regulated deficit irrigation.

Irrigation Treatments	Ca (g kg^{-1} dw)	Mg	K	Fe	Cu	Mn	Zn
ANOVA Test	***	NS	***	NS	NS	*	NS
Tukey's Multiple Range Test							
T1	2.742 a	1.867	7.043 b	0.023	0.011	0.029 ab	0.046
T2	2.170 ab	1.852	7.717 a	0.021	0.011	0.029 ab	0.045
T3	1.447 b	1.867	7.057 b	0.022	0.011	0.028 b	0.042
T4	2.814 a	1.890	6.857 b	0.024	0.011	0.029 a	0.040

†NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
‡Values (mean of 8 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

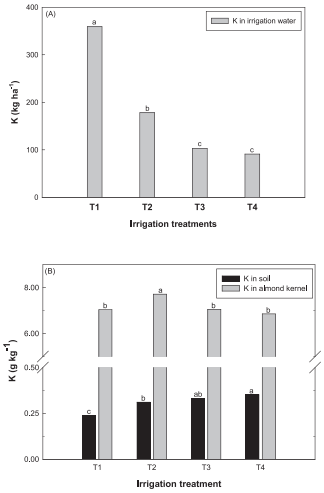

Fig. 1. Potassium content applied with irrigation water and measured in soil and almond kernel.

the treatment with moderate RDI (T2) and the opposite was shown for fully irrigated almonds (T1) as well as in severe RDI and SDI strategies. Fig. 1 showed the applied K content through irrigation (A), and the amount of K found in soil and fruit (B). A strongly negative correlation (-0.992) was observed between the amount of K administrated through irrigation treatments and the K measured at the topsoil (0–20 cm) ($R^2 = 0.98$; $p < 0.01$). A decrease in K content of fruit from fully irrigated treatment (T1) as well as for treatments which presented higher levels of stress in plant (T3 and T4), could occur due to the close relationship which exists between water availability (e.g. leaching of minerals with excess water) and minerals absorption. The soluble mineral absorption depends on the water flow in the soil path to the plant roots. Which means that deficit irrigation applied in right levels could reduce the minerals leaching but also the saturation of minerals and biocides in the root zone (Alikhani-Koupaei et al., 2018). Mineral elements measured in other fruits such as apples, tomatoes, olives and grapes were not affected by deficit irrigation strategies, when compared to the same fruits cultivated in better soil and irrigation conditions (Alikhani-Koupaei et al., 2018; Nakajima et al., 2004).

3.4. Fatty acids

Almond lipid content is manly composed by monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). As reported by literature the main fatty acids in almonds are, oleic (C18:1), linoleic (C18:2), palmitic (C16:0) stearic (C18:0) and palmitoleic (C16:1) acids, in a decreasing order (Yada et al., 2011). In the present study eighteen fatty acids were identified and quantified in all four treatments and they are all presented in Table 6. In agreement with prior studies oleic acid was the most abundant fatty acid found within this study, followed by linoleic, palmitic, stearic, *cis*-vaccenic and palmitoleic. The fatty acid composition predominantly contained MUFA and PUFA. Although there were no statistically significant differences among treatments for the oleic acid, the important finding is that mild water stress did not reduce the content and in fact a small increment could be observed. Oleic acid is a MUFA with a great interest for human's health because it can reduce the low-density-lipoprotein (LDL) cholesterol and it is demonstrated that a higher content of oleic acid in almonds can avoid the fatty acids rancidification during storage, transport and processing (Zamany et al., 2017). The mean oleic acid content of all treatments was ˜65% and similar results (˜62%) in Nonpareil almonds variety was previously reported (Zhu et al., 2015). To compare the level of oleic acid, its contents in extra virgin olive oil, peanut landrace oils, sunflower seed oil, tomato seed oil, were ˜60%, ˜48%, ˜33% and from 17 to

Table 6
The composition of fatty acids in raw almonds under water stress conditions.

Compound (FAMEs) (g kg^{-1} dw)	ANOVA Test[†]	T1	T2	T3	T4
Tukey Multiple Range Test[‡]					
C14:0 (Myristic)	*	0.13 b	0.15 a	0.14 ab	0.13 ab
C15:0 (Pentadecylic)	**NS**	0.06	0.07	0.08	0.07
C16:0 (Palmitic)	*	29.2 ab	30.9 a	29.7 ab	27.8 b
C16:1 (Palmitoleic)	**NS**	2.11	2.23	2.20	2.05
C17:0 (Margaric)	***	0.32 b	0.42 a	0.46 a	0.32 b
C17:1 cis (Heptadecenoic)	*	0.50 ab	0.54 a	0.49 ab	0.48 b
C18:0 (Stearic)	**NS**	9.68	10.7	9.97	9.31
C18:1n9 (Oleic)	**NS**	228	233	226	220
C18:1n7 (Cis-Vaccenic)	*	5.27 ab	5.63 a	5.38 ab	5.05 b
C18:2n6 (Linoleic)	**NS**	80.8	86.8	83.1	77.2
C18:3n3 (α-Linolenic)	**NS**	0.22	0.22	0.20	0.20
C20:0 (Arachidic)	*	0.58 ab	0.65 a	0.58 ab	0.55 b
C20:1 n9 (Eicosenoic)	**NS**	0.37	0.40	0.36	0.36
C20:3 n6 (Eicosatrienoic)	**NS**	0.08	0.08	0.09	0.08
C22:1 (Erucic)	**NS**	0.11	0.14	0.14	0.12
C22:2n6 (Docosadienoic)	**NS**	0.08	0.06	0.08	0.05
C23:0 (Tricosylic)	**NS**	0.05	0.08	0.07	0.06
C24:1 (Nervonic)	**NS**	0.09	0.10	0.09	0.07
Oleic:linoleic acid	**NS**	**2.82**	**2.70**	**2.73**	**2.85**
Total SFA	**NS**	**40.1**	**43.0**	**41.0**	**38.2**
Total MUFA	**NS**	**236**	**242**	**235**	**228**
Total PUFA	**NS**	**81.2**	**87.1**	**83.4**	**77.6**
PUFA : SFA	**NS**	**2.03**	**2.03**	**2.04**	**2.03**
PUFA : MUFA	**NS**	**0.34**	**0.36**	**0.36**	**0.34**
(MUFA + PUFA)/SFA	**NS**	**7.93**	**7.67**	**7.77**	**7.98**
Atherogenic Index (AI)	**NS**	**0.09**	**0.10**	**0.10**	**0.09**
Thrombogenic Index (TI)	**NS**	**0.24**	**0.25**	**0.25**	**0.24**
Total fatty acids	**NS**	**358**	**372**	**359**	**343**

[†] NS = not significant at $p < 0.05$; *. **. ***. significant at $p < 0.05$. 0.01. and 0.001. respectively.

[‡] Values (mean of 4 replications) followed by the same letter within the same raw and factor were not significantly different ($p < 0.05$). according to Tukey's least significant difference test.

26% depending on the tomato variety (Giuffrè and Capocasale, 2016; Giuffrè et al., 2017; Giuffre et al., 2017, 2016). Besides, it was observed that if a thermal stress was applied to extra virgin olive oil the oleic acid increased from 60 to 63% (Giuffrè et al., 2017). The current study revealed statistically significant differences among treatments for six fatty acids and those were myristic (C14:0), palmitic (C16:0), margaric (C17:0), *cis*-heptadecenoic (C17:1), *cis*-vaccenic (C18:1n7), and arachidic (C20:0) acids.

An increase in polyunsaturated fatty acids gives a higher functionality to *hydroSOS* almonds due to many studies in which authors state that products rich in MUFA and PUFA could contribute to cardiovascular and coronary heart diseases as well as obesity, diabetes and cancers (Bitok and Sabaté, 2018). *cis*-Vaccenic which is an omega-7 fatty acid synthetized from palmitoleic acid by way of stearoyl CoA desaturase - 1. Although the studies are very scarce, *cis* - vaccenic acid, it was associated with a lower risk of heart failure from ischemic origin (with antecedent coronary heart disease) (Djoussé et al., 2014). The present study revealed that a significant negative correlation was found between the stress integral and *cis*-vaccenic fatty acid ($R^2 = 0.70$; $p < 0.0001$) and showed that the higher the level of water stress in the plant the lower content of this unsaturated fatty acid (Fig. 2).

The MUFA content (66%) was higher in Vayro almonds than in Nonpareil ones (64%), extra virgin olive oil (49%), peanut landrace oils (49%), sunflower seed oil (33%) and tomatoes seed oil (18%) (Giuffrè and Capocasale, 2016; Giuffrè et al., 2017; Giuffre et al., 2017, 2016; Zhu et al., 2015). In addition, PUFA was also higher (23%) than those of extra virgin olive oil (19%) but lower than in Nonpareil almonds (26%), peanut landrace oils (33%), sunflower seed oil 56% and tomato seed oil (49–63 %).

Table 6 shows that T2 treatment scored the highest values of those

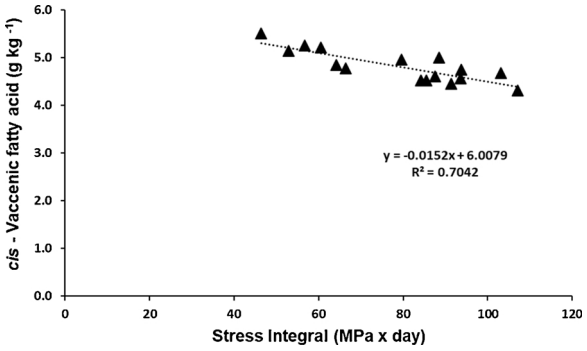

Fig. 2. Correlation between Stress Integral and *cis*–vaccenic (C18:1n7) fatty acid.

statistically significant different fatty acids. Even though, differences were small, fatty acids content increased when RDI was applied at moderate levels while the contrary was observed for the SDI treatment. This finding assented with different authors who concluded that using a moderate deficit irrigation, in almond, pistachio or olives the fatty acid content was increased and the opposite was observed when a severe water stress was applied (Cano-Lamadrid et al., 2015, 2017; Carbonell-Barrachina et al., 2015; Zhu et al., 2015). On the other hand, disagreed with Sanchez-Bel et al. (2008) and Nanos et al. (2002) which found that using irrigation lead to a superior almond oil quality than a non-irrigation treatment.

Yada et al. (2011) affirmed that both quantity and quality of the lipid content is highly dependent on the genotype, geographical area, climatic conditions of the growing season, and water applied. Irrigation usually was found to have limited effects on the lipid quantity and quality. These affirmations are actually stated by this study because no differences among treatments were observed neither for oleic:linoleic, PUFA:SFA, PUFA:MUFA ratio nor for total SFA (saturated fatty acids), MUFA and PUFA. A high oleic:linoleic ratio is an important benchmark to assess the storage stability of almond kernel and oil due to its preventive effect on lipid oxidation during processing, storage and transport as explained above (Colic et al., 2017).

PUFA/SFA ratio is usually calculated because provides information about weather a diet is atherogenic or could promote coronary heart diseases; this ratio has been calculated using only hypercholesterolemic acids (lauric, myristic, and palmitic), as required by several authors (Ulbricht and Southgate, 1991). On the other hand, thrombogenic index offer information about formation of clots in the blood vessels and is defined as the ratio between pro-thrombogenic (represented by SFA) and anti-thrombogenic (MUFAs, PUFAs-n6 and PUFAs-n3) (Batista et al., 2017). In this context, analyzing the present study results can be concluded that irrigation treatments did not influence in the atherogenic and thrombogenic index, maintaining in this way the health properties of almonds. In addition, if compared to other products it can be said that almonds have a reduced atherogenic and thrombogenic index (Batista et al., 2017).

4. Conclusions

This study is one of the first simultaneously evaluating quality parameters (morphological, physico-chemical and functional) of *hydroSOS* almonds under regulated (RDI) and sustained (SDI) deficit irrigation. As a general conclusion it can be highlighted that almost all of the measured parameters were not affected by none of the applied deficit irrigations strategies. After one-year long experiment kernel ratio was not influenced by DI treatments. However, there were some statistically significant differences among treatments and almonds from T2, observing an improvement in relation to different compounds. Thus, almonds from moderated RDI (T2) were characterized by a redder color, a higher fat, and potassium content, as well as a greater

unsaturated fatty acid (*cis*-heptadecenoic, *cis*-vaccenic). For all this reason, moderate RDI (T2) could be recommended as a suitable strategy when water availability is below to crop irrigation requirements, without committing the final yield and fruit quality.

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Nutrition Quality Parameters of Almonds as Affected by Deficit Irrigation Strategies

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Abstract: The influence of full irrigation, double-regulated (RDI) and sustained deficit irrigation (SDI) treatments on almond quality was assessed by analyzing different parameters: sugars, organic acids, antioxidant activity, total phenolic content (TPC), and volatile compounds. Almond quality studies for plants submitted to water stress are scarce, and it is essential to understand the biochemical responses of plants to water stress in maintaining fruit yield and quality. Citric acid, sucrose, antioxidant activity, and TPC were not affected by the application of studied deficit irrigation strategies (DI). An increase in malic acid and a decrease in glucose was observed for stressed samples (T3 and T4), while a higher number of total volatiles compounds was found for moderate RDI (T2). Using deficit irrigation strategies, the almond yield and quality was not changed, and in fact, some parameters, such as glucose and key volatile compounds, slightly increased under moderate RDI. This finding might encourage farmers to implement these strategies and contribute to sustainable agriculture.

Keywords: sugars; antioxidants; phenols; volatile compounds; hydroSOStainable products; *Prunus dulcis*

1. Introduction

Fresh (non-salty and adequate for irrigation) water is a limited resource and the uncertainty of the remaining amount for the next generation has it this a dramatic global risk factor [1,2]. Agriculture is the primary use of fresh water worldwide because more than 40% of food production comes from irrigated fields, and it is in a weak position due to its susceptibility to weather (temperatures and precipitation) changes [3]. Mediterranean agriculture is a perfect model of arid and semiarid farming,

in which fields must deal with limited irrigation water supplies due to scarce rain leading to periodic drought and competitiveness with other productive sectors, such as tourism [4,5]. For this reason, identifying agricultural practices that increase water use efficiency is necessary to be able to develop a sustainable agricultural system [6].

When considering the cultivated surface area, almond (*Prunus dulcis*) is the third most cultivated tree in Spain, and the major tree nut crop in the Mediterranean area [4,6]. Although it is a drought resistant species, it is believed that irrigation is needed to improve yield and fruit quality [7]. However, many authors showed that irrigation water could be reduced, maintaining or even slightly improving fruit quality by using deficit irrigation (DI) strategies [6,8–14]. Oher nuts, such as pistachio, are also considered a great alternative for arid and semiarid areas, helping farmers to increase income by saving irrigation water but having minimum impact on yield and fruit quality when the adequate irrigation strategy is applied [10,15]. A DI strategy refers to an agricultural practice in which irrigation water is applied below the crop evapotranspiration (ET; the soil evaporation and plant transpiration losses) needs. Regulated deficit irrigation (RDI) is a DI strategy in which the amount of irrigation water is reduced during a specific period when the crop is less stress sensitive. On the other hand, in sustained deficit irrigation (SDI), a uniform and reduced amount of water is applied during the whole growing cycle of the plant or tree, developing a progressive stress in the plant [6]. Products obtained from plants submitted to a controlled water stress are called hydroSOStainable (*hydroSOS*) [2,13–19].

Sugars and organic acids of "Vairo" almonds under water stress conditions are scarce in the scientific literature, and although organic acids play a limited role in the quality of almonds, sugars are essential for good flavor and taste [20]. The antioxidant activity (AA) is related to the ability of almonds to reduce pro-oxidant agents; AA is considered a key quality feature by consumers because an increase in the consumption of antioxidant compounds was associated with reduced obesity in women and also helped in reducing the risk of stroke and cardiovascular diseases, and even some cancer types [21]. Almond polyphenols are mostly found in its skin, with values ranging from 9.10 to 32.1 g kg^{-1} (skin values obtained from blanched and roasted almonds, freeze-dried or dried in a hot-air oven at 60 °C) [22]. However, almond skin is often removed by blanching for commercial reasons and this unit operation will drastically reduce the polyphenol content. Volatile compounds are responsible for the characteristic flavor properties of raw and processed almonds and contribute to their high consumer acceptance. Benzaldehyde is one of the main volatiles in bitter almond, but in general, it is found in very low amounts in sweet almonds [23].

The aim of this work was to evaluate the influence of RDI and SDI conditions on quality parameters of almonds, namely on the content of sugars, organic acids, total phenolic and volatile compounds, and on the antioxidant activity. Optimizing the water resources in almond farming in the Mediterranean area of Spain is essential for the sustainability of Spanish agriculture.

2. Results and Discussion

2.1. Irrigation

Four irrigation treatments, including a control one, were applied at different stress levels. The amount of irrigation water applied within this study ranged from 100 to 433 mm (T1 = 433 ± 26; T2 = 148 ± 24, T3 = 103 ± 3.0, and T4 = 114 ± 13 mm). Figure 1 shows the almond yield for each treatment (Figure 1a), the minimum values of the stem water potential (Figure 1b), and the waters stress integral (Figure 1c) for each treatment. It is very important to highlight that almond yield was not significantly ($p < 0.05$) affected by any of the treatments under study. These results agreed with previous studies on almonds and pistachios grown under regulated deficit irrigation [10,24]. Regarding almonds, the study was done for the "Marta" genotype over five years, and although a yield decrease was observed for the first and fourth years, no differences were seen for second, third, and fifth years [24]. Moreover, authors working with pistachio from Kerman cultivar concluded that the nut yield was not affected by RDI in any of the two seasons under the studied conditions [10].

The implementation of deficit irrigation DI strategies was possible by controlling the crop water status through midday stem water potential. As observed in Figure 1b,c, T1 showed the lowest levels of stress with a value of −1.55 MPa for the minimum stem water potential (SWP) and a stress integral (SI) of 54.2 MPa × day, while T3 (severe RDI) displayed the highest levels of stress for both parameters (MinSWP = −2.08 MPa; SI = 94.9 MPa × day).

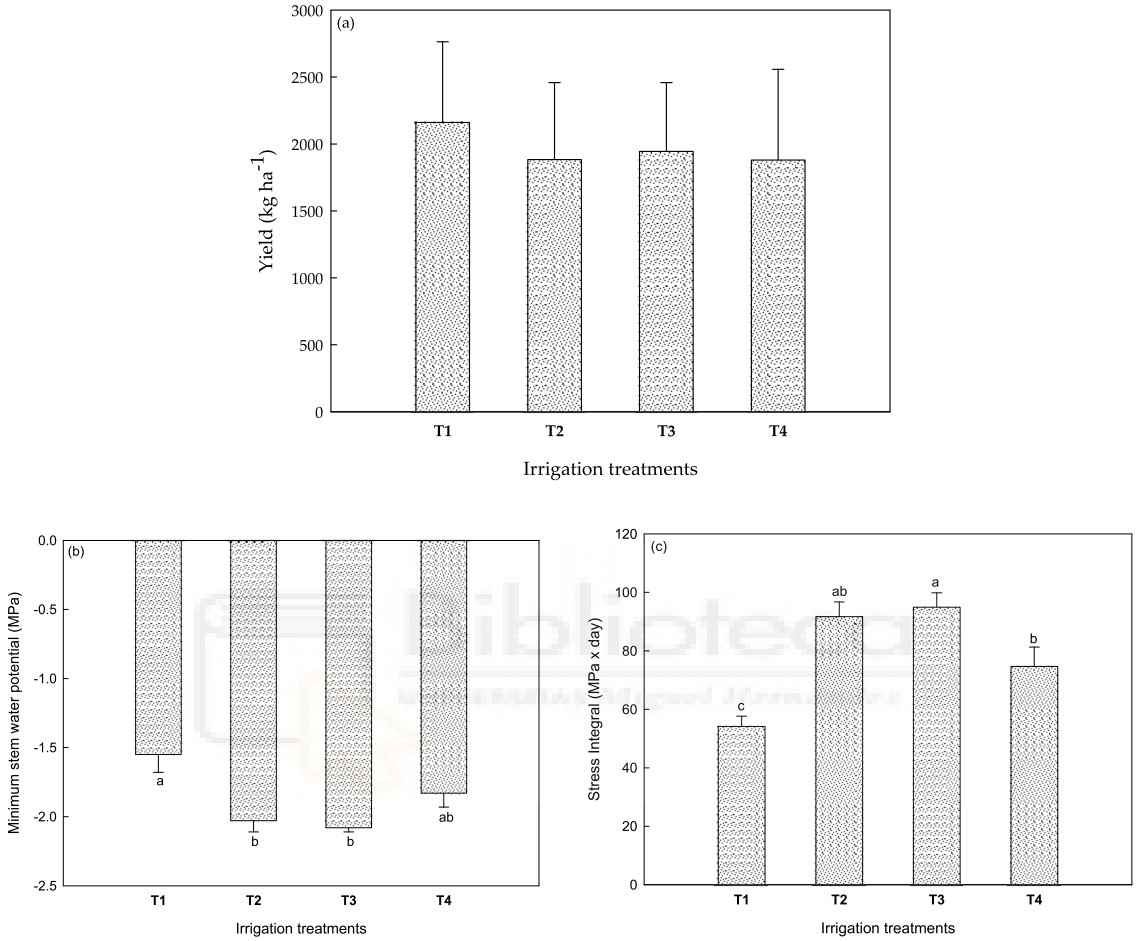

Figure 1. Almond yield (**a**), minimum stem water potential (**b**), and water stress integral (**c**) for each treatment. Bars with the same letter were not significantly different ((**a**) $p < 0.001$; (**b**) $p < 0.01$; (**c**) $p < 0.001$), according to Tukey's least significant difference test.

2.2. Sugars and Organic Acids

Sucrose was the main soluble sugar found in almonds, representing 85–91% of the total carbohydrates, due to its accumulation during ripening and because many other reducing sugars are substrates for its synthesis [25]. Table 1 shows that the results of the sucrose content ranged from 33 to 35 g kg^{-1}, being significantly similar among treatments. Previous studies reported a sucrose increase with irrigation (total water supply of ~10 m^3 $tree^{-1}$ during summer) in almonds from "Ferragnes", "Texas", and "Guara" genotypes, but different irrigation strategies were applied [20,25]. On the contrary, other authors observed a higher sucrose content in non-irrigated almonds from "Marta" variety and linked it to the plant adaptive physiological responses to water stress, while a significant similarity between those irrigated at full (110%) ET, 50% ET periodically supplied to only one side of the root system for the entire year (PRD), and RDI (irrigation at 100% of ET for the entire year and at 30% of ET from early June until harvest) was observed [26].

Table 1. Sugars and organic acid profiles and contents (g kg^{-1} dry weight (dw)) of raw almonds as affected by deficit irrigation treatments.

Treatment	Sugars		Organic Acids	
	Sucrose	Glucose	Citric	Malic
	(g kg^{-1} dw)			
	ANOVA Test †			
	NS	***	NS	**
	Tukey's Multiple Range Test ‡			
T1	33.31 ± 0.69	12.03 ± 0.53 a	2.27 ± 0.04	8.81 ± 0.47 b
T2	34.29 ± 0.49	11.78 ± 0.33 a	2.23 ± 0.04	9.02 ± 0.51 b
T3	35.47 ± 0.68	5.58 ± 0.28 b	2.31 ± 0.05	11.18 ± 0.96 ab
T4	35.12 ± 2.31	6.38 ± 0.53 b	2.36 ± 0.07	13.02 ± 1.33 a

Note: † NS = not significant at $p < 0.05$; * and ** significant at $p < 0.05$ and 0.01, respectively; ‡ Values (mean of 8 replications) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), according to Tukey's multiple range test.

Glucose, fructose, sorbitol, raffinose, and inositol were other monosaccharides found previously in almonds [25], however in this study, glucose and fructose were the only reducing sugars identified. While fructose was found only in trace amounts (data not shown), glucose content presented statistically significant higher values for T1 and T2 (moderate RDI) treatments. Sánchez Bel et al. [25] in studies about almonds ("Guara" genotype), and Nahar et al. [27] in tomatoes, concluded that water stress enhanced the sweetness of tomatoes by increasing their glucose content. These results might be related to the osmotic adjustment, which can be activated by accumulation of solutes (rich in hydroxyl (-OH) groups, such as sugars, proline, etc.) in the cytoplasm under stress conditions. This biochemical mechanism aids plants in naturalizing to dry and saline conditions by protecting the cellular membrane, protein, and enzymes against dehydration [28]. Consequently, the osmotic adjustment enhances the capacity to maintain positive turgor, increasing the sugars and organic acid [27]. Egea et al. [6] and Cornacchia et al. [26] obtained no statistically significant differences among fully irrigated and DI strategies in almond trees.

The main identified organic acids (Table 1) were citric and malic. Citric acid was not affected by DI, as other authors have previously reported [6]. On the contrary, 0.4% increase of citric acid content was observed in drip–irrigated "Guara" almonds (16.8 m^3 $year^{-1}$) with respect to non-irrigated almonds [25]. An increase of 2.4 and 4.2 g kg^{-1} of malic acid content for T3 and T4 (SDI), respectively, was observed; this experimental finding agreed with previous studies on tomatoes and grapes, in which water stress enhanced their quality by raising the concentration of important organic acids [27]. However, a decrease of malic acid content in "Guara" almonds under non-irrigated conditions [11] and in "Marta" almonds under partial root zone (PRD) conditions (irrigation supplied at 50% ET during the whole growing season) was observed [25]. Malic acid was stable for moderate RDI and similar results were reported in "Marta" almonds under RDI (trees were irrigated at 50% ET during kernel filling and at 100% ET for the rest growth period) [6]. Studies in organic acid content in almonds under water stress conditions are scarce, although it seems that organic acids may play a limited role in the quality of almonds.

2.3. Antioxidant Activity ($ABTS^+$, $DPPH^•$, and FRAP methods) and Total Phenolic Content

Table 2 shows the results of antioxidant activity and total phenolic content (TPC) analyzed in raw kernel, blanched kernels, and almond skin. An enormous difference in antioxidant activity and polyphenols for raw almonds, blanched, and skin was observed, and mean values of all treatments were: (i) $ABTS^+$ (2.2-azino-bis) = 9.5, 1.5, and 36 mmol Trolox kg^{-1}, respectively; (ii) $DPPH^•$ (2,2-diphenil-1-picrylhydrazyl) = 28, 20, and 33 mmol Trolox kg^{-1}, respectively; (iii) FRAP (ferric reducing ability of plasma) = 3.9, 0.5, and 62 mmol Trolox kg^{-1}, respectively.

Table 2. Antioxidant activity (mmol Trolox kg^{-1}) and total phenolic content (mg gallic acid equivalents, GAE kg^{-1}) of almond, as affected by deficit irrigation treatments.

	ABTS^{+} (mmol Trolox kg^{-1})	**DPPH$^{\bullet}$ (mmol Trolox kg^{-1})**	**FRAP (mmol Trolox kg^{-1})**	**TPC (g GAE kg^{-1})**
		ANOVA †		
Raw almond	NS	NS	NS	NS
Blanched almond	*	NS	*	*
Skin	NS	NS	***	NS
Raw almonds ‡				
T1	9.24 ± 0.84	26.1 ± 1.10	5.00 ± 0.56	5.06 ± 0.22
T2	10.24 ± 0.66	29.3 ± 0.58	3.05 ± 0.47	5.83 ± 0.28
T3	9.04 ± 0.68	27.8 ± 0.10	3.22 ± 0.53	5.80 ± 0.34
T4	9.61 ± 0.33	27.4 ± 0.96	4.38 ± 0.32	5.39 ± 0.23
Blanched almonds ‡				
T1	1.65 ± 0.06 a	19.8 ± 0.52	0.50 ± 0.01 b	0.49 ± 0.06 b
T2	1.44 ± 0.07 ab	19.6 ± 1.00	0.53 ± 0.01 ab	0.71 ± 0.05 a
T3	1.71 ± 0.01 a	21.9 ± 0.56	0.60 ± 0.02 a	0.53 ± 0.01 b
T4	1.30 ± 0.12 b	19.0 ± 0.09	0.57 ± 0.03 ab	0.42 ± 0.06 c
Almond skin ‡				
T1	35.8 ± 1.42	32.4 ± 1.54	67.6 ± 1.05 a	13.0 ± 0.34
T2	37.2 ± 0.33	33.6 ± 1.15	64.2 ± 0.62 ab	12.6 ± 0.16
T3	35.1 ± 0.92	34.9 ± 1.56	62.4 ± 0.94 b	12.7 ± 0.18
T4	36.3 ± 1.43	36.5 ± 1.45	54.4 ± 1.41 c	12.3 ± 0.02

Note: † NS = not significant at $p < 0.05$ and * significant at $p < 0.05$; ‡ Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), according to Tukey's multiple range test.

The TPC found in this study for raw kernel (5.5 g gallic acid equivalents (GAE) kg^{-1}, mean value of all treatments) was higher than those previously reported by other authors (range from 0.6 to 1.9 g GAE kg^{-1}) but lower than that reported by Lin et al. [29] (7.5 g GAE kg^{-1}) for the almonds from Almond Board of California. The difference could be attributed to factors such as variety, geographical area, or agricultural practices [21,30]. Blanched kernel TPC (0.5 g GAE kg^{-1}) was similar to that reported in literature (0.7 g GAE kg^{-1}) for almonds in general [30]. Finally, a mean TPC value of 13 g GAE kg^{-1} was found on almond skin and similar values were reported for American almonds (11–17 g GAE kg^{-1}), but lower values were found for Spanish almonds (26 g GAE kg^{-1}) [22].

The total dietary intake of polyphenols is estimated to be around 1 g [31]. The results of the present study showed that, for example, ~30 g of almonds (~20 kernels of "Vairo" genotype cultivated in Sevilla, Spain) provides ~170 mg of total phenols and represents almost 20% of the total dietary polyphenols intake.

Antioxidant activity and TPC were not significantly affected by the irrigation treatment in the raw almond. Regarding the blanched almonds, T4 was found to have the lowest antioxidant activity measured with ABTS and the lowest TPC. Blanched samples from the moderate RDI (T2) showed the highest TPC and the antioxidant activity was similar to the control (for the ABTS and DPPH methods). Regarding the almond skin, the TPC and antioxidant activity (ABTS and DPPH) were not affected by the irrigation treatments, except for FRAP method, which showed that T2 was similar to the control and T4 had the lowest antioxidant activity. These results agreed with Cano Lamadrid et al. [32] and Sánchez-Rodríguez et al. [12] in studies investigating effects of deficit irrigation on quality and functional profiles of table olives (hydroSOStainable olives) and pomegranates, respectively [9,12,33]. An increase, however, in polyphenols and antioxidant activity was observed for lettuce growing under water stress conditions [34] and grapes from clusters exposed to RDI (compared with SDI), which might happen due to the reduction of canopy leaf area of vines under RDI conditions [35].

2.4. Volatile Compounds

Twenty-six compounds were identified and quantified in the volatile profile of "Vairo" almonds. A total of 10 alcohols, 9 alkanes, 3 aldehydes, 1 terpene, 1 ketone, and 1 organic acid were identified and are presented in Table 3, together with their retention time, retention indices, and their odor descriptors, while Table 4 presents the contents of the volatile compounds. Alcohols, which were the most abundant volatiles (0.82 mg kg^{-1}), are released by enzymatic reactions in raw almond and contribute to the characteristic sweet aroma and to the consumer acceptance [23]. Most of the alcohols were not affected by the studied treatments, except 1-hexanol, which had the highest content, 0.40 mg kg^{-1}, in T2 almonds. High levels of hexanol content was also found by other authors in Nonpareil almonds extracted with a similar method [23]. It is known that hexanol increases with the almond ripening and is associated with herbal odor (fruity, alcoholic, sweet, green notes) and green flavor (fruity, apple skin, oily) [11,36]. Garcia Esparza et al. [11] in studies about DI in grapes concluded that watering during post-veraison (the change of grapes color) at 75% of the crop ET (irrigation was applied to replace 75% of crop ET) compared to rain fed decreased the alcohol content (from 4.08 to 3.91 mg g^{-1}) and increased that of the aldehydes (hexanal from 0.43 to 0.50 mg g^{-1}), which produced herbaceous (non-desirable) aromas in wines [11].

Table 3. Profile of volatile compounds in almonds, retention index, and main odor and aroma descriptors.

Compound	Chemical Family	Code	RT (min)	Retention Index †		Odor Descriptor
				Experimental	Literature ‡	
Ethanol	Alcohol	V1	2.235		489	Strong alcoholic ethereal medical [36]
2-Butanol	Alcohol	V2	2.363		608	Sweet apricot [36]
Hexane	Alkane	V3	2.861		600	Petroleum like [37]
3-Methyl furan	Furan	V4	2.986		646	
Acetic acid	Acid	V5	3.217		663	Pungent acidic cheesy vinegar [36]
3-Methyl-2-butenol	Alcohol	V6	4.864	761	770	Sweet fruity, green lavender [36]
1-Pentanol	Alcohol	V7	4.959	763	761	Pungent, fermented, bready, yeasty, winey, solvent [36]
2-Methyl-1-butanol	Alcohol	V8	5.048	765	748	Roasted, wine, onion, fruity, fusel, alcoholic, whiskey [36]
3-Methyl-1-butanol	Alcohol	V9	5.190	768	768	Fusel, alcoholic, cognac, fruity, banana, molasses [36]
2,3,3-Trimethyl pentane ¥	Alkane	V10	5.361	771	768	
2,2,5-Trimethyl hexane ¥	Alkane	V11	6.059	787	789	
2-Hexanol	Alcohol	V12	6.675	801	801	Chemical, winey, fruity, fatty, terpenic, cauliflower [36]
Hexanal	Aldehyde	V13	6.763	803	803	Fresh green fatty aldehydic grassy leafy fruity sweaty [36]
1-Hexanol	Alcohol	V14	9.795	870	869	Ethereal, fusel, oily, fruity, alcoholic, sweet, green [36]
Nonane	Alkane	V15	11.198	901	900	Gasoline [36]
2-Heptanol	Alcohol	V16	11.551	907	906	Fresh, lemongrass, herbal, sweet, floral, fruity, green [36]
Benzaldehyde	Aldehyde	V17	14.982	962	962	Almond, fruity, powdery, nutty, cherry, sweet, bitter [36]
2,2,4,6,6-Pentamethylheptane ¥	Alkane	V18	16.650	989	997	
Decane	Alkane	V19	17.461	1001	1000	
Limonene	Terpene	V20	19.416	1029	1029	Citrus, orange, sweet, fresh, peely [36]

Table 3. *Cont.*

Compound	Chemical Family	Code	RT (min)	Retention Index †		Odor Descriptor
				Experimental	Literature ‡	
Benzyl alcohol	Alcohol	V21	20.062	1038	1040	Sweet, floral, fruity, rose, balsamic nuances [36]
Undecane	Alkane	V22	24.615	1101	1100	
Nonanal	Aldehyde	V23	24.994	1107	1107	Waxy, aldehydic, citrus, green lemon peel, orange peel [36]
Dodecane	Alkane	V24	31.945	1201	1200	
Tridecane	Alkane	V25	39.090	1301	1300	
β-Damascone ¥	Ketone	V26	44.816	1385	1383	Fruity, floral, berry, plum, black currant, honey, rose, tobacco [36]

Note: ¥ = tentatively identified (identification only based on spectral database); † RT = retention time; ‡ = NIST (National Institute of Standards and Technology) [38].

Table 4. Volatile compounds (mg kg^{-1}) found in raw almonds as affected by water stress. The quantification of these volatile compounds is based on the use of β-ionone as internal standard.

Code	Chemical	ANOVA †	T1	T2	T3	T4
			(mg kg^{-1})			
V1	Ethanol	NS	0.11	0.21	0.17	0.16
V2	2-Butanol	NS	0.06	0.08	0.09	0.07
V3	Hexane ¥	NS	0.02	0.05	0.04	0.02
V4	3-Methyl furan	*	0.01 b	0.02 a	0.01 b	0.01 b
V5	Acetic acid	NS	0.03	0.05	0.04	0.02
V6	3-Methyl-2-butenol	NS	0.02	0.04	0.04	0.02
V7	1-Pentanol	NS	0.05	0.10	0.07	0.05
V8	2-Methyl-1-butanol	NS	0.05	0.08	0.05	0.04
V9	3-Methyl-1-butanol	NS	0.01	0.02	0.02	0.01
V10	2.3.3-Trimethyl pentane ¥	NS	0.01	0.02	0.03	0.02
V11	2.2.5-Trimethyl hexane ¥	NS	0.03	0.07	0.09	0.06
V12	2-Hexanol	NS	0.04	0.09	0.07	0.12
V13	Hexanal	NS	0.08	0.11	0.13	0.06
V14	1-Hexanol	***	0.31 ab	0.40 a	0.16 c	0.25 bc
V15	Nonane	NS	0.03	0.06	0.09	0.08
V16	2-Heptanol	NS	0.01	0.03	0.03	0.01
V17	Benzaldehyde	NS	0.03	0.06	0.04	0.04
V18	2,2,4,6,6-Pentamethyl heptane ¥	**	0.11 b	0.23 ab	0.46 a	0.50 a
V19	Decane	NS	0.23	0.48	0.39	0.42
V20	Limonene	*	0.04 ab	0.09 a	0.05 ab	0.03 b
V21	Benzyl alcohol	NS	0.02	0.05	0.02	0.03
V22	Undecane	NS	0.52	0.66	0.52	0.53
V23	Nonanal	NS	0.18	0.32	0.23	0.20
V24	Dodecane	NS	0.29	0.54	0.39	0.45
V25	Tridecane	***	0.16 c	0.45 ab	0.54 a	0.25 bc
V26	β-Damascone ¥	NS	0.06	0.08	0.06	0.06
	TOTAL	**	2.50 b	4.39 a	3.85 ab	3.52 ab

Note: † NS = not significant at $p < 0.05$; *. **. ***, significant at $p < 0.05$., 0.01., and 0.001, respectively; ‡ values (mean of 4 replications) followed by the same letter within the same row were not significantly different ($p < 0.05$), according to Tukey's least significant difference test; ¥ = tentatively identified.

From the alkanes (2.2 mg kg^{-1} mean values), statistically significant differences among studied treatments were found for pentamethyl heptane and tridecane. Pentamethyl heptane increased in T3 and T4 samples and tridecane in T2 and T3 samples. Previous studies showed that tridecane was formed by decarboxylation of myristic fatty acid (C14:0) [39] and this fact could be explained by the

higher content of tridecane in T2 and T3, which can be induced by the water stress produced in the almond trees by RDI and could affect the plant metabolism.

Limonene was the only terpene found, and statistically significant differences were observed among the samples. Moderate RDI (T2) increased the limonene content, which is associated with fresh, citrus, and sweet notes. This result agreed with Carbonell et al. [10], who observed a higher content of limonene (from 12.4% to 14.8%) in pistachio under moderate RDI [10].

Benzaldehyde, which is the major volatile compound in bitter almond, was not predominant in these almonds, and this might be attributed to the low content of amygdalin [23]. Amygdalin is a cyanogenic glycoside naturally produced in almond, and it is the benzaldehyde precursor [23]. No statistically significant differences between the control and DI treatments were observed, implying that the sensory quality might not be affected by DI strategies. The finding agreed with other authors in studies of pistachio under RDI [10].

The total content of volatile compounds was significantly different among samples. Almonds from moderate RDI showed a higher total content (4.39 mg kg^{-1}) as compared to the control samples (2.50 mg kg^{-1}). Similar results (4.36 mg kg^{-1}) were observed for raw almonds (mixture of "Butte" and "Padre" varieties) [40]. The aroma of raw almonds is, in general, weak, and a low total content of volatile compounds is usually expected. However, this content can be increased during roasting (6.17 mg kg^{-1} after 28 min of roasting; 11.4 mg kg^{-1} after 33 min of roasting, and 16.0 mg kg^{-1} after 38 min of roasting) [40] due to the Maillard and lipid oxidation reactions [40–42].

In this study, a reduced number and content of aldehydes was found, confirming the freshness of the studied almonds. In previous studies, aldehydes (e.g., hexanal) levels significantly increased, from 0.42 mg kg^{-1} in raw Butte and Padre almonds to 0.98 mg kg^{-1} in almonds roasted for 28 min at 138 °C and to 1.63 mg kg^{-1} after 24 weeks of storage at 35 °C [43]. During storage, and due to lipid oxidation reactions [44], hexanal increased, and this is why this compound (hexanal) is considered an indicator of oxidation or rancidity (low degree of freshness) in nuts and nut oils. The freshness (low level of rancidity) of all four almond samples was confirmed by the low content of hexanal and nonanal found [44,45]. Both hexanal and nonanal values showed no statistical significant differences among treatments, as well as a low content of hexanal and a similar content of nonanal when compared to the literature [46].

2.5. Pearson's Correlation Coefficients

Table 5 shows the Pearson's correlation coefficients among studied variables with significant differences among treatments. A positive and significant correlation was observed between stress integral (SI) and (i) TPC ($R = 0.72$; $p < 0.01$), (ii) 2-butanol ($R = 0.57$; $p < 0.05$), and (iii) total volatile content ($R = 0.75$; $p < 0.001$). A positive correlation between the TPC and water stress levels in leaves of *Solanum villosum* and roots of *Solanum scabrum* has been reported [47]; this same positive correlation was also observed in tomatoes plants and maize [48]. Water stress can create damage in plants due to formation of reactive oxygen species (ROS) and to the alteration of the water–plant relationship [48]. The degree to which the plant can avoid or soften the physiological processes determines the resistance degree to water stress of each plant species [48]. Volatile compounds, which confers the typical almond aroma, were also found to be positively correlated with water deficit in different plants, such as grapevines, apples, tomatoes, and strawberry [49]. The increase in the total volatile compound value might be associated with the metabolic responses that deal with (i) high levels of light energy and (ii) formation of oxidative compounds under drought [49].

Table 5. Pearson's correlation coefficients (*R*) among parameters.

	SI	Glucose	Malic Acid	Oleic Acid	Linoleic Acid	TPC	2-Butanol	Hexanal	Nonanal	TVC
Stress integral (SI)	**1.00**									
Glucose	−0.30	**1.00**								
Malic acid	0.21	−0.89	**1.00**							
Oleic acid (C18:1n9)	0.25	0.76	−0.89	**1.00**						
Linoleic acid (C18:2 n6)	0.57	0.50	−0.68	0.94 *	**1.00**					
Total phenolic Content (TPC)	0.72 **	−0.06	0.06	−0.08	0.18	**1.00**				
2-Butanol (V2)	0.57 *	−0.12	−0.20	0.19	0.18	0.29	**1.00**			
Hexanal (V13)	0.74	−0.01	−0.36	0.65 *	0.83 *	0.53	0.79	**1.00**		
Nonanal (V23)	0.75	0.32	−0.30	0.68 *	0.84 *	0.61	0.65	0.61	**1.00**	
Total volatile compounds (TVC)	0.75 ***	−0.02	0.23	0.02	0.37	0.76 ***	0.53 *	0.65 **	0.53 *	**1.00**

Note: *, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.

A negative correlation was found between the contents of glucose and malic acid (R = −0.89; $p > 0.05$). Partially similar results were reported in other studies on plums, apricots, and apples [50,51]. This negative correlation is normally observed during the ripening process, because glucose and fructose increased due to the reaction of invertase enzyme through glycolysis, while the organic acids decreased because they are used in respiration and are converted into sugars [52].

A significant correlation was observed between the TPC and the total content of volatile compounds (R = 0.76; $p < 0.001$); this same trend was also found in olives [53].

A statistically significant correlation was found between the contents of linoleic acid and hexanal (R = 0.83; $p < 0.05$), and between oleic acid and nonanal (R = 0.68; $p < 0.05$). This agreed with previous studies, in which positive correlations were reported for the content of linoleic acid and the production of aldehydes ((*E*)-2-heptenal, (*E*)-2-octenal, (*E*,*E*)-2,4-decadienal and (*E*,*E*)-2,4-nonadienal) [44]. The oils containing linoleic (C18:2) and linolenic (C18:3) acids are converted into hexanal, (*E*)-2-hexenal, or (*Z*)-3-hexenal through the enzymatic pathway of lipoxygenase and hydroperoxide lyase [46]. Thus, hexanal is derived from 13-hydroperoxide, which is one of the most abundant hydroperoxides produced by autooxidation of linoleic acid, and it is directly linked to the development of oxidative off-flavors [44]. On the other hand, nonanal is produced from the oleic acid (C18:1) [44,45].

3. Materials and Methods

3.1. Plant Material, Growing Conditions and Experimental Design

The experiment was performed during the 2017 season at the commercial farm "La Florida" (37.23° N, −5.91° W, Dos Hermanas, Seville, Spain). The almond (*Prunus dulcis*) orchard was 7 years-old at the beginning of the experiment. There were 2 almond cultivars in the orchard, "Guara" and "Vairo", and the tree spacing for both cultivars was 6 m × 8 m. The experimental plots had 4 lines of 3 trees and measurements were performed in the central trees of the "Vairo". The trees were irrigated with a line of drip emitters (3.8 L h^{-1}) separated by 0.4 m. Irrigation scheduling was performed daily.

The seasonal weather data were obtained from the "Instituto de Investigación y Formación Agraria (IFAPA) Los Palacios" station, in the Andalusian weather stations network (Figure 2). This station is about 6 km away from the experimental orchard. The data for 2017 was typical of Mediterranean zones, with null rainfall during the summer period and warm winters.

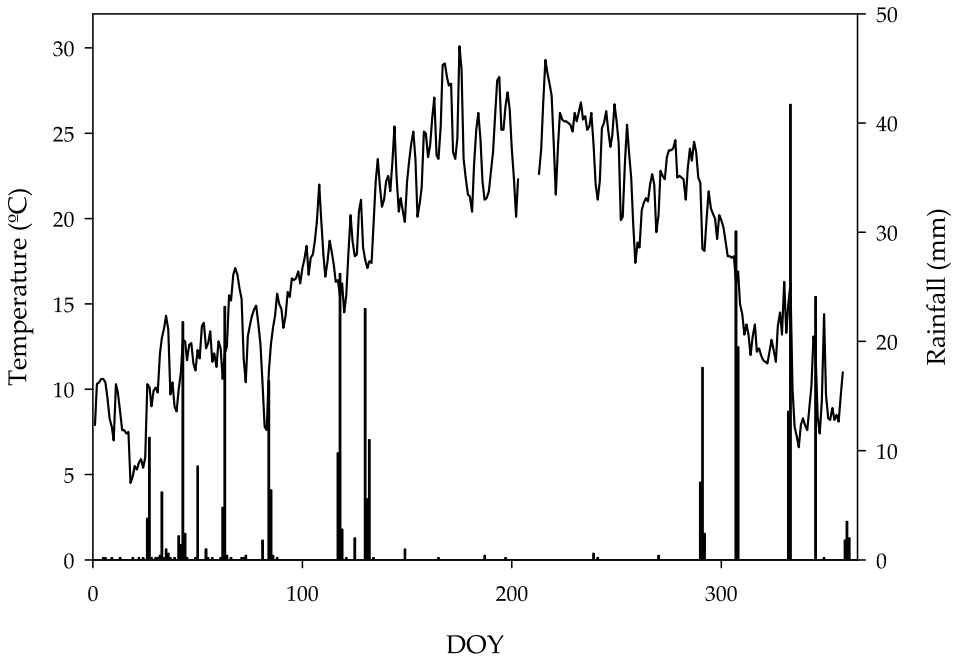

Figure 2. Annual pattern of daily mean air temperature and rainfall. Data were obtained from the "IFAPA Los Palacios" station, which is approximately 6 km away from the experiment site. This meteorological station is part of the Andalusian agroclimatic stations network (Junta de Andalucía).

The irrigation was scheduled according to measurements performed using a pressure chamber (PMS Instrument Company, Albany, OR, USA) and the threshold values of midday stem water potential (SWP) were measured to evaluate the level of plant stress. Three irrigations treatments were established together with a control treatment:

- Full irrigation (T1), to assure the estimated ET during the entire growing season.
- Moderate RDI (T2); in the period of kernel filling, almonds were irrigated when SWP < −1.5 MPa, and for the rest of the time, trees were irrigated to keep a SWP as the baseline proposed by McCutchan and A Shackel [54].
- Severe RDI (T3); the same as T2, except trees were irrigated when SWP < −2 MPa during kernel filling.
- SDI (T4); a lower amount of water was distributed uniformly throughout the year.

Equation (1) was used to calculate the stress integral (SI) and min ψ_{stem} represented the average of minimum SWP for any interval, while *n* was the number of days interval:

$$SI = |\sum(\min \psi_{stem} - (-0.2)) \times n \quad (1)$$

The harvest was done in August using a self-propelled trunk shaker with collector. Each treatment was harvested separately, and almonds were sun-dried to reach a moisture content below 5%, and then delivered to Miguel Hernández University for quality analyses. Almonds were shelled, and the kernels were ground, vacuum packed, and frozen until analysis.

3.2. Sugars and Organic Acids

High-performance liquid chromatography (HPLC) equipment was used to identify and quantify the sugars and organic acids as previously described by Lipan et al. [2] with some modification. Almond finely ground (1 g) in a Moulinex grinder (AR110830) for 10 s was homogenized with 5 mL of phosphate buffer 50 mM (pH = 7.8) with an homogenizer (Ultra Turrax T18 Basic) during 2 min at 11,300 rpm, while the tube was maintained in an ice bath, then was centrifuged (Sigma 3–18 K;

Sigma Laborzentrifugen, Osterode and Harz, Germany) for 20 min at 15,000 rpm and 4 °C and was filtered (0.45 µm Millipore membrane filter). The filtered supernatant (10 µL) was injected into a Hewlett Packard (Wilmington DE) series 1100 (HPLC) using 0.1% ortophosphoric acid elution buffer. Sugars were measured using a Supelcogel TM C-610H column (30 cm × 7.8 mm) with a pre-column (Supelguard 5 cm × 4.6 mm; Supelco, Bellefonte, PA) and the detection was carried out with a refractive index detector (RID). Organic acids were separated in the same HPLC condition as sugars and absorbance was measured at 210 nm with a diode-array detector (DAD). Calibration curves were run in triplicate injection using standards of different organic acids and sugars provided by Sigma (Poole, UK). Analyses were run in triplicate and results were expressed as g kg^{-1} dw.

3.3. Fatty acids Analysis

Fatty acids methyl esters (FAMEs) were prepared as described by Lipan et al. [2] with some modification, while identification and quantification were done according to Tuberoso et al. [55]. Briefly, 40 mg of ground almond were saponified with 100 µL of dichloromethane (Cl_2CH_2) and 1 mL of sodium methoxide solution and kept for 10 min at 90 °C. Boron trifluoride (BF_3) methanolic (1 mL) was added, followed by 30 min of reaction in darkness. FAME extraction from the mixture was done with 1.5 mL hexane. The separation of FAMEs was conducted using a Shimadzu GC17A gas chromatography coupled with a flame ionization detector and a DB-23 capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) J&W Scientific, Agilent Technologies. Helium gas was used as the carrier with a flow rate of 1.1 mL min^{-1}, and 35 mL min^{-1} at the make-up point. The temperatures of the injector and detector were 240 and 260 °C, respectively. The injection volume was 0.8 µL (split ratio 1:20). Finally, the temperature program was: initial temperature 100 °C held for 1 min, temperature gradient of 3 °C min^{-1} until 220 °C, followed by a gradient of 5 °C min^{-1} until 245 °C, and kept at 245 °C for 1 min. FAME peaks identification was performed by comparing the retention times of the FAME Supelco MIX-37 standards and the results were expressed quantitatively as g kg^{-1} concentration using methyl nonadecanoate as internal standard.

3.4. Antioxidant Activity and Total Phenolic Content

The antioxidant activity and total phenolic content was carried out not only for raw kernel but also for blanched almond and its skin. The blanching process consisted of almond immersion in boiling water (100 °C) for 2 min, followed by manual skin removal. The method of extraction consisted of 0.5 g of sample being sonicated with 10 mL of extractant (MeOH/water (80:20, *v*/*v*) + 1% HCl at 20 °C) for 15 min and stored for 24 h at 4 °C. The next day, the mixture was sonicated again under the above-mentioned conditions, then, it was centrifuged at 10,000 rpm for 10 min.

The antioxidant activity of the obtained extract was measured using 3 methods: $DPPH^{\bullet}$, $ABTS^{+}$, and FRAP. $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) free radical was used to determine the radical scavenging activity of the sample as described by Brand-Williams et al. [56]. A brief description of the process is that 10 µL of the sample were mixed with 40 µL of MeOH and 990 µL of the free radical solution, shaken, and placed in darkness for 15 min. Later, the sample absorbance was measured at 515 nm. Moreover, 10 µL of the sample supernatant was mixed with 990 µL solution of $ABTS^{+}$ (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) or FRAP (ferric reducing antioxidant power) free radicals to determine the free radical scavenging capacity of the sample. After 10 min of reaction, the sample absorbance was read at 734 nm for $ABTS^{+}$ method and 593 nm for FRAP. All measurements were carried out in an ultraviolet-visible (UV-vis) spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK). The quantification was done according to the calibration curve of Trolox, prepared in a concentration ranging from 0.5 to 5.0 mmol Trolox L^{-1}. The linearity was above $R^2 = 0.998$ and results were expressed in mmol Trolox kg^{-1} [9,56].

Total polyphenolic content (TPC) was determined using the Folin-Ciocâlteu colorimetric method, in which 100 µL of sample supernatant were mixed with 200 µL Folin-Ciocâlteu reagent and 2 mL of H_2O_2. This mixture was stored at 22 °C for 3 min and 1 mL of 20% sodium carbonate was added,

followed by incubation for 1 h at room temperature. Later, the mixture absorbance was measured at 765 nm in the above-mentioned equipment. The results were calculated according to the gallic acid calibration curve and were expressed as gallic acid equivalents (GAE), g GAE kg^{-1} [9,56].

3.5. Volatile Compounds Analysis

Volatile compounds were extracted using headspace solid phase microextraction (HS-SPME). For the extraction, 2 g of grinded almond and 50 µL of β-ionone (100 mg L^{-1}) were placed in a hermetic vial with polypropylene cap and PTFE(polytetrafluoroethylene)/silicone septa and was used as an internal standard; this internal standard was used for the semi-quantification of the volatile compounds, as no calibration curve was done for each of the compounds reported in this study. The vial was placed in a water bath at controlled temperature to assure the vial was 40 °C, which was needed to simulate the mouth temperature when chewing almonds. Once the temperature was reached and was constant, a 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber was introduced in the headspace of the vial for 50 min. This fiber is characterized by high capacity of trapping volatile compounds from fruits and nuts. Moreover, the fiber was desorbed for 3 min in the injector port of a gas chromatograph Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) coupled with mass spectrometer (MS) detector Shimadzu GC-MS QP-5050A used for the volatile compounds identification. The GC-MS was equipped with a SLB-5ms Fused Silica Capillary Column of 30 m × 0.25 mm × 0.25 µm film thickness, 5% diphenyl, and 95% dimethyl siloxane (Supelco Analytical). For the analyses, helium was used as gas carrier at a flow rate of 0.7 mL min^{-1} in splitless mode. The oven program was: (a) initial temperature 40 °C, (b) rate of 2.0 °C min^{-1} to 145 °C, (c) rate of 25 °C min^{-1} from 145 to 300 °C and hold for 90 s. In addition, the injector was kept at 230 °C, while the detector at 320 °C. The volatile compounds identification was done by using 3 methods: (a) retention indices, (b) Gas Chromatography - Mass Spectrometry (GC-MS) retention times of authentic chemicals, and (c) mass spectra (authentic chemicals and NIST05 spectral library collection) [38].

4. Statistical Analysis

The statistical analyses were done by using one-way analysis of variance (ANOVA), and data were submitted to Tukey's multiple range test to compare means. Statistically significant differences were considered when $p < 0.05$ and were studied using XLSTAT Premium 2016 (Addinsoft, New York, USA). Pearson's correlation was carried out with the same program in which data were subjected to Correlation Tests. For figures preparation, Sigma Plot 11 was used.

5. Conclusions

Based on the literature search, this study was the first to evaluate the quality parameters (sugars, organic acids, antioxidant activity, and total contents of phenolic and volatile compounds) of hydroSOStainable "Vairo" almonds under regulated (RDI) and sustained (SDI) deficit irrigation. Almond yield was not affected either by RDI or SDI. Almonds are a very good source of polyphenols (5.5 g GAE kg^{-1}; mean values for all treatments), and regarding the antioxidant activity and total phenolic compounds, there were no significant differences among irrigation treatments. Moderate regulated deficit irrigation led to almonds with high glucose content (potentially linked with almond sweetness) and total content of volatile compounds (potentially linked with almond odor, aroma, and flavor), implying a high sensory quality. Consequently, almond quality can be improved if the water stress in the plant is induced in a controlled way, contributing to sustainable agriculture with greater benefits for the farmers through commercialization of these almonds as hydroSOS products.

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Sample Availability: Samples of the compounds are available from the authors until the end of 2019. The samples are stored at 4 °C in darkness, and it is planned to store them until the end of 2019.

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PHYTOPROSTANES AND PHYTOFURANS –OXIDATIVE STRESS AND BIOACTIVE COMPOUNDS– IN ALMONDS ARE AFFECTED BY DEFICIT IRRIGATION IN ALMOND TREES

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Phytoprostanes and Phytofurans—Oxidative Stress and Bioactive Compounds—in Almonds are Affected by Deficit Irrigation in Almond Trees

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ABSTRACT: Almonds have gained consumers' attention due to their health benefits (they are rich in bioactive compounds) and sensory properties. Nevertheless, information about phytoprostanes (PhytoPs) and phytofurans (PhytoFs) (new plant markers of oxidative stress and compounds with biological properties for human health) in almonds under deficit irrigation is scarce or does not exist. These compounds are plant oxylipins synthesized by the oxidation of α-linolenic acid (ALA). Besides, they are biomarkers of plant oxidative degradation and biologically active molecules involved in several plant defense mechanisms. hydroSOStainable or hydroSOS mean plant foods made from from plants under controlled water stress. Almonds are a good source of polyunsaturated fatty (PUFAs) acids, including a high content of ALA. This paper aimed to describe the influence of diverse irrigation treatments on *in vitro* anti-oxidant activity (AAc) and total phenolic content (TPC), as well as on the level of ALA, PhytoP, and PhytoF in "Vairo" almonds. The AAc and TPC were not affected by the irrigation strategy, while the *in vivo* oxidative stress makers, PhytoPs and PhytoFs, exhibited significant differences in response to water shortage. The total PhytoP and PhytoF contents ranged from 4551 to 8151 ng/100 g dry weight (dw) and from 33 to 56 ng/100 g dw, respectively. The PhytoP and PhytoF profiles identified in almonds showed significant differences among treatments. Individual PhytoPs and PhytoFs were present above the limit of detection only in almonds obtained from trees maintained under deficit irrigation (DI) conditions (regulated deficit irrigation, RDI, and sustained deficit irrigation, SDI) but not in control almonds obtained from fully irrigated trees. Therefore, these results confirm PhytoPs and PhytoFs as valuable biomarkers to detect whether an almond-based product is hydroSOStainable. As a final conclusion, it can be stated that almond quality and functionality can be improved and water irrigation consumption can be reduced if controlled DI strategies are applied in almond orchards.

KEYWORDS: *α-linolenic acid, stress biomarkers, Prunus dulcis, regulated deficit irrigation, sustained deficit irrigation, hydroSOStainable products*

■ INTRODUCTION

Numerous scientific studies about climate change have reported the current impact on global water resources and, although water management has mainly been locally confronted, issues about water scarcity are certainly global.[1] In this respect, insufficient water for farming practices in arid and semiarid countries can severely affect manufacturing sectors in other countries along the global supply chains.

Almond (*Prunus dulcis* Mill.) is the major tree nut crop in the Mediterranean Basin. Although this crop is considered as a drought-resistant crop, irrigation is necessary to obtain a profitable yield of high-quality nuts.[2,3] However, the volume of irrigation water can be significantly reduced by using deficit irrigation strategies (DI) in almonds, pistachios, table and olive oil olives, and pear−jujube fruit orchards, among others, without jeopardizing or even improving the quality and functional profiles of the final products.[4−10] A DI agricultural strategy is used with irrigation water applied below the crop evapotranspiration (ET). Regulated deficit irrigation strategy (RDI) in almond trees is referred to as the reduction of the amount of irrigation water applied during the kernel filling phase, which is the less sensitive phenological stage of the almond tree to water stress.[11,12] Besides, sustained deficit irrigation (SDI) is focused on applying a uniform but reduced volume of water (always below the optimal value) along the whole growing season, causing gradual stress in the trees.[11]

Supported by the results published by recent studies (regarding both composition and capacity to modulate pathophysiological situations) the inclusion of almonds in a diet has been evaluated, encouraged by their feature as an outstanding dietary source of vitamin E, fiber, mono- and polyunsaturated fatty acids, polyphenols, amino acids (highlighting among others: glutamic acid, aspartic acid, leucine, and

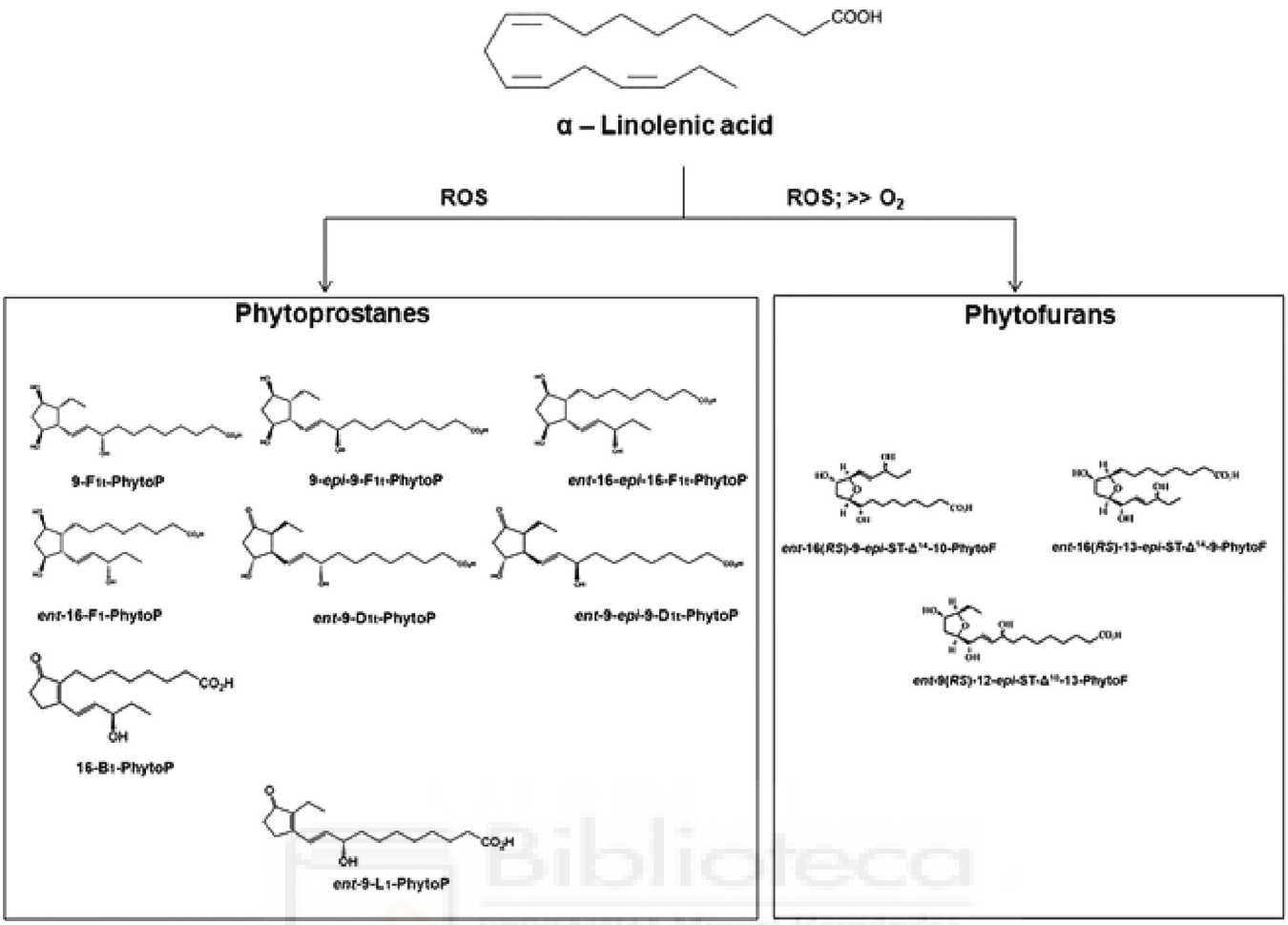

Figure 1. Chemical structures of the phytoprostanes and phytofurans standards used in the study according to Collado-González et al. and Pinciroli et al. using the Taber/Roberts nomenclature.[38,46,76]

arginine), and minerals, while their composition has been associated with high hypocholesterolemic indices and the reduction of atherogenic and thrombogenic events.[5,6,13−15] Hence, the proximate and phytochemical compositions of almonds have been associated with the prevention of heart diseases, cancer, diabetes, DNA damage, and oxidative stress in smokers.[15−20]

Since plants do not present chain elongation and desaturase enzymes needed to form arachidonic acid (AA), higher plants cannot produce isoprostanes, which are molecules produced in mammals from AA under oxidative conditions.[14,21−28] However, plants produce important amounts of other oxylipins generated from α-linolenic acid (ALA), from which the phytoprostanes (PhytoPs) stand out from the crowd.[29−33] These compounds are generated from ALA bounded to plant membranes by a nonenzymatic peroxidation process initiated by reactive oxygenated species (ROS), and they have been described as biomarkers of oxidative stress (OS) condition in plant tissues (Figure 1).[34−38] On the contrary, phytofurans (PhytoFs) are biosynthesized under 21% oxygen pressure and also represent valuable biomarkers in higher plants.[36−41] Both types of plant oxylipins can be found free or esterified in plant foods.[41]

Besides being reliable biomarkers of OS, PhytoPs and PhytoFs are also lipid mediators.[39] PhytoPs have many health benefits such as (i) protecting cells against the damage produced under OS conditions, playing an important role in the immune and neuronal function regulation; (ii) interacting with gut microflora; and (iii) developing anti-inflammatory and apoptosis-inducing activities and (iv) as anti-oxidants[7,21,29,42−44] These actions have been recently associated with the structural similarities of PhytoPs and PhytoFs vs human oxylipins[44−46]

In the present, only a few studies on PhytoPs in almonds, nuts, and olives have been reported[7,8,13,14,47] and scarce relationships have been established between agricultural practices like water management and these plant oxylipins. We hypothesize that water constrains/water stress leads to the synthesis of phytoprostanes and phytofurans on almonds and that such bioproducts may serve as indicators of water stress. Therefore, this study aimed to evaluate, for the first time, the effect of different DI strategies (regulated and sustained) on the anti-oxidant activity and total phenolic content, as well as on the profile and PhytoPs and PhytoFs content in almonds (kernel) "Vairo" cv. and their potential role as biomarkers of RDI and SDI.

MATERIALS AND METHODS

Materials and Design of the Experiment. The assay was carried out for 2017 season in a 7 year old commercial farm called "La Florida" (37.23° N, −5.91° W, Dos Hermanas, Seville, Spain). The almond (*Prunus dulcis* Mill.) orchard included the Vairo cultivar, following a 6 m × 8 m square pattern with a tree soil cover of 25% for T1, 27% for T2, 23% for T3, and 22% for T4. The experimental

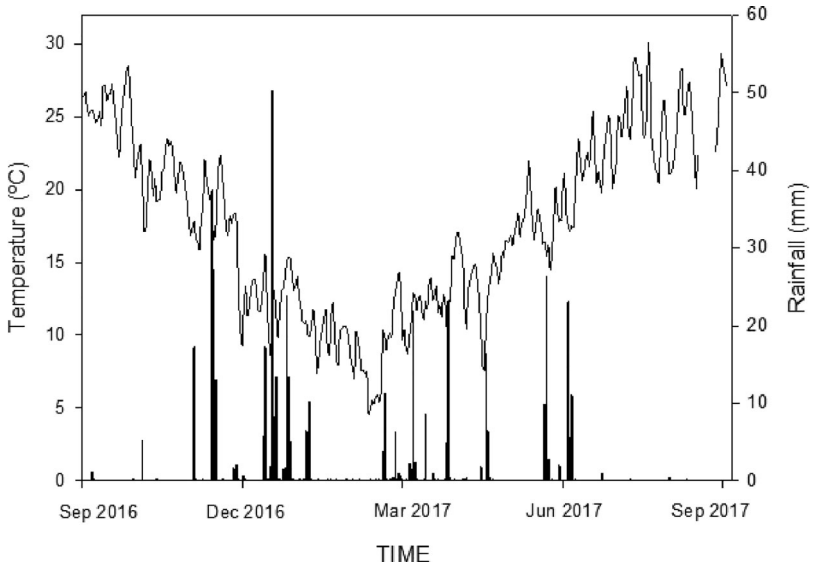

Figure 2. Annual pattern of daily mean air temperature and rainfall. Data were obtained from the "IFAPA Los Palacios" station, which is approximately 6 km away from the experiment site. This meteorological station is part of the Andalusian agroclimatic stations network (Junta de Andalucía).

blocks included 4 lines of 12 trees per row, and data collection were carried out in the central trees. The irrigation was carried out with a line of drip emitters (3.8 L/h) separated by 0.4 m; irrigation scheduling was performed daily.

Irrigation Treatments. Three irrigations treatments (T2−T4) were evaluated together with a control treatment (T1). The characteristics of each irrigation conditions were as follows: T1, full irrigation, to ensure the estimated ET (the soil evaporation and the plant transpiration losses) during the entire growing season; T2, moderate RDI, during the kernel filling, trees were irrigated when the stem water potential (SWP) was below −1.5 MPa, while during the remaining time, trees were irrigated to maintain an SWP as the baseline in agreement with McCutchan and Shackel;[48] T3, severe RD, identical conditions as those described for T2 but trees were irrigated when SWP was below −2.0 MPa during the kernel filling stage; T4, SDI, an equal proportion of ET wa applied through almond phenological development, producing a constantly increasing water stress along the growing season.

Climate, Plant Water Status, and Yield. Seasonal weather data were obtained from a station situated about 6 km away from the experimental field, which belongs to the "Instituto de Investigación y Formación Agraria (IFAPA) Los Palacios" of the Andalusian weather stations network (Figure 2).[8] The data for this season was characteristic of Mediterranean regions, with no rainfall in summer and warm winters.

Irrigation was planned in agreement with the data provided by a pressure chamber (PMS Instrument Company, Albany, OR, USA), and the threshold values of midday (12:00 solar time) SWP were taken into account to measure the level of plant stress. The SWP was measured in 2 trees of each replicate, on the middle third of the trees in fully developed leaves. The leaves were introduced into small plastic bags covered aluminum film for 2 h before the measurements. Four flowmeters per each treatment were used to calculate the applied water on each plot. Only leaves were analyzed for SWP, all other parameters were measured on almonds.

Equation 1 was used to measure the stress integral (SI), in which min ψ_{stem} represented the average of minimum SWP for any interval and n was the number of days in that interval:

$$SI = \left|\left|\sum (\min \psi_{stem} - (-0.2)) \times n \right.\right. \quad (1)$$

At the end of season (August, 2017), monitored trees (4 trees per treatment) were harvested (28 weeks after blossom) with a self-propelled trunk shaker with collector, monitoring each tree separately. Around 6 kg of in-shell almonds per each tree were sent to the laboratory for quality analysis. Previously, each sample set extended horizontally until getting a moisture content below 5%. Finally, the almonds were analyzed in the university laboratory. Almonds were shelled, and the kernels were ground, vacuum packed, and frozen until analysis.

Anti-Oxidant Activity and Total Phenolic Content. The anti-oxidant activity (AAc) and total phenolic content (TPC) were analyzed in agreement with Lipan et al. with some modifications.[49]

The extraction protocol for the analysis of AAc was started by sonicating 1 g of ground almond in 7 mL of the extracting solution [MeOH/water (80:20, v/v) + 1% HCl at 20 °C] for 15 min. Mixtures were left overnight at 4 °C. Samples were sonicated again for 15 min in the water at room temperature and centrifuged at 12 500 rpm for 5 min. The supernatants extracted for the evaluation of the AAc were measured using two methods: (i) 2,2-azinobis (3-ethylbenzothiazo-line-6-sulfonic acid ($ABTS^+$) at 734 nm and (ii) ferric reducing anti-oxidant power (FRAP) at 593 nm. A Trolox calibration curve was prepared, and the data were detailed in millimols of Trolox equivalent per kilogram of dry weight (mmol TE/kg dw).

The TPC extraction consisted of the sonication of 0.5 g of ground sample in 10 mL of extractant for 15 min. After 24 h at 4 °C, samples were sonicated again for 15 min in the water at room temperature and centrifuged at 12 500 rpm for 5 min. The supernatant was measured using Folin−Ciocâlteu colorimetric method. The quantification of TPC was done using a gallic calibration curve freshly prepared each day of analysis, and results were expressed in grams of gallic acid equivalents per kilogram of dry weight (g GAE/kg dw). All measurements were run in an ultraviolet−visible (UV−vis) spectrophotometer (Helios γ model, UVG 1002E; Helios, Cambridge, UK).

Determination of α-Linolenic Content. ALA fatty acid was determined as previously described in hydroSOStainable almonds.[5] Ground almond (~40 mg), 100 μL of dichloromethane, and 1 mL of sodium methoxide solution were refluxed at 90 °C for 10 min. The sample was cooled in an ice bath for 3 min and then 1 mL of boron trifluoride (BF_3) methanolic solution (13−15% BF_3 basis) was added, and the mixture was kept under darkness for a 30 min reaction. Hexane (1.5 mL) was used for ALA extraction. α-Linolenic acid was detected in agreement with Tuberoso et al.[50] after being separated using a gas chromatograph (Shimadzu GC17 A) with a flame ionization detector and a DB-23 capillary column (30 m length × 0.25 mm internal diameter × 0.25 μm film thickness; J&W Scientific, Agilent Technologies). The carrier gas was helium (He) (at a

Table 1. Total Phenolic Content, Anti-Oxidant Activity ($ABTS^+$ and FRAP), and α-Linolenic Acid Content in Vairo Almonds Produced under Different Irrigation Conditions

irrigation treatments	TPC (g GAE/kg dw)	$ABTS^+$ (mmol TE/kg dw)	FRAP (mmol TE/kg dw)	ALA (g/kg dw)
		ANOVA test[a]		
	N.s.	N.s.	N.s.	N.s.
		Tukey's multiple range test[b]		
T1	5.12 ± 0.22	4.40 ± 0.26	1.40 ± 0.06	0.21 ± 0.01
T2	6.17 ± 0.26	5.81 ± 0.30	1.85 ± 0.08	0.18 ± 0.01
T3	6.10 ± 0.31	6.24 ± 1.12	1.93 ± 0.29	0.19 ± 0.01
T4	5.53 ± 0.22	4.03 ± 1.14	1.31 ± 0.30	0.20 ± 0.01

[a]N.s. = not significant at $p < 0.05$. [b]Values (mean of 12 replications) followed by the same letter within the same column and factor were not significantly different ($p < 0.05$) according to Tukey's least significant difference test. $ABTS^+$ = 2.2-azino-bis; FRAP = ferric reducing ability of plasma; TPC = total phenolic compounds; dw = dry weight; ALA = α-linolenic fatty acid. T1 = full irrigated, T2 = moderate regulated deficit irrigation (RDI), T3 = severe RDI, T4 = sustained deficit irrigation.

constant pressure of 316 KPa, initial flow of 1.1 mL/min), and detector gases were H_2 (30 mL/min) and air (350 mL/min), with He (35 mL/min) as makeup gas. The injector and the detector temperatures were 240 and 260 °C, respectively, while the injection volume was 0.8 μL and the split ratio was 1:20. The oven temperature program was as follows: (i) 100 °C held for 1 min; (ii) 3 °C/min until 220 °C; (iii) 5 °C/min until 245 °C, and held at 245 °C for 1 min. The peak corresponding to ALA was identified by comparing the retention times with those of Supelco MIX-37, the authentic standard, and methyl nonadecanoate was used as an internal standard. The results of α-linolenic acid were expressed as grams per kilogram of dw.

Phytoprostane and Phytofuran Analysis. Ground almond (~1 g) was suspended in 2.5 mL of a solution containing MeOH/BHA (1 g/L) (99.9:0.1, v/v). Then, the mixture was stirred for 10 min in an ice bath, sonicated for 10 min in an ultrasound bath, and again stirred for 10 min under the same conditions. The mix was centrifuged at 2000 rpm for 10 min, and then, it was extracted and filtered through a Sep-Pack C_{18} cartridge (Waters, Milford, MA).[36,51] The PhytoPs and PhytoFs were diluted and extracted by solid-phase extraction (SPE). Briefly, 0.5 mL of the extracted and filtered sample was dissolved in 5 mL of hexane, 1 mL of MeOH, and 1 mL of bis-tris buffer (0.02 M HCl, pH = 7). This emulsion was extracted into a Strata X-AW cartridge (100 mg per 3 mL) preactivated with 8, 2, and 2 mL of hexane, MeOH, and Milli-Q water, respectively. To wash the cartridge with the sample, hexane, Milli-Q water, MeOH, Milli-Q water (1:3, v/v), and acetonitrile (2 mL of each one) were used. The analytes were recovered with 1 mL of MeOH, and then, they were concentrated to dryness by means of a SpeedVac concentrator (Savant SPD121P, Thermo Scientific). The dried samples were dissolved into 200 μL (90:10 (v/v)) of water/acetic acid (99.99:0.01, v/v) and methanol/acetic acid (99.95:0.01, v/v). Then, they were filtered through 0.45 μm PVDF filters (Millipore) and 20 μL were injected in triplicate. The results were expressed in ng/100 g dw.

UHPLC Coupled to Triple Quadrupole Mass Spectrometry Analysis. The separation of the almond oxylipins was developed in agreement with Collado-González et al. and Domínguez-Perles et al.[36,51] by using UHPLC coupled to a 6460 triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany) with a BEH C_{18} column (2.1 mm × 50 mm, 1.7 μm) (Waters, Milford, MA). The column temperature was 6 °C. Water/acetic acid (99.99:0.01, v/v) (solvent A) and methanol/acetic acid (99.99:0.01, v/v) (solvent B) were used as mobile phases at a flow rate of 0.2 mL min^{-1}. The injection volume was 20 μL. The gradient program used was: 60% B at 0 min, 62% B at 2 min, 62.5% B at 4 min, 65.0% B at 8 min, and 60% B at 8.01 and 1.5 min of column equilibration. The electrospray interface was set up in the negative mode, and the mass spectrometry analysis were run in the multiple reaction monitoring mode (MRM). The quantitative evaluation of PhytoPs and PhytoFs in almonds was carried out using authentic standards. The PhytoP standards included the following PhytoPs: 9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, 9-D_{1t}-PhytoP, 9-*epi*-9-D_{1t}-PhytoP, 16-B_1−PhytoP, and 9-L_1-PhytoP. The PhytoFs standards studied were: *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF. Both PhytoPs and PhytoFs were obtained in agreement with Cuyamendous et al.[40,52−54] (Figure 1).

Statistical Analysis. The design of experiments was randomized with four replications per treatment, and data were analyzed using a one-way analysis of variance (ANOVA) and a Tukey's multiple range test. Statistically significant differences were considered at $p < 0.05$. Pearson's correlation coefficients were calculated to measure associations between two variables. All statistical analyses were performed using XLSTAT Premium 2016 (Addinsoft New York), while SigmaPlot 11 was used for figures preparation.

RESULTS AND DISCUSSION

Plant Water Status. During the experimental growing season, a total of 433.3 mm of water was applied for T1, 148.0 mm for T2, 103.3 mm for T3, and 114.2 mm for T4. Thus, reductions of 66, 76, and 74% in irrigation water for T2, T3, and T4 almond treatments were observed, respectively. However, no statistical differences were found among the 3 DI treatments (T2−T4), meaning that T2, T3, and T4 almond trees received significantly lower volumes of irrigation water as compared to the optimum irrigation treatment (T1) but similar among them. The minimum SWP was significantly different among treatments with values ranging from −1.55 (T1) to −2.08 MPa (T3). Regarding the almonds stress integral control trees and DI treatments, they showed the following decreasing order: T3 (95.0 MPa/day) > T2 (92.0 MPa/day) > by T4 (75.0 MPa/day) > control and T1 (54.0 MPa/day on average).

Total Phenolic Content (TPC) and Anti-Oxidant Activity ($ABTS^{\bullet+}$ and FRAP methods). As the mechanism of action of anti-oxidants is diverse depending on the dominant features of the anti-oxidant composition, two methods of measurement were used to analyze the anti-oxidant activity of the polyphenolic almonds extracts ($ABTS^{\bullet+}$ and FRAP).

The TPC and AAc of almonds of Vairo *cv.* were investigated. As provided, no significant differences among the irrigation treatments were found neither for TPC nor for anti-oxidant activity (Table 1). In this aspect, the mean value for all treatments for TPC was 5.73 g GAE/kg dw, while the $ABTS^{\bullet+}$ and FRAP values were of 5.13 and 1.62 mmol TE/kg dw, respectively. These results are in agreement with other authors working with *cv.* Vairo, although they found a concomitant correlation between the TPC and the stress integral.[49] Moreover, other authors working with olives of *cv.* "Manzanilla", under moderate RDI, reported an increase of TPC in both raw and table olives as a result of water shortage.[55] Besides, a significant impact on the functionality of

pistachio from *cv.* "Kerman" was also described after the application of a moderate RDI, which caused an increase of TPC.[56] In this regard, many studies reported an increase in TPC values in plants grown under stress conditions, in agreement with their role as plant molecules against biotic and abiotic stress.[6] This argument has been justified by the fact that when the carbohydrates exceed the amount used for growth needs, the excess of CO_2 assimilated under stressing conditions is used for the biosynthesis of carbon-based secondary metabolites.[57] The similarities between the treatments of the present study may occur due to the level of stress applied, which may not be enough to affect the TPC and AAc of the studied almonds. For instance, in SDI, the stress is created progressively in plants through the whole season,[11] and similarities in results between the control and SDI treatment were also reported in grape berries and decreasing of TPC and AAc in pomegranates. This trend was associated with a reduction in polyphenolic biosynthesis[10,58]

α-Linolenic Fatty Acid Content. In parallel to the assessment of samples TPC and AAc, the content of the ALA was determined . This compound showed a mean value of 0.19 g/kg dw and was not significantly affected by the irrigation treatment (Table 1). These results are in agreement with those previously described in Vairo, Marta, Guara, and Lauranne cultivars of tree almond growth under hydric stress conditions.[5,59] However, a slight increase of the ALA content was reported recently in "Nonpareil" *cv.* almonds cultivated under 55 and 70% RDI relative to wet and SDI (55, 70, and 85% irrigation) samples.[60] The lipid fraction is essential for building elements of the cell membrane and storage products, showing modifications in chemical and physiological properties of the environment.[61] As the plasma membrane is the first place to receive water stress damage, it results in changes in the fatty acid composition of the cell membrane.[61] Higher plants produce this polyunsaturated fatty acid, from which PhytoPs and PhytoFs are generated through nonenzymatic autoxidation.[62]

Qualitative and Quantitative PhytoP and PhytoF Profile. PhytoPs and PhytoFs are plant oxylipins produced by radical catalyzed oxidation of ALA, as explained in the previous section and detected by UHPLC-QqQ-MS/MS (Supporting Information).

In this study, eight PhytoPs belonging to two series (9 and 16) of the D_{1t}, F_{1t}, L_1, and B_1 classes were found. Besides, two PhytoFs were also identified and quantified in hydroSOStainable almonds: *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF. Both types of plant oxylipins were found below the limits of quantification in almonds of T1 trees, irrigated to guarantee the ETc during the growing season. The total mean value (T2, T3, and T4) of the PhytoP content was 5050 ng/100 g dw, with contents of individual compounds oscillating from 9.3 to 2078.1 ng/100 g dw and with 9-*epi*-9-F_{1t}-PhytoPs being the dominant compound, while *ent*-16-*epi*-16-F_{1t}-PhytoPs and *ent*-16-F_{1t}-PhytoPs were the compounds found at the lowest concentration (Table 2). It should be highlighted that the latter compounds (*ent*-16-*epi*-16-F_{1t}-PhytoPs and *ent*-16-F_{1t}-PhytoPs) represent the mixture of two epimers, whose signals were not distinguishable under the present chromatographic conditions. The present results were consistent with those previously reported in almonds of "Planeta", "Colorada", "Garriges", "Atocha", "Ferraduel", and "Avellanera" cultivars[14]

Table 2. Phytoprostanes Content (ng/100 g dw) in hydroSOStainable Almonds (Vairo Cultivar)

irrigation treatments	*ent*-16-*epi*-16-F_{1t}-PhytoP + *ent*-16-F_{1t}-PhytoP	9-F_{1t}-PhytoP	9-*epi*-9-F_{1t}-PhytoP	9-*epi*-9-D_{1t}-PhytoP	9-D_{1t}-PhytoP	16-B_1-PhytoP	9-L_1-PhytoP	total PhytoP
ANOVA test[a]	***	***	***	***	***	***	***	***
Tukey's multiple range test[b]								
T1	<LOQ c	<LOQ c	<LOQ c	<LOQ c	<LOQ c	<LOQ c	<LOQ d	<LOQ c
T2	14.8 ± 1.6 b	1369.0 ± 162.0 b	2411.0 ± 91.8 b	45.9 ± 5.2 b	35.1 ± 2.8 b	411.0 ± 32.7 b	264.0 ± 21.3 c	4551.0 ± 305.0 b
T3	<LOQ c	2202.0 ± 230.0 a	2871.0 ± 134.0 a	57.5 ± 11.6 a	50.4 ± 1. ab9	1228.0 ± 111.0 b	969.0 ± 50.4 b	7377.0 ± 324.0 a
T4	22.3 ± 1.5 a	2127.0 ± 173.0 a	3030.0 ± 31.1 a	102.0 ± 6.6 a	60.1 ± 6.5 a	1455.0 ± 45.7 a	1355.0 ± 70.8 a	8152.0 ± 189.0 a

[a]Significant at $p < 0.001$***. [b]Values (mean of four replications) followed by the same letter within the same column were not significantly different ($p < 0.05$), according to Tukey's least significant difference test. <LOQ = detected under the limit of quantitation.

Table 3. Phytofurans Content (ng/100 g dw) in hydroSOStainable Almonds (Vairo Cultivar)

irrigation treatments	*ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF	*ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF	*ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF	total PhytoFs
		ANOVA test[a]		
	***	***	***	***
		Tukey's multiple range test[b]		
T1	<LOQ c	<LOQ	<LOQ b	<LOQ c
T2	55.5 ± 6.0 a	<LOQ	0.8 ± 0.1 a	56.3 ± 6.0 a
T3	32.2 ± 4.8 b	<LOQ	0.8 ± 0.1 a	33.0 ± 4.8 b
T4	38.5 ± 5.0 ab	<LOQ	0.9 ± 0.1 a	39.4 ± 5.0 ab

[a]Significant at $p < 0.001$***. [b]Values (mean of four replications) followed by the same letter within the same column and factor were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

and other nuts such as macadamia and pecans[8] and table olives.[7]

The total PhytoFs mean value of T2, T3, and T4 was 32 ng/100 g dw, with contents of individual compounds between 0.63 and 31.55 ng/100 g dw. *Ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF was the compound with the lowest content, with *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF having the highest one; *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF was below its detection threshold (Table 3). High contents of PhytoFs have been reported in seeds and nuts such as pine nuts, walnuts, chia, and flaxseed (30, 1000, 400, and 60 ng/100 g),[40] in chocolate (~363 ng/100 g), and overall in legumes (up to 479 μg/100g dw).[63,64]

Water Deficit Effect on Free Phytoprostane and Phytofuran Content. As shown in Table 2, the PhytoPs content increased as consequence of water deficiency with significant differences among the four treatments under study. The total content of free PhytoPs in the Vairo almonds from the different irrigation treatments ranged from under the limit of quantification (LOQ) in T1 nuts up to 8151 ng/100 g in fruits of almond trees under T4 irrigation conditions. Regarding individual PhytoPs, 9-*epi*-9-F_{1t}-PhytoP and 9-F_{1t}-PhytoP were the dominant compounds with concentrations ranging between 2411.0 and 3030.0 ng/100 g dw and between 1369.0 and 2202.0 ng/100 g dw, respectively. The PhytoPs 16-B_1-PhytoP and 9-L_1-PhytoP were found at an intermediate level, exhibiting the average concentrations of 1031.3 and 862.7 ng/100 g dw, respectively. Finally, the coeluting *ent*-16-*epi*-16-F_{1t}-PhytoP + *ent*-16- F_{1t}-PhytoP, 9-D_{1t}-PhytoP, and 9-*epi*-9- D_{1t}-PhytoP were found at the lowest concentrations (12.4, 48.5, and 68.5 ng/100 g dw, on average, respectively) (Table 2). When comparing the obtained data with previous reports, it was stated that PhytoPs content in Vairo almonds was higher than in raw Manzanilla green table olive fruits (581−999 ng/100 g),[7] olive oil, sunflower oil (1500−3900 ng/100 g),[51] and algae from several species (6−1381 ng/100 g).[34] Similar contents were found, however, in refined sunflower oil[51] and table olives conserved using the "Spanish style".[7] This finding seems to indicate that the OS of the almonds under DI was comparable to that caused in plant foods by the industrial process. Regarding the diverse PhytoP classes, the predominant one was the 9-series of F_{1t}-PhytoP in all treatments except in full irrigated conditions (T1). The importance of this specific class is based on its molecules for esterified compounds in plant materials, including those found in plant membranes, which means that this could be the first compound involved in plant defense against different types of stress.[65] In this aspect, similar results were also observed in almonds of different cultivars,[14] also in nuts such as walnuts, macadamias, and pecans,[8] as well as in green table olives[7] and macroalgae.[34] It is important to highlight that, usually, in oils, the predominant classes were 9-D_{1t}-PhytoPs[50] and 9-F_{1t}-PhytoPs;[66,67] however, in refined oils the dominant PhytoPs class was 16-B_1-PhytoPs. That could be considered a marker of harsh treatment during a refining process, which implies very high temperatures that can induce a higher ALA oxidation.[51]

In respect to the impact of the diverse regimes of DI applied, T2 samples were featured by the lowest amount of PhytoPs, while T3 and T4 nuts were the highest and were statistically similar. On the contrary, PhytoPs were found below the LOQ in almonds belonging to the fully irrigated treatment (T1). This statement agreed with authors working with "Cornicabra" extra virgin olive oil (EVOO) obtained from deficit irrigated olives,[8] while disagreeing with other authors working on almonds, which reported higher values of PhytoPs in irrigated products relative to those from rain-fed almond trees.[14] However, the mentioned study analyzed different cultivars under diverse irrigation conditions. In the present study, the stress was controlled in the plant, while in the reported work about rain-fed almonds, the plants only depend on the rainwater. The same authors also reported that water scarcity induced the production of the 16-series of F_{1t}-PhytoP class, which were not detected in kernels from irrigated almond trees. The same trend was observed in the present study, in which none of the eight compounds found in almonds obtained from deficit irrigated trees were detected when applying full irrigation. The response of each PhytoP to water stress was, in general, the same for the separate treatments, having a very low concentration in T1 almonds, the lowest content in kernels from moderate RDI, and the highest contents in nuts grown under severe RDI and SDI.

According to the scientific literature, and to the best of our knowledge, PhytoFs content in almonds has never been described. Nevertheless, some papers have described the concentration of PhytoFs in several plant-based foods and foodstuffs, such as chocolate, vegetable oils, pine nuts, walnuts, chia, peas, and flaxseed[36,40,63,64] In this sense, in chocolate and vegetable oils, three compounds were identified: *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF, while in nuts and seeds, only *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF was found.

The PhytoF profile of Vairo almonds (Table 3), after cultivation under full irrigation and deficit irrigation conditions, evidenced that two PhytoFs are found when applying RDI, with *ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF being the most abundant compound followed by *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF. Similar to PhytoPs, PhytoFs were also detected under the limit of quantification in control almonds (fully irrigated trees). Total and individual contents of free PhytoFs in almonds presented significant differences among treatments. In

Table 4. Pearson's Correlation Coefficients (R) among Parameters[a,b]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
SI	1.00															
2	−0.13	1.00														
3	−0.19	0.98***	1.00													
4	0.19	0.14	0.18	1.00												
5	−0.17	−0.28	−0.27	−0.44	1.00											
6	0.65**	−0.42	−0.39	0.32	−0.10	1.00										
7	0.50*	−0.10	−0.09	0.42	−0.01	0.79***	1.00									
8	0.33	0.13	0.18	0.54*	−0.09	0.72**	0.79***	1.00								
9	0.56*	0.19	0.21	0.56*	−0.19	0.69**	0.76***	0.82***	1.00							
10	0.56*	−0.13	−0.11	0.20	0.01	0.77***	0.71**	0.59*	0.83***	1.00						
11	0.56*	0.14	0.08	0.40	−0.11	0.52*	0.63**	0.52*	0.82***	0.79***	1.00					
12	0.33	−0.25	−0.18	−0.11	−0.02	0.38	0.37	0.23	0.38	0.45	0.20	1.00				
13	0.55*	0.11	0.13	0.52*	−0.13	0.76***	0.82***	0.87***	0.98***	0.86***	0.83***	0.35	1.00			
14	0.34	0.04	0.01	0.69**	−0.24	0.38	0.59*	0.61*	0.55*	0.17	0.43	0.08	0.54*	1.00		
15	0.49*	0.07	0.13	0.55*	−0.48	0.76***	0.71**	0.78***	0.83***	0.67**	0.51*	0.49	0.82***	0.51*	1.00	
16	0.34	0.04	0.01	0.69**	−0.25	0.39	0.60*	0.62*	0.56*	0.18	0.43	0.09	0.55*	1.00***	0.52*	1.00

[a]SI = Stress Integral; 2 = $ABTS^+$, 3 = FRAP; 4 = TPC; 5 = α-linolenic acid (C18:3n3); 6 = *ent*-9-*epi*-9-D_{1t}-PhytoP; 7 = *ent*-9-D_{1t}-PhytoP; 8 = 9-F_{1t}-PhytoP; 9 = 9-*epi*-9-F_{1t}-PhytoP; 10 = 9-L_1-PhytoP; 11 = 16-B_1-PhytoP; 12 = *ent*-16-*epi*-16-F_{1t}-PhytoP + *ent*-16-F_{1t}-PhytoP; 13 = total PhytoPs; 14 = *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF; 15= *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF; 16 = total PhytoFs. [b]Note: *, **, and *** are significant at $p < 0.05$, 0.01, and 0.001, respectively.

this regard, it was observed that *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF was significantly higher in T2 (55.5 ng/100 g dw) than in both T3 (32.2 ng/100 g dw) and T4 (38.5 ng/100 g dw) irrigation treatments, implying that this compound was mostly synthesized under mild water deficiency, but its concentration decreased under more severe ID conditions. However, no significant differences were observed among T2, T3, and T4 with respect to the content of *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF (0.8−0.9 ng/100 g dw). Consequently, the total PhytoFs were significantly different among treatments with almonds obtained from trees maintained under T2 and T3 irrigation conditions displaying the highest (56.3 ng/100 g dw) and the lowest (33.0 ng/100 g dw) contents, respectively.

The evaluation of the almond under DI strategies situates PhytoPs and PhytoFs as candidate markers of abiotic stress in almond trees.

Pearson's Correlation Coefficients. When analyzing the correlation existing among the studied variables with significant differences among treatments (Table 4), a significant and positive correlation was observed among the stress integral (SI) and the following PhytoPs and PhytoFs: (i) 9-*epi*-9-D_{1t}-PhytoPs (R = 0.65; p < 0.01); (ii) 9-D_{1t}-PhytoPs (R = 0.50; p < 0.05); (iii) 9-*epi*-9-F_{1t}-PhytoPs (R = 0.56; p < 0.05); (iv) 9-L_1-PhytoPs (R = 0.56; p < 0.05); (v) 16-B_1-PhytoPs (R = 0.56; p < 0.05); (vi) total PhytoPs (R = 0.55; p < 0.05); and (vii) *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoFs (R = 0.49; p < 0.05). Many PhytoPs increased as a result of water stress, which agrees with other authors working with Manzanilla olives and extra virgin olive oil of Cornicabra cultivar under deficit irrigation.[7,51] This trend could be related to an enhancement of the production of reactive oxygen species (ROS) in the different cellular compartments as a result of the drought stress induced in the plant.[68] Consequently, ROS enhancement leads to the generation of lipid peroxidation products, including structural analogues of jasmonates, the PhytoPs, and also PhytoFs.[69]

Moreover, a significant and positive correlation was also observed among the TPC and the following PhytoPs and PhytoFs: (i) 9-F_{1t}-PhytoPs (R = 0.54; p < 0.05); (ii) 9-*epi*-9-F_{1t}-PhytoPs (R = 0.56; p < 0.05); (iii) total PhytoPs (R = 0.52; p < 0.05); (iv) *ent*-9(RS)-12-*epi*-ST-Δ^{10}-13-PhytoFs (R = 0.69; p < 0.01); (v) *ent*-16(RS)-13-*epi*-ST-Δ^{14}-9-PhytoFs (R = 0.55; p < 0.05), and (vi) total PhytoFs (R = 0.69; p < 0.01). This correlation was expected because it has been reported that PhytoPs, PhytoFs, and other oxylipins can induce the biosynthesis of secondary metabolites of response to biotic and abiotic stress.[35,70]

Drought stress and salinity are the major environmental factors affecting plant productivity. Under water deficiency, numerous chemical and physiological responses are produced by activating the signals transduction pathways. Plants can perceive stimuli by producing and transmitting signals, helping them to develop tolerance mechanisms.[71] Water stress constraints the photosynthetic activity by producing an imbalance between the generation and electrons consumption, which results in electron transport to oxygen producing ROS.[72] The relief of the oxidative harm caused by ROS and the increased resistance to environmental stress is regularly correlated with an effective anti-oxidant defense system used by plants to cope with ROS. Moreover, plants also accumulate phytohormones under water stress conditions, which are responsible for the initiation of many defense mechanisms, including the increase in anti-oxidants to enhance plant tolerance to water stress.

A similarity between plant and mammalian stress response markers has been reported in that PhytoPs and PhytoFs could affect human pathophysiological mechanisms, similarly to human oxylipins,[73] such as the activation of the mammalian transcription factor Nrf2 (nuclear factor erythroid-2).[74] The Nrf2 factor is known for being a regulator of the cell resistance to oxidants, besides controlling and inducing expressions of the anti-oxidant response. The activation of this factor leads to numerous health-promoting properties of natural compounds. In this way, the significant and positive correlation among oxylipins and TPC suggests a possible synergic effect. In connection with this, the benefits of consuming almonds might be related to the interaction of both types of bioactive compounds, since they might activate the Nrf2 factor pathway and increase the expression of the anti-oxidant response.[75]

No correlation was observed among the ALA and other parameters, and this statement agrees with other authors who reported that the relative concentration of the oxylipins did not necessarily mirror the relative ALA concentration in nuts and seeds.[40] This phenomenon might occur either because part of the PhytoPs are diverted to the production of jasmonic acid or other fatty acids can be a direct or indirect source of PhytoPs by direct oxidation or by isomerization reaction toward linolenic acid.[8,34,36,43,70]

Finally, a positive and significant correlation (R = 0.55; p = 0.02) was also observed between total PhytoPs and total PhytoFs. This could indicate that these compounds present common biosynthetic pathways that lead to another type of oxidative compound linked to nonenzymatic oxidation at different oxygen tension.[40,76,77]

In summary, water scarcity is affecting many agricultural systems; thus, the implementation of sustainable irrigation strategies is urgent, especially in semiarid regions. In this sense, the present study evaluated the functionality of almonds cultivated under different irrigation treatments aimed to combat water scarcity. This study presents the AAc, TPC, and quantitative and qualitative profiles of PhytoPs in Vairo almonds under different DI strategies. Besides, it reveals, for the first time, the profile and content of PhytoFs in almonds. Overall, deficit irrigation increased the PhytoPs and PhytoFs with no effect on the TPC and AAc. It is important to highlight that these oxylipins were present only under the LOQ in fully irrigated almond trees. The importance of PhytoPs and PhytoFs is explained by plant physiology, their interaction as technological mediators, and biological effect in humans because they are consumed through diet, and their presence in human biofluids has been already reported. Thus, DI conditions can be considered as an important strategy to reduce the water consumption and, simultaneously, to enhance the PhytoPs and PhytoFs content, as compounds with a potential beneficial effect on human health, since there is evidence on their effect on the regulation of immune function and anti-inflammatory and apoptosis-inducing activities. Almonds consumed whole or processed are a well-known healthy food, and their bioactivity has been attributed to oxygenate ALA and potentially to PhytoPs. However, the concentration of PhytoFs found in almonds could also have functional implications relative to human health. In this sense, *in vivo* and/or *in vitro* studies might be carried out to validate the full effects of these oxylipins. Consequently, the most important conclusion is that PhytoPs and PhytoFs are

important biomarkers to detect whether a product is hydroSOStainable or not. Accordingly, water productivity can be increased in the almond crop if regulated deficit irrigation strategies are applied, generating fruits with high functional interest. Further research during several growing cycles is needed to check other important quality markers of hydroSOStainable almonds.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c02268.

Table of UHPLC/MS/MS parameters (PDF)

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Notes

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Supplemental Table 1 . UHPLC/MS/MS parameters for the quantification and confirmation of PhytoPs and PhytoFs in almonds of “Vairo” cultivar under water stress.

Compound	Retention time (min)	MRM transition (*m/z*)
Phytoprostanes		
ent-16-*epi*-16-F_{1t}-PhytoP	1.807	327.1>283.2
ent-16-F_{1t}-PhytoP	1.807	327.1>283.2
9-F_{1t}-PhytoP	1.913	327.2>171.2
9-*epi*-9-F_{1t}-PhytoP	2.132	327.2>171.2
ent-9-*epi*-9-D_{1t}-PhytoP	2.133	325.2>307.2
ent-9-D_{1t}-PhytoP	2.491	325.2>307.2
16-B_1-PhytoP	3.230	307.2>235.2
9-L_1-PhytoP	3.507	307.2>185.1
Phytofurans		
ent-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF	1.650	344.0>300.0
ent-16(*RS*)-9-*epi*-ST- Δ^{14}-10-PhytoF	1.667	343.9>201.1
ent-16(*RS*)-13-*epi*-ST- Δ^{14}-9-PhytoF	1692	343.0>171.1

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How does water stress affect the low molecular weight phenolics of hydroSOStainable almonds?

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ABSTRACT

Water scarcity is a threat for food production because, water, is more and more limited and force farmers to use new deficit irrigation (DI) strategies without affecting fruit yield and quality. No information exists on almond polyphenols and proanthocyanidins (PAs) produced under DI. The present work studied the effect of 2 regulated DI (RDI) and one sustained (SDI) on the low molecular weight phenolics together with the antioxidant activity (AA) in almonds. Fifteen phenolic compounds were identified (13 flavonoids and 2 non-flavonoids) and 10 PAs. Kaempferol-3-*O*-galactoside was the predominant compound in almond skin and whole kernel but it was not found in deskinned kernels. The use of moderate RDI significantly increased the total phenolic content in skin (~9.8%), PAs, and the AA. Consequently, after one season the application of DI positively affected the almond cv. Vairo phenols, however, several seasons must be evaluated in order to corroborate the present results.

1. Introduction

Water has a critical importance in the Mediterranean Basin due to the limited rainfall and high temperatures, which result in a deficit water balance, normally alleviated with irrigation (García Tejero & Duran Zuazo, 2018). Thus, Mediterranean agro-systems must cope with the lack of water, because any policy of continuous expansion of the supply is unsustainable. For this, efficient strategies for deficit irrigation (DI) management are necessary. In this aspect, regulated deficit irrigation (RDI) is an irrigation strategy designed to save water with minimal impact on yield and fruit quality (Egea et al., 2013; Lipan et al., 2019). The RDI is based on the principle of applying less volume of water in the most resistant period of the plant phenological stages (Egea et al., 2013). On the other hand, sustained deficit irrigation (SDI) is a strategy upon which a reduced water volume (below the optimum) is uniformly applied along the entire growing season (Egea et al., 2013). Using RDI strategies a new generation of products (almonds, pistachio, olives) has been developed, and they are called hydroSOStainable (hydroSOS) products (Lipan et al., 2019; Noguera-Artiaga, Pérez-López, Burgos-Hernández, Wojdyło, & Carbonell-Barrachina, 2018; Sanchez-Rodriguez, Cano-Lamadrid et al., 2019). They are characterized by an augmented concentration of bioactive compounds that simultaneously allow optimizing water use, impact the almonds functionality and increase the intensity of key sensory attributes.

Almond (*Prunus dulcis*) is the principal tree nut species and even it is drought resistant its yield and profitability are extremely reliant on irrigation (Egea et al., 2013). However, despite this drought resistance, irrigation is mainly needed in the Mediterranean Basin due to the low rainfall and high evaporative demand throughout the almond growing period.

Almonds are one of the most common edible nuts and can be consumed either as snacks (raw, roasted or fried) or as ingredient in confectionery products [especially *turrón* (Spain), *torrone* (Italy) and *nougat* (France)]. Almonds are considered healthy components in a diet due to their bioactive compounds (mono- and poly-unsaturated fatty acids,

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minerals, tocopherols, phytosterols, phytoprostanes and polyphenols, among others) (Carrasco-Del Amor et al., 2015; Lipan et al., 2019). In respect to the contribution to human health, almonds have been associated with improvements in cardiovascular health and diabetes (Bitok & Sabaté, 2018; Jiang et al., 2002), reduction of DNA damage, and oxidative stress in smokers (Jia et al., 2006), as well as with antiproliferative activity on liver and colon cancers in humans (Medina, Domínguez-Perles, Ferreres, Tomás-Barberán, & Gil-Izquierdo, 2013).

These beneficial effects are mainly attributed to the lipid profile, vitamin E, fibre, arginine, and polyphenols, among others (Monagas, Garrido, Lebron-Aguilar, Bartolome, & Gomez-Cordoves, 2007). The skin has a very low economic value and it is often removed during the industrial processing of almonds. Although this by-product represents only ~4% of the total almond weight, the skin contains between 70 and 100% of total phenols in almonds (Monagas et al., 2007). Polyphenols are plant secondary metabolites classified in two groups (i) flavonoids and (ii) non-flavonoids, both of them found in almonds (Milbury, Chen, Dolnikowski, & Blumberg, 2006; Monagas et al., 2007). In almonds, the most abundant non-flavonoids compounds reported are procatechuic, vanillic and hydroxy-benzoic acids, while the predominant flavonoids include flavanols, flavonols, and flavanones, among others (Milbury et al., 2006; Monagas et al., 2007). Proanthocyanidins (PAs) are formed as a result of the polymerization of the flavanols (+)-catechin and (−)-epicatechin, and are classified based on their monomeric unit linkage in homo- and hetero-polymers (Collado-González et al., 2013; Rue, Rush, & van Breemen, 2018); thus, PAs are also known as condensed tannins, which naturally occur in plants and are found in different concentrations in common foods such as fruits, vegetable, legumes, grains, and nuts (Collado-González et al., 2013; Rue et al., 2018). The antiproliferative and antitumoral effect of PAs on human cancer cell is dependent on their degree of polymerization because, for instance, the antiulcer activity increases with high degree of polymerization of catechin unit (Mao et al., 2000). Moreover, as a natural antioxidant, PAs are also used to stabilize food colours and to prevent rancidity (Rue et al., 2018). Authors postulated an increase in phenolic compounds under water stress conditions up to certain level from which it start to decrease (Horner, 1990).

Overall, as water scarcity is the most limiting factor in Mediterranean basin and the world food production mainly depend on water availability, is unconditionally necessary to use draught resistant species and irrigation strategies to reduce the amount of water maintaining or even improving the fruit quality. In the light of these antecedents, the aim of this study was to evaluate the profile of phenolic compounds and proanthocyanidins (PAs), the PAs polymerization degree, and the antioxidant activity of almonds cultivated under water stress conditions.

2. Material and methods

2.1. Irrigation treatments and plant material

The experiment was carried out during the 2017 growing season in the commercial farm “La Florida”, located in Dos Hermanas (Seville, Spain). The soil and climatological conditions were as previously described by Martín-Palomo et al. (2019). The seasonal weather data was typical of Mediterranean area, with no rainfall during summer and warm winters. These data were obtained from a station situated about 6 km away from the experimental orchard and belonging to “Instituto de Investigación y Formación Agraria Los Palacios”. The almond trees (*Prunus dulcis*, cultivar Vairo) were 7 years-old and were planted in a 6 × 8 m framework. Trees were irrigated with one line of drips (3.8 L/h) separated 0.4 m. The statistical design used randomized complete blocks with 4 repetitions and 4 irrigation treatments. Two types of RDI (T2 and T3) depending on the stress level and the phenological stage of the trees, 1 sustained DI (T4), and 1 control treatment (T1) were evaluated:

- T1, *full irrigation* or *control (400 mm of water applied annual)*. Irrigation was supplied to cover the estimated crop evapotranspiration.
- T2, *deficit irrigation (RDI) treatment during kernel filling (150 mm of wate applied annual)*. Irrigation scheduling was based on measurements of midday stem water potential (SWP) and maximum daily shrinkage (MDS). During kernel filling, T2 trees were irrigated when SWP was below −1.5 MPa or MDS signal was higher than 1.75. Out of this period, trees were irrigated to a SWP as the baseline suggested for McCutchan and Shackel (1992) or MDS signal equal to 1.
- T3, *deficit irrigation (RDI) treatment during kernel filling with a total seasonal water applied of 103 mm*. During kernel filling, T3 trees were irrigated when SWP was below −2.0 MPa or MDS signal was higher than 2.75. The rest of the time irrigation was the same as T2.
- T4, *sustained deficit irrigation (SDI) in which 114 mm of water was applied during whole year, in this sense the stress was gradually created in plant instead of focusing only in one period such as T2 and T3.*

Midday stem water potential (SWP) was measured to schedule the irrigation by using a pressure chamber (PMS Instrument Company, USA). Moreover, the values of SWP were used to calculate the stress integral (SI) according to the following Eq. (1):

$$SI = \left| \sum (\min\psi_{stem} - (-0.2)) \times n \right|$$

where min ψ_{stem} represented the average of minimum SWP for any interval while *n* was the day numbers interval.

Almonds were harvested on August 2017 at their mature stage (when 95% of the fruits have the hull open); they were sun-dried to reach a moisture content below 5% and were sent to the Miguel Hernández University facilities for quality analysis. Around 6 kg of in-shell almonds were used for each treatment. Almonds were manually opened to obtain edible kernels. For each treatment, 4 batches of 100 g almonds were randomly prepared and divided into two groups (i) whole kernels with skin and (ii) almonds to be deskinned leading to skinned almonds and skins. In order to remove the kernel skin, almonds were submerged in room temperature water for 20 min and then, the obtained samples (skinned almonds and skins) were dried during 24 h at room temperature. All samples (skins, deskinned kernels, and whole kernels) were finely ground in a Moulinex MC 3001 grinder (Moulinex, Écully, France) for 20 s and stored at −20 °C until further processing or analysis.

2.2. Chemicals

2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$), 6-hydroxy 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2pyridyl)-s-triazine (TPTZ), acetic acid, phloroglucinol, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). (−)-Epicatechin, (+)-catechin, quercetin, kaempferol-3-*O*-glucoside, and procyanidins B1 and B2 were purchased from Extrasynthese (Lyon, France). Chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, and 3,5-dicaffeoylquinic acid were purchased from TRANS MIT GmbH (Giessen, Germany). Acetonitrile for UPLC (Gradien grade) and ascorbic acid were from Merck (Darmstadt, Germany). UPLC grade water, prepared by using an HLPSMART 1000 s system (Hydrolab, Gdańsk, Poland), then filtered through a 0.22 µm membrane filter just prior to use. The standard for proanthocyanidins quantification epigallocatechin, was purchased from Phytoplan (Heidelberg, Germany). Acetonitrile and methanol LC-MS grade, and acetone of HPLC grade were purchased from Panreac Química S.A. (Barcelona, Spain), while acetic acid of LC-MS grade was from Scharlau (Sentmenant, Spain).

2.3. Extraction, identification, and quantification of phenolic compounds by LC-PDA-MS-QTOF

Polyphenols analysis was performed following the protocol previously described by Noguera-Artiaga et al. (2018). Briefly, 1.0 g of deskinned or whole kernels or 0.5 g of skin were weighed and 5 mL of aqueous methanol (30:70, *v/v*) with 1% of ascorbic acid (*v/w*) were

added. The suspension was stirred and sonicated for 15 min in an ultrasonic bath (JP Selecta S.A, model 3000512, Barcelona, Spain). The homogenized mixture was kept in darkness, at room temperature, during 24 h. Afterwards, the homogenized mixture was centrifuged (MPW-150R centrifuge, MPW Med. Instruments, Warsaw, Poland) at 20.878 × *g*, 4 °C for 10 min. The supernatant was filtered through a 0.45 μm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) and was directly injected into an UPLC system.

All analyses were performed in triplicate. Separation of phenolic compounds was performed using an ultra-performance liquid chromatography (UPLC) coupled with a photodiode detector (PDA; Waters, Milford, MA, USA) quadrupole and tandem time-of-flight mass spectrometry (QTof) (Waters, Manchester, UK), equipped with an electrospray ionization (ESI) source. A satisfactory chromatographic separation was achieved by using a UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm, Waters Corp.; Milford, USA) at 30 °C. The mobile phases consisted of water/formic acid (99.0:1.0, *v/v*) (A) and acetonitrile (B). The injection volume was 10 μL and elution was performed at a flow rate of 0.45 mL/min. The chromatographic separation was obtained by applying the gradient as follows: 99% A at 0.0 min, isocratic elution for 1 min; a linear gradient until minute 12.0 achieving 0% A; and, finally, from 12.0 min to 13.5 min, it was achieved again the initial conditions (99% A). The initial conditions were maintained for 15 min to re-equilibrate the column. The analyses were performed in negative mode and full scan from *m/z* 100 to 1500 was used. The MS parameters of the fragmentor were optimized, being capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 130 °C, desolvation temperature of 350 °C and desolvation gas (N_2) flow rate of 300 L/h. The internal reference compound (leucine encephalin) was introduced via the LockSpray channel and the lock mass correction was ± 1.000 for the mass window.

Chromatograms were recorded at 280 nm (flavan-3-ols), 320 nm (phenolic acids), and 360 nm (flavonols and flavanones). Quantification was performed by injecting calibration curves of standards according to Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016). This analysis was run in quadruplicate, and results were expressed as mg *per* 100 g dry weight [mg $(100\ g)^{-1}$ dw] of sample.

2.4. Extraction and determination of proanthocyanidins by HPLC–PDA–ESI/MSn

This work pays attention to low molecular weight procyanidins since these compounds are sensitive to water stress conditions (increasing) according to previous studies and higher molecular weight procyanidins tend to self-aggregate (Collado-González et al., 2013) into compounds that they cannot be absorbed by the human gastrointestinal tract due to their big molecular size (Tomas-Barberán et al., 2007)

Proanthocyanidins were characterized and quantified by using two different approaches. On the one hand, through a direct analysis of individual procyanidin oligomers in the almonds methanol extract at room temperature separated by HPLC according to Gironés-Vilaplana et al. (2014) but only in whole kernels. Thus, 100 mg of ground kernels were accurately weighed. Then, 1 mL of methanol/water (70:30, *v/v*) was added to each of the 4 replicates. For the HPLC analysis, samples were acidified with 1% of formic acid. The solution was vortexed and sonicated in an ultrasonic bath for 60 min. Later, samples were kept at 4 °C overnight and sonicated again for 60 min. In order to separate the supernatant from the solid residue, the solutions were centrifuged (model EBA 21, Hettich Zentrifugen) at 9500 × *g*, for 5 min. This supernatant was filtered through a 0.45 μm PVDF filter (Millex HV13, Millipore, Bedford, MA, USA) and afterwards was directly injected into a HPLC system. All analyses were run in quadruplicate.

Samples (30 μL) were analysed on an Agilent HPLC 1100 series model equipped with a photodiode array (PDA) detector and a mass detector in series (Agilent Technologies, Waldbronn, Germany). The equipment consisted of a binary pump (model G1312A), an autosampler (model G1313A), a degasser (model G1322A), and a PDA detector, model G1315B. The mass detector used was an ion trap spectrometer (model G2445A) equipped with an ESI interface. The HPLC system was controlled by Chem-Station software (Agilent, version 08.03) and the ion trap spectrometer was controlled by LCMSD software (Agilent, version 4.1). Chromatographic separations were achieved on a Luna C18 column (250 × 4.6 mm, 5 mm particle size; Phenomenex, Macclesfield, UK). The mobile phase was a mixture of water/formic acid (99:1, *v/v*) (A) and acetonitrile (B), and the flow rate used was of 1 mL min^{-1}. Samples were eluted by applying the following linear gradient: 0 min, 8% B; 25 min, 15% B; 55 min, 22% B; and 60 min, 40% B. The 40% B was maintained until 70 min to re-equilibrate the column. The capillary and voltage were maintained at 350 °C and 4 Kv, respectively. Mass scan (MS) and daughter (MS-MS) spectra were measured from *m/z* 100 to 1200. Collision induced fragmentation experiments were done in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Proanthocyanidins were identified and quantified by using their UV spectra recorded at 280 nm, their molecular mass and daughter ions acquired in the negative mode on the mass spectrometer. The MSn was carried out in the automatic mode on the more abundant fragment ion in MS2 (*m/z* $[M-H]^{-1}$). All analyses were run in quadruplicate and results were expressed as mg/100 g dw of almonds kernel.

2.5. Analysis of polymeric procyanidins by procedure using phloroglucinol method by UPLC-FL

On the other hand, an acidic cleavage method in the presence of an excess of phloroglucinol was also carried out. This method leads to the depolymerization of PC structures by converting the flavanols extension units into carbocations and the terminal units into monomeric flavanols (Fernández, Kennedy, & Agosin, 2007; Hammouda, Chérif, Trabelsi-Ayadi, Baron, & Guyot, 2013; Kennedy & Jones, 2001). The carbocations directly mix with phloroglucinol, and forms flavanyl phloroglucinol adducts. The concentration of total PAs and their degree of polymerization can be determined by HPLC reaction media and calculated from the sum of total units (extension and terminal units) (Hammouda et al., 2013). The phloroglucinolysis of almonds samples were carried out according to Wojdyło et al. (2016). Briefly, samples of ~50 mg of skin, deskinned, and whole kernel powders were measured in 1.5 mL polypropylene microtubes and were treated with a solution of 0.3 M HCl in MeOH containing phloroglucinol (75 g/L) and ascorbic acid (10 g/L) at room temperature. This mixture was stirred by vortex during 30 min and, subsequently, cooled during 5 min. Immediately, in order to stop the reaction, 0.6 mL of aqueous sodium acetate (0.2 mol/L) was added. Later, solution was again cooled during 5 min and centrifuged. Chromatographic separation was achieved on a BEH Shield RP C18 (2.1 mm × 50 mm; 1.7 m) with a precolumn (Waters Corp.) working at 15 °C. Two types of eluents were used to separate the gradients: distilled water/acetic acid (97.5:2.5, *v/v*) as solvent A and acetonitrile as solvent B. The injection volume and the flow rate were 5 μL and 0.5 mL min^{-1}, respectively. The linear gradient started with 0% B, reaching 2% B at 0.60 min, 3% B at 2.17 min, 10% B at 3.22 min, 15% B at 5.00 min, and finally a 100% B at 6.00 min, with a post-run of 1.5 min for the column re-equilibration. The liquid chromatograph used for analysis of polymeric proanthocyanidins was a UPLC Waters; Milford, USA) system equipped with fluorescence detectors (FL). Fluorescence was recorded at the excitation wavelength of 278 nm and emission wavelength of 360 nm. (−)-Epicatechin and (+)-catechin, and procyanidin B_2 after phloroglucinol reaction as (+)-catechin-catechin-epicatechin-phloroglucinol adduct standards were used as a reference compound. To calculate the apparent mean degree of polymerization (DP), all subunits (flavan-3-ol monomer and phloroglucinol adduct, in moles) were summed and divided by the total flavan-3-ol monomers (in moles) according to Kennedy & Jones (2013). The conversion yield information was calculated from sum of the mass of all subunits (except

phloroglucinol portion) divided by the weighed mass of the proanthocyanidin reacted (Wojdyło, Oszmiański, Teleszko, & Sokół-Łetowska, 2013). The average degree of polymerisation (DP) was determined by calculating the molar ratio of all flavanol units (phloroglucinol adduct + terminal units) to (−)-epicatechin and (+)-catechin, which correspond to terminal units. Results were expressed as mg/100 g dw of almonds kernel. All analyses were performed in quadruplicate.

2.6. Antioxidant activity ($ABTS^{\cdot+}$ and FRAP)

The free radical scavenging activity of the diverse extracts was determined using the $ABTS^{\cdot+}$ ([2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation) and FRAP (ferric reducing antioxidant power) methods according to Re et al. (1999). To obtain the extracts to be assessed on the radical scavenging capacity, 0.5 g of milled skin or 1.0 g of deskinned and whole kernel were mixed with 10 mL of MeOH/water (80:20, *v/v*) containing 1% HCl, and the mixture was sonicated at 20 °C for 15 min, and left overnight at 4 °C. Then, the extracts were sonicated for 15 min, and centrifuged at 10.000 × *g* for 10 min. In case of skin samples, extracts were re-diluted 1/10 with distilled water. The antioxidant activity was evaluated by measuring the variation in absorbance at 734 nm after reaction with the radical ($ABTS^{\cdot+}$) and 593 nm for FRAP using a UV–*vis* spectrophotometer (Helios Gamma model, UVG 1002E, Mercers Row, Cambridge, UK). The quantification was based on the standard curve (with a linearity of $R^2 = 0.998$) prepared using different concentrations of Trolox. All the determinations were carried out in triplicate and the results were expressed in mmol Trolox/kg.

2.7. Statistical analyses

The data was subjected to one-way analysis of variance (ANOVA) using XLSTAT Premium 2016software (Addinsoft, New York, NY) and later all means were separated using a Tukeýs multiple range test. Differences were considered statistically significant at $p < 0.05$. Pearson's correlation coefficients were also calculated for 16 individual data points (4 per each treatment) and significant correlation were set up at $p < 0.05$.

3. Results and discussion

3.1. Plant water status

As mentioned above, the present study involved 4 irrigation treatments that represented an array of water stress conditions. In this regard, Fig. 1 shows the water volume applied together with the stress integral values. The applied water values ranged from 100 to 433 mm (1000–4330 m^3/ha), with T1 receiving the highest amount of water, while no significant differences were found among the deficit irrigated treatments. As expected, stress integral values (ranging from 54 to 95 MPa × day mean values of each treatment) were significantly different among treatments with T3 being the most stressed one, while T2 was statistically similar to both T3 and T4. Almonds from T3 were irrigated, during the kernel filling, when SWP was below −2.0 MPa. Finally, T1 almond trees were the less stressed ones.

3.2. Qualitative analysis of phenolic compounds

Fifteen phenolic compounds were identified in the methanolic extract of almonds using LC-PDA-MS-QTof: 13 flavonoids (6 flavanols, 6 flavonols, and 1 flavanone) and 2 non-flavonoids (phenolic acids) (Table 1). The phenolic compounds were identified according to their retention time, molecular mass, fragmentation patterns, and UV–*vis* characteristic spectra in comparison with the information available in the literature (Bolling, Dolnikowski, Blumberg, & Oliver Chen, 2009; Milbury et al., 2006). Electrospray ionization mass spectrometry

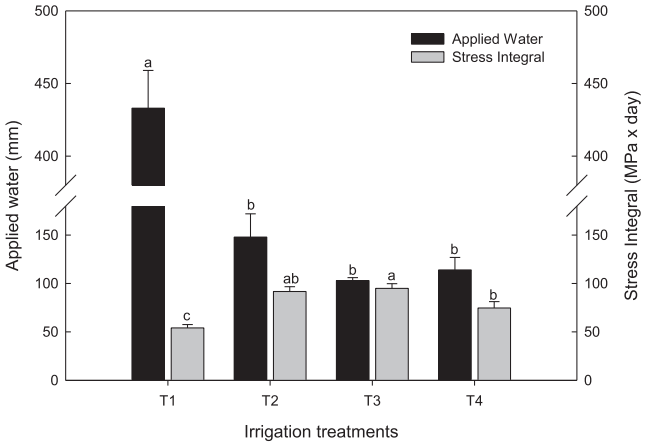

Fig. 1. Applied water along the season (mm) and the stress integral (MPa × day) of the almonds grown under water stress conditions.

combined with LC-PDA-MS-QTof allowed identifying appropriately all phenolic compounds found in the studied parts of almond. Flavonoids are divided in different groups, namely anthocyanins, flavanols, flavones, flavanones, and flavonols. In almonds skin the predominant group was flavonols, being the major compound identified kaempferol-3-*O*-galactoside (peak at 8.75 min), with a $[M-H]^-$ ion at *m/z* 447.09 atomic mass unit (amu) and MS/MS $[M-H]^-$ fragment at *m/z* 285.04 amu. In the flavanols group, the major compound found in skin was procyanidin B-type dimer (peak at 4.89 min) with a $(M-H)^-$ ion at *m/z* 577.13 amu and MS/MS $[M-H]^-$ fragments at *m/z* 289.07 and 245.08 amu. The irrigation treatment did not influence the phenolic profile of almonds, with all 15 compounds being found in all samples.

3.3. Effect of irrigation on the concentration of phenolic compounds

Table 2 shows the contents of each of the 15 phenolic compounds found in the different parts of the almonds grown under different irrigation conditions. The total phenolic content (TPC) ranged from an average content of 37.3 mg/100 g dw for all irrigation treatments in skin to 1.13 mg/100 g dw in deskinned kernel, with the whole almonds representing an intermediate position with 4.77 mg/100 g dw. Values of 41.3 mg/100 g dw have been previously reported in almond skin from Spanish varieties, while 1.7-fold less polyphenolic concentration (24.2 mg/100 g dw) has been found in skin of a mixture of American varieties (Milbury et al., 2006; Monagas et al., 2007). The present results suggest that TPC in skin was significantly affected by the irrigation treatment (mainly the phenolic acids), by increasing in a moderate RDI treatment (T2) and maintained similar to the control in severe RDI (T3) and SDI (T4). However, no significant differences among irrigation treatments for the kernel and deskinned kernels were observed. The water stress mechanism in plant involve a reduction on turgor pressure, enhancement of the ion toxicity and inhibition of the photosynthesis (Ali & Baek, 2020). Therefore, the phytohormones rise the total carbohydrates and polysaccharides, and activates the antioxidant system to help the plant to fight against reactive oxygen species. This fact has been recently demonstrated in almonds cultivated in lands receiving little rainfall where phytoprostanes (oxidative stress and jasmonic acid precursor) were increased compared to full irrigated almond trees (Carrasco-Del Amor et al., 2015). Because when the amount of the carbohydrates exceed the total used for growth, the excess of CO_2 assimilated could increase the biosynthesis of carbon-based secondary metabolites (Horner, 1990). Besides, authors also attributed the increase in phenolic compounds in plants under waters stress conditions to the raise in levels of their precursor free phenylalanine (Horner, 1990).

p-Hydroxy-benzoic and vanillic (compounds 1 and 2, respectively, Table 2) acids were the only phenolic acids found within this study and

Table 1
Identification of single phenolic compounds found in almonds under water stress using LC-MS-QTof/PDA.

Peak	Compound	Retention time (min)	UV λ (nm)	Parent ion (m/z $[M-H]^-$)	Primary fragment (MS2 m/z $[M-H]^-$)
1	*p*-Hydroxy-benzoic acid	2.60	250	137.01	
2	Vanillic acid	2.84	251/291	167.03	
3	Procyanidin B-type dimer	4.89	280	577.13	289.07/245.08
4	(-)-Epicatechin	4.51	279	289.07	245.09
5	Procyanidin B-type dimer	5.86	280	577.12	425.08/289.07
6	Procyanidin B-type trimer	6.10	280	867.03	577.12/289.07
7	Procyanidin B-type tetramer	6.29	280	1153.25	865.19/577.13/289.07
8	Procyanidin B-type trimer	7.21	280	867.04	289.07
9	Naringenin-7-*O*-glucoside	8.26	–	433.11	271.05
10	Kaempferol-3-*O*-rutinoside (isomer 1)	8.64	267/346	593.14	285.05
11	Isorhamnetin-3-*O*-rutinoside	8.64	265/349	623.16	315.04
12	Kaempferol-3-*O*-rutinoside (isomer 2)	8.70	267/346	593.14	285.04
13	Kaempferol-3-*O*-galactoside	8.75	351/271	447.09	285.04
14	Isorhamnetin-3-*O*-glucoside	9.00	269/350	477.10	315.03
15	Kaempferol-3-*O*-glucoside	9.06	272/349	447.09	285.03

represented 12% of TPC in skin. This is in agreement with other authors, who reported the same compounds in almond skin (Milbury et al., 2006; Monagas et al., 2007; Prgomet et al., 2019). The phenolic acids were significantly affected by both DI and skin removal, increasing their concentration in samples obtained under water stress conditions in plant and decreasing or even completely disappearing in the kernels (*p*-hydroxy-benzoic acid) as a result of the skin removal.

Flavonoids, a group of polyphenols with two or more aromatic rings, are present in all plant organs and are an integral part of the human diet (Bolling, 2017); it is important for their recognition when eating foods that they are contributing to the sensory profile of foods, particularly to their astringency and bitterness (Milbury et al., 2006). These phytochemicals were the major phenolic group within this study in both skin and whole kernel (mean value among the treatments of 32.51 and 2.87 mg/100 g dw, respectively), but they were not found in the deskinned kernels, evidencing that these polyphenols are only found in the almond skin. Regarding their relative abundance, the individual flavonoid kaempferol-3-*O*-galactoside (compound 13, Table 2) was the predominant phenolic compound in both almond skin and whole kernel [mean values for all treatments of 20.00 and 1.78 mg/100 g dw, respectively]; however, this flavonol was not found in deskinned kernels. In general, the irrigation factor significantly affected compounds, with moderate RDI (T2) increasing the contents of several compounds (*p*-hydroxy-benzoic acid, vanillic acid, procyanidin B-type dimer, procyanidin B-type dimer, procyanidin B-type trimer, procyanidin B-type tetramer, procyanidin B-type trimer, naringenin-7-*O*-glucoside, and kaempferol-3-*O*-rutinoside) in almond skin. To the present date, there are many studies dealing with the phenolic composition of almonds, but no previous characterization has evaluated the effect of water stress. In this aspect, Prgomet et al. (2019) studied the effect of water stress on the phenolic content of almond by-products such as hull, skin, and blanching water, and reported that irrigation water lowered significantly the phenolic content in hulls (Prgomet et al., 2019), which is consistency with the findings reported in the present work, in which the TPC was higher under moderate water stress conditions (T2). This might be a result of the polyphenols accumulation as a response of a plant environmental stress (Prgomet et al., 2019). However, in the above-mentioned study the phenolic content in skin was not reported to change due to the irrigation conditions but with the season since the cultivar was not a factor in their experimental design. These differences could be attributed to the irrigation treatments applied and also to the methodology applied during samples processing; for instance, in the previous study almonds were blanched at 95 °C, while in the current experiment, the skin removal was done with water at room temperature. It was reported that the temperature of water increased the extraction from skin to blanching water as the phenolics content was 15 times higher in the latter one (Prgomet et al., 2019). Moreover, the authors, also analysed phenolics in hulls and blanching water, and reported a higher content in non-irrigated trees for the hulls and a reverse trend for blanching water, being consistent among the seasons. Only 6 out of the total 15 phenolic compounds were kept in the deskinned almonds: vanillic acid (2), procyanidin B-type dimer (3), (−)-epicatechin (4), procyanidin B-type dimer (5), naringenin-7-*O*-glucoside (9), and kaempferol-3-*O*-rutinoside (12), the rest of them were lost during the skin removal.

3.4. Proanthocyanidins profile and content

Proanthocyanidins, also named condensed tannins, are abundant flavonoids present in our diet and the most abundant polyphenol family in almonds; however, they are less studied due to their polymeric nature and high structural complexity (Bolling, 2017; Deprez, Mila, Huneau, Tome, & Scalbert, 2001). For instance, in almonds only two quantitative studies were reported and indexed in nutrient databases, as reported by Bolling (2017). The PAs are responsible for the astringency and bitterness in foods when react with salivary proteins (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004).

The HPLC–PDA–ESI/MSn is a useful analytical technique to identify small oligomeric proanthocyanidins (PAs), which can be easily quantified by using authentic standards. Ten oligomeric PAs were identified and quantified within this study and they are presented as monomeric units in Table 3. In this study, only low molecular weight PAs up to tetramers could be isolated and characterized as pure compounds. All these low molecular weight PAs had B-type linkage (C–C bond between monomers) although, other authors reported also type-A oligomers in almonds skin (mixture of Spanish and American varieties) (Monagas et al., 2007). The reason of these differences might be the matrix analysed and the extraction method. For instance, in the present study the whole kernel was used to measure the Pas, while in the cited work only the skin was analysed. Out of the 10 PAs found in almonds, the 3 most abundant were trimers (mean values of all treatments: trimer 1 = 10.50 mg/100 g dw, trimer 2 = 10.00 mg/100 g dw and trimer 5 = 6.27 mg/100 g dw) and 1 tetramer (7 = 6.16 mg/100 g dw). Most of the identified and quantified PAs were also reported in almond blanching water (Pérez-Jiménez & Torres, 2012).

Regarding water deficit, two two low molecular weight PAs (one trimer and one tetramer) were significantly different among the treatments (Table 3). A higher amount of the trimer PA 5 (Rt = 13.5 min) was attributed to a controlled water deficit effect. In this sense, the samples obtained after moderate RDI (T2 = 6.38 mg/100 dw) and SDI (T4 = 8.84 mg/100 g dw) were significantly higher than the control (T1 = 4.80 mg/100 g dw). However, when the stress (RDI) in plant increased (T3 = 5.06 mg/100 g dw), the concentration of this trimer was reduced, displaying similar values to the full irrigation treatment (T1). On the other hand, an increase in the concentration of the tetramer PA 10 was also observed as a consequence of water stress, with

Table 2
Quantification of phenolic compounds (mg/100 g dw) found in almonds cultivated in deficit irrigation conditions.

C N° [ψ]	ANOVA Test[†]			Skin				Deskinned kernel				Whole kernel			
	S	DK	WK	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
				mg/100 g dw				mg/100 g dw				mg/100 g dw			
				Tukey Multiple Range Test[‡]				Tukey Multiple Range Test[‡]				Tukey Multiple Range Test[‡]			
1	***	–	***	0.39 ± 0.10^{bc}	0.65 ± 0.21^{a}	0.54 ± 0.12^{ab}	0.33 ± 0.07^{c}	N.d.	N.d.	N.d.	N.d.	0.03 ± 0.008^{bc}	0.04 ± 0.010^{a}	0.04 ± 0.004^{a}	0.02 ± 0.004^{c}
2	*	*	N.s	0.26 ± 0.08^{ab}	0.37 ± 0.10^{a}	0.29 ± 0.15^{b}	0.20 ± 0.07^{b}	0.04 ± 0.013^{a}	0.03 ± 006^{ab}	0.02 ± 0.005^{b}	0.03 ± 0.011^{ab}	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.02
3	***	N.s	*	1.19 ± 0.27^{a}	1.30 ± 0.15^{a}	0.89 ± 0.22^{b}	0.81 ± 0.23^{b}	0.62 ± 0.11	0.56 ± 0.14	0.47 ± 0.07	0.47 ± 0.21	0.70 ± 0.16^{ab}	0.76 ± 0.22^{ab}	0.56 ± 0.21^{b}	0.88 ± 0.18^{a}
4	N.s	N.s	N.s	0.15 ± 0.06	0.16 ± 0.10	0.16 ± 0.11	0.10 ± 0.03	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.02	0.06 ± 0.03	0.07 ± 0.02	0.07 ± 0.03	0.06 ± 0.03	0.08 ± 0.02
5	***	***	N.s	0.37 ± 0.04^{b}	0.81 ± 0.31^{a}	0.38 ± 0.18^{b}	0.24 ± 0.08^{b}	0.08 ± 0.02^{a}	0.06 ± 0.01^{b}	0.06 ± 0.01^{b}	0.07 ± 0.02^{ab}	0.21 ± 0.13	0.19 ± 0.10	0.18 ± 0.09	0.17 ± 0.06
6	**	–	*	0.53 ± 0.24^{ab}	0.80 ± 0.14^{a}	0.58 ± 0.27^{ab}	0.35 ± 0.12^{b}	N.d.	N.d.	N.d.	N.d.	0.09 ± 0.03^{ab}	0.09 ± 0.03^{ab}	0.07 ± 0.04^{b}	0.12 ± 0.03^{a}
7	**	–	N.s	0.52 ± 0.17^{b}	0.79 ± 0.22^{a}	0.44 ± 0.18^{b}	0.43 ± 0.17^{b}	N.d.	N.d.	N.d.	N.d.	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
8	***	–	–	0.31 ± 0.15^{b}	0.74 ± 0.31^{a}	0.20 ± 0.10^{b}	0.19 ± 0.15^{b}	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.
9	***	N.s	N.s	0.46 ± 0.27^{b}	0.99 ± 0.36^{a}	0.28 ± 0.10^{b}	0.21 ± 0.07^{b}	0.05 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.08 ± 0.02	0.08 ± 0.03	0.07 ± 0.03	0.11 ± 0.03
10	***	N.s	*	0.55 ± 0.11^{a}	0.75 ± 0.25^{a}	0.20 ± 0.04^{b}	0.27 ± 0.11^{b}	0.41 ± 0.08	0.38 ± 0.05	0.41 ± 0.13	0.38 ± 0.15	0.48 ± 0.15^{ab}	0.40 ± 0.17^{ab}	0.37 ± 0.08^{b}	0.57 ± 0.12^{a}
11	N.s	–	**	0.31 ± 0.08	0.33 ± 0.04	0.35 ± 0.07	0.27 ± 0.08	N.d.	N.d.	N.d.	N.d.	0.04 ± 0.02b	0.06 ± 0.01^{ab}	0.08 ± 0.03^{a}	0.07 ± 0.02^{a}
12	N.s	–	N.s	8.04 ± 1.56	7.64 ± 1.32	7.59 ± 1.31	7.90 ± 1.34	N.d.	N.d.	N.d.	N.d.	0.60 ± 0.21	0.71 ± 0.21	0.68 ± 0.19	0.74 ± 0.16
13	N.s	–	N.s	19.78 ± 4.70	20.77 ± 3.75	19.63 ± 2.72	20.01 ± 3.04	N.d.	N.d.	N.d.	N.d.	1.50 ± 0.44	1.86 ± 0.58	1.79 ± 0.49	1.97 ± 0.41
14	N.s	–	N.s	0.45 ± 0.14	0.48 ± 0.07	0.59 ± 0.11	0.52 ± 0.12	N.d.	N.d.	N.d.	N.d.	0.06 ± 0.01	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.02
15	N.s	–	N.s	3.39 ± 0.79	3.73 ± 0.56	4.22 ± 0.61	4.06 ± 0.63	N.d.	N.d.	N.d.	N.d.	0.22 ± 0.06	0.29 ± 0.07	0.29 ± 0.05	0.29 ± 0.07
Σ PA	***	N.s	*	4.73 ± 0.8^{6b}	7.35 ± 1.73^{a}	3.96 ± 1.06^{bc}	3.12 ± 0.57^{c}	1.27 ± 0.21	1.13 ± 0.19	1.06 ± 0.21	1.07 ± 0.41	1.77 ± 0.41	1.72 ± 0.47	1.44 ± 0.36	2.04 ± 0.41
Σ F	N.s	–	N.s	31.97 ± 6.85	32.95 ± 5.49	32.37 ± 4.66	32.75 ± 4.98	–	–	–	–	2.43 ± 0.69	3.00 ± 0.87	2.93 ± 0.69	3.13 ± 0.64
Total	***	N.s	N.s	36.70 ± 6.60^{b}	40.30 ± 5.59^{a}	36.33 ± 5.14^{b}	35.87 ± 5.35^{c}	1.27 ± 0.21	1.13 ± 0.19	1.06 ± 0.21	1.07 ± 0.41	4.19 ± 1.09	4.72 ± 1.27	4.36 ± 0.97	5.17 ± 0.96

[†] *N.s = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.* [‡]*Values (mean of 8 replications) followed by the same letter, within the same row were not significantly different ($p < 0.05$), according to the Tukey's least significant difference test. N.d. = not detected.* [ψ] *C = compound; S = skin; DK = deskinned kernel; WK = whole kernel; PA = phenolic acids; F = flavonols; dw = dry weight; 1 = p-hydroxy-benzoic acid, 2 = Vanillic acid, 3 = Procyanidin B-type dimer, 4 = (-)-Epicatechin, 5 = Procyanidin B-type dimer, 6 = Procyanidin B-type trimer, 7 = Procyanidin B-type tetramer, 8 = Procyanidin B-type trimer, 9 = Naringenin-7-O-glucoside, 10 = Kaempferol-3-O-rutinoside, 11 = Isorhamnetin-3-O-rutinoside, 12- Kaempferol-3-O-rutinoside, 13- Kaempferol-3-O-galactoside, 14- Isorhamnetin-3-O-glucoside, 15- Kaempferol-3-O-glucoside; T1 = Full irrigation, T2 = Moderate regulated deficit irrigation (RDI), T3 = Severe RDI, T4 = Sustained deficit irrigation.*

Table 3
Effect of irrigation treatments in proanthocyanidin oligomeric species content (mg/100 g dw) in whole kernel.

Peak	Proanthocyanidin	Rt (min)	ANOVA Test[†]	T1[ψ]	T2	T3	T4
				mg/100 g dw			
				Tukey Multiple Range Test[‡]			
–	Dimer B type [(E)C-B-(E)C]	5.3	–	Traces	Traces	Traces	Traces
1	Trimer B type [(E)C-B-(E)C- B-(E)C]	9.4	N.s	10.45 ± 2.28	8.67 ± 1.12	9.77 ± 2.23	13.10 ± 1.87
2	Trimer B type [(E)C-B-(E)C- B-(E)C]	11.3	N.s	10.62 ± 2.42	7.90 ± 1.97	10.89 ± 1.79	10.69 ± 1.24
3	Trimer B type [(E)C-B-(E)C- B-(E)C]	12.2	N.s	1.72 ± 0.31	1.53 ± 0.21	1.79 ± 0.11	1.70 ± 0.15
4	Trimer B type [(E)C-B-(E)C- B-(E)C]	12.9	N.s	0.63 ± 0.09	0.88 ± 0.32	0.71 ± 0.36	1.27 ± 0.52
5	Trimer B type [(E)C-B-(E)C- B-(E)C]	13.5	**	4.80 ± 0.28^{b}	6.38 ± 1.51^{ab}	5.06 ± 1.37^{b}	8.84 ± 1.53^{a}
6	Tetramer B type [(E)C-B-(E)C- B-(E)C- B-(E)C]	16.6	N.s	1.71 ± 0.48	2.17 ± 0.50	1.80 ± 0.54	1.57 ± 0.57
7	Tetramer B type [(E)C-B-(E)C- B-(E)C- B-(E)C]	17.1	N.s	6.34 ± 1.09	5.77 ± 0.64	6.33 ± 0.96	6.21 ± 0.92
8	Tetramer B type [(E)C-B-(E)C- B-(E)C- B-(E)C]	18.1	N.s	0.81 ± 0.21	1.04 ± 0.20	1.02 ± 0.19	1.21 ± 0.27
9	Tetramer B type [(E)C-B-(E)C- B-(E)C- B-(E)C]	18.3	N.s	1.82 ± 0.18	1.15 ± 0.17	1.21 ± 0.57	1.54 ± 0.60
10	Tetramer B type [(E)C-B-(E)C- B-(E)C- B-(E)C]	34.7	***	2.79 ± 0.01^{b}	4.00 ± 0.59^{a}	4.44 ± 0.75^{a}	3.62 ± 0.62^{ab}
	Total		N.s	41.69 ± 6.27	39.49 ± 3.10	43.01 ± 3.94	49.76 ± 3.12

[†] *N.s = not significant at $p < 0.05$; *. **. ***, significant at $p < 0.05$. 0.01. and 0.001, respectively.* [‡] *Values (mean of 4 replications) followed by the same letter within the same row were not significantly different ($p < 0.05$), according to Tukey's least significant difference test;* [ψ]*dw = dry weight; traces = detected but below the limit of quantification; T1 = Full irrigation, T2 = Moderate regulated deficit irrigation (RDI), T3 = Severe RDI, T4 = Sustained deficit irrigation.*

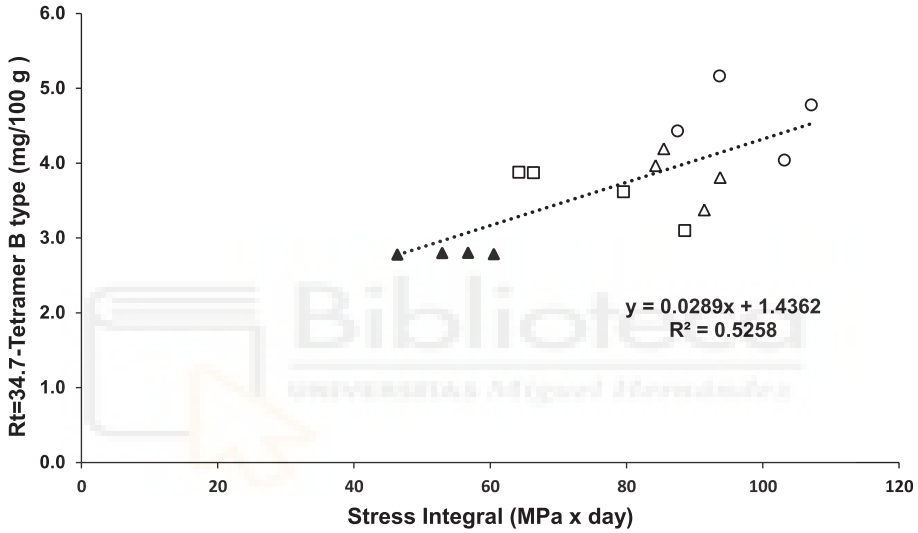

Fig. 2. Pearson's correlation between stress integral and the content of tetramer B type (Rt = 34.7 min) proanthocyanidin. *Note: ▲ = Full irrigated (T1); □ = Sustained deficit irrigation (T4); Δ = Moderate regulated deficit irrigation (T3); ○ = Severe regulated deficit irrigation.*

the DI irrigation treatments (T2 = 4.00 and T3 = 4.44 mg/100 g dw, respectively) leading to higher PA 10 concentration than the control (T1 = 2.79 mg/100 g dw). A significant negative correlation (R = −0.73; $p = 0.001$) was observed between the content of tetramer PA 9 (Rt = 18.3 min) and the stress integral in which 16 data (4 × treatments) points have been used. However, a significant positive correlation (R = 0.73; $p = 0.001$) was observed between the content of PA 10 (Rt = 34.7 min) and the stress integral with a coefficient of determination (correspondent to the squared correlation coefficient) of $R^2 = 0.53$ (Fig. 2). This relationship agreed with authors working on analysis of flavan-3-ols and PAs in tea plants growth under water stress conditions (Hernández, Alegre, & Munné-Bosch, 2006); they observed an increase of 10-fold in (−)-epicatechin in tea plants exposed to 19 days of water deficit. This compound serves to form PAs building blocks; thus, it may finally polymerize and accumulate PAs in vacuoles. It is possible that the oxidation of (−)-epicatechin to its quinone, observed in water stress samples, is a step of an oxidative process leading to the biosynthesis of PAs (Hernández et al., 2006). Proanthocyanidins has a significant protective role linked to biotic stress resistance such as defence against different external factors (Hernández et al., 2006).

The total low molecular weight PAs concentration found in almond kernels ranged from 39.5 mg/100 g dw (T2) to 49.8 mg/100 g dw in (T4), but no statistically significant differences were observed among the irrigation treatments. The current values are consistent and even higher than those reported by Bittner, Rzeppa, and Humpf (2013) in almond kernels (7.09–30.40 mg/100 g dw), in almond blanching water (32.2 mg/100 g) (Pérez-Jiménez & Torres, 2012), and in roasted almond skin (49.3 mg/100 g) (Monagas et al., 2009). However, higher values of PAs (184 mg/100 g fresh weight) in almonds were reported by Gu et al. (2004) in their study about the concentration of the PAs in US foods and their daily intake. In this aspect, it should be said that differences in PAs contents may be influenced by the sample origin, variety, stage of ripeness, post-harvesting storage or extraction method (de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2000). For instance, in a previous reported study in nuts (hazelnuts, pecans, pistachios, almonds, walnuts, peanuts, and cashews) samples were defatted prior to the PAs extraction (Gu et al., 2004), while in the present study the extraction was made directly with the almond powder. Plant metabolites such as fat, protein, and carbohydrates can form complexes with PAs, interfering with their extraction and isolation (Rue et al., 2018). Also other authors found a high degree of variability among the PAs content of different almond cultivars (Butte, Carmel, and Nonpareil), ranging from 32.2 and 111 mg/100 g (Bolling, 2017). The blanched Spanish and US almond skin was also reported as having

significant differences among the proanthocyanidin dimers and trimers, with total contents being 10.6 and 4.10 mg/100 g, respectively (Monagas et al., 2007).

3.5. Proanthocyanidins content after phloroglucinolysis and degree of polymerization

The *phloroglucinolysis* method was used because this is the only quantification method which allowed to measure the total PAs concentration including both extractable and non-extractable PAs and can better reflect the real concentration of total PAs (Kennedy & Jones, 2001). Besides, this method enabled to determine the average degree of polymerization (DP) and nature and proportion of the constitutive flavanols units to be determined. The *phloroglucinolysis* was carried out in the all three parts of the edible almond: skin, deskinned and whole kernel. The results obtained on total PAs in skin, deskinned, and whole kernel (Table 4) evidenced the highest PAs content in skin (7796 mg/100 g dw), on average], followed by that of the whole kernels (774 mg/100 g dw, on average), while in the deskinned almonds, the PAs were below the limit of quantification. Regarding the irrigation treatments, the PAs content increased in moderate RDI (T2) and severe RDI (T3) in whole kernels. This is in accordance with previous studies detailing an up-regulation of the PA synthesis (overall towards PA dimers and simple units like catechin or epicatechin) as consequence of water deficit treatments (Genebra et al., 2014; Hernández et al., 2006). Lower values of PAs content were reported in pistachios nuts (352–427 mg/100 g), although the same tendency was observed regarding the deficit irrigation; the PAs content was increased with the water stress in plant (Noguera-Artiaga et al., 2018). These results are important from a nutritional and medical point of view and show the importance of the almond skin, which is often removed to make almonds less bitter and astringent. Authors demonstrated the antioxidant defence and hepatoprotection of almond skin procyanidins by regulating the protein expression of phase II detoxifying and antioxidant enzymes in HepG2 cells and acetaminophen treated hepatotoxic mice (Truong et al., 2014).

Table 4
Phloroglucinolysis method data showing polymeric proanthocyanidins (mg/100 g dw) the degree of polymerisation and antioxidant activity (mmol Trolox/kg dw) of almonds as affected by irrigation strategies.

	Pas mg/100 g dw	DP	$ABTS^{\cdot+}$ (mmol Trolox/kg dw)	FRAP (mmol Trolox/kg dw)
ANOVA†				
Skin	***	*	N.s	**
Deskinned kernel	–	–	N.s	N.s
Whole kernel	*	N.s	**	***
Tukey's multiple range test‡ for skin				
T1	7749 ± 589^{a}	4.7 ± 0.1^{ab}	113 ± 31.33	19.6 ± 6.70^{b}
T2	6497 ± 1354^{b}	4.8 ± 0.2^{a}	119 ± 33.24	22.8 ± 9.32^{ab}
T3	8463 ± 554^{a}	4.6 ± 0.1^{b}	133 ± 13.55	27.3 ± 2.89^{a}
T4	8475 ± 666^{a}	4.6 ± 0.1^{b}	140 ± 24.29	28.9 ± 6.61^{a}
Tukey's multiple range test‡ for deskinned kernel				
T1	N.d.	N.d.	0.68 ± 0.32	1.35 ± 0.13^{b}
T2	N.d.	N.d.	0.97 ± 0.60	1.77 ± 0.18^{a}
T3	N.d.	N.d.	0.58 ± 0.28	1.86 ± 0.51^{a}
T4	N.d.	N.d.	0.79 ± 0.44	1.26 ± 0.53^{b}
Tukey's multiple range test‡ for whole kernel				
T1	953 ± 123^{b}	5.1 ± 0.2	4.23 ± 0.59^{bc}	2.93 ± 1.66
T2	1115 ± 264^{a}	5.1 ± 0.1	5.61 ± 0.64^{ab}	3.91 ± 1.24
T3	1150 ± 243^{a}	5.2 ± 0.2	6.01 ± 2.00^{a}	3.76 ± 0.69
T4	878 ± 159^{c}	5.1 ± 0.2	3.87 ± 2.05^{c}	3.63 ± 0.81

† *N.s. = not significant at $p < 0.05$ and * significant at $p < 0.05$.* ‡*Values (mean of 3 replications) followed by the same letter, within the same column and factor, were not significantly different ($p < 0.05$), according to Tukey's multiple range test.* ¥*N.d = not detected; dw = dry weight; T1 = Full irrigation, T2 = Moderate regulated deficit irrigation (RDI), T3 = Severe RDI, T4 = Sustained deficit irrigation; PAs = Polymeric proanthocyanidins; DP = degree of polymerization. $ABTS^{\cdot+}$ = 2.2-azino-bis* (3-ethylbenzothiazoline-6-sulfonic acid)*; FRAP = ferric reducing ability of plasma.*

Antioxidant, anticarcinogenic, cardio-protective, antimicrobial, and neuroprotective activities were reported for flavanol compounds (Monagas et al., 2009). Moreover, all these biological properties are strongly related to their degree of polymerization (DP), because it influences the bioavailability *in vivo* (Manach et al., 2004). Proanthocyanidins are different from other polyphenols precisely due to their polymeric nature and high molecular weight, which limit their absorption through gastrointestinal tract (Manach et al., 2004). For instance, only dimers and trimers crossed the intestinal epithelium, while oligomers higher than trimers are not absorbed in their natural form (Deprez et al., 2001). However, authors using single layers of Caco-2 cells as a model of absorption in the small intestine reported that PAs with high DP were retained in Caco-2 cells due to their affinity to proteins (which also explain the long aftertaste of astringency in mouth after consuming high PAs foods) (Deprez et al., 2001). As said, PAs with high DP are retained in the small intestine, which help to protect mucosa against aggression by toxic compounds in food products (Deprez et al., 2001).

Generally, the average of DP was reported to vary between 3 and 11, but also might reach values as high as 17 in some matrixes, such as cider apple extract (Santos-Buelga & Scalbert, 2000). Values on DP above 10 have been previously reported in almonds (Gu et al., 2004). However, current data only shows PAs reaching up to tetramers and a DP measured by *phloroglucinolysis* up to 5.1 in whole kernel and up to 4.7 in skin. This difference might be related to the equipment sensibility and the extraction method. Authors reported PAs with a DP greater than 4 in almonds when different equipment (MALDI–TOF MS and MALDI–TOF/TOF MS) was used (Hernandez, Alegre, & Munnebosch, 2006). This might also be associated to a poor solubility or the secondary chemical reactions with the insoluble matrices (Santos-Buelga & Scalbert, 2000). For instance, PAs with higher DP were better extracted with aqueous acetone than aqueous methanol, because high-molecular-weight PAs are better absorbed in a polar matrix than lower molecular weight PAs (Santos-Buelga & Scalbert, 2000). Regarding irrigation treatments, a slightly higher DP was found in almond skin under moderate RDI, while no differences were observed for the whole kernels. The daily intake of PAs has been previously estimated between 100 and 500 mg (Deprez et al., 2001). The present study, showed a content of 13.5 mg/100 g of the tetramer PAs for the control almonds (T1) and an average of 14.4 mg/100 g for the almonds cultivated under DI (T2 = 14.1, T3 = 14.8 and T4 = 14.1 mg/100 g). Thus, consuming 43 g of control almonds (as recommended by Food and Drug Administration health claim) represents 5.81% daily intake of the total (only tetramers) polymeric PAs content, while hydroSOStainable almonds represents 6.19%. The increase of polymeric PAs in hydroSOStainable almonds is slight but is greater, and the PAs concentration might be significantly increase when considering that proposed daily consumption is up to 500 mg/day. Moreover, the PAs polymers were reported not to be absorbed through the gut epithelium due to their high molecular weight and so their affinity to protein (Deprez et al., 2001). In this sense, this increase in polymeric PAs in hydroSOStainable almonds will be on benefits, since PAs retained by mucosa may protect it against aggression of those toxic compounds ingested from food.

3.6. Antioxidant activity ($ABTS^{\cdot+}$ and FRAP)

The antioxidant activity of almonds under DI was evaluated using two different methods: $ABTS^{\cdot+}$ and FRAP (Table 4). By analysing AA of the different parts of the almond cultivated under different levels of drought, it was demonstrated that all almond parts had significant capacity to scavenge $ABTS^{\cdot+}$ free radicals, as well as the ability to reduce

to the ferric complex (FRAP), but, at different levels. For instance, $ABTS^{\cdot+}$ scavenging was significantly higher in skins (mean value for all treatments of 126 mmol Trolox/kg dw) than in the whole kernel (mean of 4.9 mmol Trolox/kg dw) and deskinned almonds (0.8 mmol Trolox kg^{-1} dw). The same trend was observed for the FRAP data. Regarding water deficit, the $ABTS^{\cdot+}$ scavenging was higher in whole kernels cultivated under moderate and severe RDI (5.61 and 6.01 mmol Trolox/kg dw for T2 and T3 samples, respectively) than in the other treatments; this same trend was also confirmed by the FRAP data. Similar type of responses against water deficit have been previously reported in "Vairo" almonds (Lipan et al., 2019), "Manzanilla" raw and processed table olives (Sánchez-Rodríguez, Lipan et al., 2019), and cumin (Bettaieb Rebey et al., 2012). The free radical scavenging properties of almonds have been directly linked to their almonds polyphenolic content (Huang, Chang, & Shao, 2006). For instance, a positive correlation was reported between the stress integral and TPC in almonds (Lipan et al., 2019). The exposure of plants to water stress increases the production of ROS, to protect themselves by activating the antioxidant defence system (Sánchez-Rodríguez et al., 2010). The activity of low molecular antioxidants can successfully scavenge damaging radicals and may prevent potential damage (Bettaieb Rebey et al., 2012). As expected, a significant positive correlation was observed in the current study among the $ABTS^{\cdot+}$ and FRAP values with the total of procyanidins obtained after *phloroglucinolysis* in whole almonds ($R = 0.612$; $p = 0.012$ and $R = 0.666$; $p = 0.005$), confirming that PAs might be of great interest in nutrition and medicine due to their high antioxidant capacity and consequently a valuable defensive effect on human health (Santos-Buelga & Scalbert, 2000). Moreover, positive correlations between the antioxidant activity ($ABTS^{\cdot+}$ and FRAP) and different phenolic compounds were also observed in almond skin such as (i) $ABTS^{\cdot+}$ and FRAP with Isorhamnetin-3-*O*-glucoside ($R = 0.656$; $p = 0.006$ and $R = 0.660$; $p = 0.005$); (ii) FRAP with Kaempferol-3-*O*-glucoside ($R = 0.508$; $p = 0.045$); and (iii) $ABTS^{\cdot+}$ with total flavonols ($R = 0.533$; $p = 0.034$).

4. Conclusions

The present study was the first one identifying and quantifying the ow molecular weight phenolics in almonds, cultivar Vairo, cultivated under water stress conditions. After a careful study of the experimental data, it can be concluded that reducing the amount of irrigation water during the kernel filling presented a significant impact on the almond functionality. For instance, the application of moderate DI (T2) significantly increased the total phenolic content, the content of individual compounds (phenols *p*-hydroxy-benzoic acid, vanillic acid, procyanidin B-type dimer, procyanidin B-type tetramer, procyanidin B-type trimer, and naringenin-7-*O*-glucoside), individual PAs (one trimer B-type and one tetramer B-type), the degree of polymerization and the antioxidant activity. Further research during several seasons of water stress is required to check the present results repeatability.

CRediT authorship contribution statement

Leontina Lipan: Conceptualization, Data curation, Investigation, Methodology, Software, Writing - original draft, Writing - review & editing. **Jacinta Collado-González:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Aneta Wojdyło:** Methodology, Supervision, Validation, Writing - review & editing. **Raúl Domínguez-Perles:** Data curation, Methodology. **Ángel Gil-Izquierdo:** Funding acquisition, Validation. **Mireia Corell:** Methodology. **Alfonso Moriana:** Funding acquisition, Project administration, Resources. **Marina Cano-Lamadrid:** Formal analysis, Writing - review & editing. **Ángel Carbonell-Barrachina:** Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing - review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127756.

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Table 1 S. Pearson's correlation coefficients *(R)* among antioxidant activity, total proanthocyanidins after phloroglucinolysis, degree of polymerization and phenolic compounds in skin.

Skin	ABTS	FRAP	Σ PP	DP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Σ PhA	Σ FL	Σ PC
ABTS•+	**1.00**																					
FRAP	0.98***	**1.00**																				
∑ PP	0.03	0.07	**1.00**																			
DP	0.17	0.17	-0.78***	**1.00**																		
1	0.05	0.06	-0.72**	0.49	**1.00**																	
2	-0.24	-0.20	-0.52*	0.42	0.72**	**1.00**																
3	-0.51*	-0.49	-0.51*	0.42	0.46	0.78***	**1.00**															
4	0.25	0.25	-0.29	0.09	0.56*	0.60*	0.36	**1.00**														
5	-0.19	-0.18	-0.82***	0.53*	0.85***	0.81***	0.70**	0.54*	**1.00**													
6	0.16	0.10	-0.64**	0.49	0.73***	0.69**	0.51*	0.69**	0.76***	**1.00**												
7	-0.07	-0.08	-0.77***	0.51*	0.74***	0.67**	0.67**	0.50*	0.88***	0.74***	**1.00**											
8	-0.16	-0.14	-0.84***	0.62*	0.73***	0.73***	0.65**	0.31	0.89***	0.54*	0.83***	**1.00**										
9	-0.29	-0.28	-0.80**	0.52	0.71**	0.73***	0.72**	0.34	0.91***	0.58*	0.85***	0.95***	**1.00**									
10	-0.27	-0.32	-0.78**	0.58*	0.52*	0.58*	0.73***	0.21	0.75***	0.52*	0.77***	0.86***	0.87***	**1.00**								
11	0.00	-0.05	-0.09	-0.12	0.32	0.44	0.11	0.48	0.33	0.52*	0.11	0.13	0.26	0.12	**1.00**							
12	0.48	0.39	0.06	-0.12	0.01	-0.04	-0.16	0.58*	-0.04	0.30	-0.09	-0.15	-0.16	-0.01	0.38	**1.00**						
13	0.49	0.43	-0.15	0.05	0.22	0.13	-0.09	0.68**	0.23	0.48	0.14	0.08	0.08	0.13	0.48	0.93***	**1.00**					
14	0.66**	0.66**	0.25	-0.23	0.09	0.00	-0.44	0.44	-0.08	0.20	-0.15	-0.24	-0.31	-0.39	0.49	0.55*	0.59*	**1.00**				
15	0.46	0.51*	0.31	-0.14	0.00	0.01	-0.52*	0.13	-0.23	-0.07	-0.42	-0.20	-0.34	-0.45	0.24	0.39	0.41	0.65**	**1.00**			
Σ PhA	-0.21	-0.21	-0.82***	0.58*	0.81***	0.84***	0.80***	0.51*	0.96***	0.76***	0.92***	0.93***	0.95***	0.86***	0.29	-0.04	0.18	-0.19	-0.31	**1.00**		
Σ FL	0.53*	0.47	-0.04	-0.02	0.16	0.09	-0.18	0.64**	0.12	0.41	0.01	-0.01	-0.04	0.02	0.47	0.95***	0.99***	0.66**	0.53*	0.07	**1.00**	
Σ PC	0.37	0.32	-0.40	0.24	0.50	0.45	0.20	0.79***	0.53*	0.69**	0.42	0.40	0.39	0.40	0.54*	0.80***	0.93***	0.48	0.32	0.50*	0.90***	**1.00**

*Note: *, **, ***, significant at p < 0.05, 0.01, and 0.001, respectively. ABTS•+=2.2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP=ferric reducing ability of plasma; Σ PP=total proanthocyanidins after phloroglucinolysis; DP=degree of polymerization 1= p-hydroxy-benzoic acid, 2= Vanillic acid, 3= Procyanidin B-type dimer, 4=(-)-Epicatechin, 5= Procyanidin B-type dimer, 6= Procyanidin B-type trimer, 7=Procyanidin B-type tetramer, 8= Procyanidin B-type trimer, 9= Naringenin-7-O-glucoside, 10= Kaempferol-3-O-rutinoside, 11= Isorhamnetin-3-O-rutinoside, 12- Kaempferol-3-O-rutinoside, 13- Kaempferol-3-O-galactoside, 14- Isorhamnetin-3-O-glucoside, 15- Kaempferol-3-O-glucoside; Σ PhA=phenolic acids, Σ FL=flavonols, Σ PC=total phenolic compounds.*

Table 2 S. Pearson's correlation coefficients *(R)* among antioxidant activity, total proanthocyanidins after phloroglucinolysis, degree of polymerization and phenolic compounds in whole kernel.

WK	ABTS	FRAP	Σ PP	DP	1	2	3	4	5	6	7	9	10	11	12	13	14	15	Σ PhA	Σ FL	Σ PC
ABTS•+	**1.00**																				
FRAP	0.98***	**1.00**																			
Σ PP	0.61*	0.67**	**1.00**																		
DP	0.32	0.40	0.50*	**1.00**																	
1	0.40	0.47	0.57*	0.51*	**1.00**																
2	-0.12	-0.14	-0.51*	-0.39	-0.32	**1.00**															
3	-0.26	-0.29	-0.15	-0.31	-0.56*	-0.12	**1.00**														
4	-0.47	-0.41	-0.30	-0.18	-0.34	-0.05	0.72**	**1.00**													
5	-0.22	-0.30	-0.31	-0.25	-0.27	-0.09	0.25	0.46	**1.00**												
6	-0.43	-0.48	-0.42	-0.22	-0.48	-0.11	0.86***	0.71**	0.34	**1.00**											
7	-0.36	-0.37	-0.47	-0.14	-0.05	0.25	0.25	0.42	0.22	0.46	**1.00**										
9	-0.55*	-0.59*	-0.48	-0.37	-0.61*	-0.13	0.80***	0.71**	0.46	0.89***	0.43	**1.00**									
10	-0.48	-0.51*	-0.39	-0.33	-0.64**	-0.04	0.86***	0.75***	0.41	0.85***	0.48	0.87***	**1.00**								
11	-0.13	-0.12	0.06	0.21	0.20	-0.34	-0.30	-0.33	0.05	-0.13	-0.19	0.10	-0.21	**1.00**							
12	0.13	0.16	0.08	0.04	0.06	-0.12	0.56*	0.67**	0.27	0.54*	0.40	0.48	0.49	-0.24	**1.00**						
13	0.10	0.12	-0.04	-0.05	-0.07	-0.15	0.55*	0.67**	0.35	0.54*	0.36	0.57*	0.52*	-0.11	0.96***	**1.00**					
14	0.39	0.41	0.14	-0.17	0.11	-0.12	0.01	0.09	0.05	-0.12	-0.04	-0.01	-0.04	-0.11	0.51*	0.61*	**1.00**				
15	0.10	0.12	0.15	0.00	0.18	-0.30	0.35	0.44	0.30	0.37	0.28	0.48	0.32	0.21	0.86***	0.89***	0.62*	**1.00**			
Σ PhA	-0.41	-0.45	-0.34	-0.35	-0.60	-0.08	0.94***	0.81***	0.50*	0.91***	0.43	0.90***	0.95***	-0.23	0.58*	0.60*	-0.01	0.41	**1.00**		
Σ FL	0.11	0.14	0.01	-0.02	-0.01	-0.17	0.53*	0.64**	0.33	0.52*	0.35	0.54*	0.48	-0.08	0.97***	1.00***	0.61***	0.92***	0.57*	**1.00**	
Σ PC	-0.12	-0.13	-0.16	-0.18	-0.29	-0.15	0.79***	0.80***	0.45	0.77***	0.43	0.78***	0.77***	-0.16	0.90***	0.93***	0.39	0.79***	0.85***	0.92***	**1.00**

*Note: *, **, ***, significant at p < 0.05, 0.01, and 0.001, respectively. WK= whole kernel; ABTS•+=2.2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP=ferric reducing ability of plasma; Σ PP=total proanthocyanidins after phloroglucinolysis; DP=degree of polymerization 1= p-hydroxy-benzoic acid, 2= Vanillic acid, 3= Procyanidin B-type dimer, 4=(-)-Epicatechin, 5= Procyanidin B-type dimer, 6= Procyanidin B-type trimer, 7=Procyanidin B-type tetramer, 9= Naringenin-7-O-glucoside, 10= Kaempferol-3-O-rutinoside, 11= Isorhamnetin-3-O-rutinoside, 12- Kaempferol-3-O-rutinoside, 13- Kaempferol-3-O-galactoside, 14- Isorhamnetin-3-O-glucoside, 15- Kaempferol-3-O-glucoside; Σ PhA=phenolic acids, Σ FL=flavonols, Σ PC=total phenolic compounds.*

PUBLICATION 5 (Open Access):

SENSORY PROFILE AND ACCEPTABILITY OF HYDROSOSTAINABLE ALMONDS

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Sensory Profile and Acceptability of HydroSOStainable Almonds

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Abstract: Fresh water availability is considered highly risky because it is a finite resource, and a deficiency in water leads to numerous economic and environmental issues. Agriculture is one of the main consumers of fresh water in practices such as irrigation and fertilization. In this context, the main objectives of this study were (i) to determine the descriptive sensory profiles of four almond types grown using different irrigation strategies and (ii) to study their acceptance in a cross-cultural study (Romania and Spain). Consumers' willingness to pay for hydroSOS almonds was also evaluated. The four irrigation strategies evaluated were a control sample, two samples grown under regulated deficit irrigation strategies (RDI), and a sample grown under a sustained deficit irrigation strategy (SDI). The main conclusion was that neither descriptive nor affective sensory results showed significant differences among treatments. These findings should encourage farmers to reduce their water usage by demonstrating that sensory quality was not significantly affected by any of the studied treatments, compared to the control. Regarding willingness to pay, both Spanish and Romanian consumers were willing to pay a higher price for the hydroSOS almonds.

Keywords: cross-cultural affective test; descriptive sensory analysis; hydroSOStainable products; *Prunus dulcis*; willingness to pay

1. Introduction

Fresh water is a finite resource, and uncertainty regarding the remaining level of water for future generations has led the world to seek sustainability as a compulsory issue for future economic

development and healthy ecosystems [1,2]. The World Economic Forum (WEF) placed water scarcity as the main global risk of the economy regarding impact, because a shortage of water means a stoppage of factories and food production, leading to the decline of the global economy [2]. The population growth drives to an augmentation in intensive food production that alters the environment due to greenhouse gas emissions, soil deterioration, and water stress [3].

Agriculture is one of the biggest consumers of fresh water, mainly due to the large volume necessary for irrigation (70–80% of the total) [1,4]. Opinions about irrigation in agriculture are divided about whether irrigation is necessary or not. Some believe water irrigation is required to produce enough food in the future due to world population growth, while others find irrigation agriculture wasteful because it creates "water-guzzling crops" [4]. For this reason, agriculture, particularly in the Mediterranean (semi-arid) region, must evaluate water use sustainability by implementing plans and irrigation strategies capable of reducing water irrigation but maintaining the quality of products [5].

Almonds are the major tree nut crop in the Mediterranean basin, which is defined by low rainfall and elevated evaporated demand during the almond growing cycle [6]. Although it is considered a drought-resistant crop, the almond tree (*Prunus dulcis*) needs irrigation to produce yield and profitability [6,7]. Numerous studies in fruits, such as almonds, olives, pistachio, apples, and grapes, have proven that fruit quality could be increased by controlling and reducing the amount of water irrigation [8–14]. Therefore, the development of deficit irrigation strategies (DI), such as regulated and sustained deficit irrigation, might be useful to increase the water productivity maintaining fruit quality.

Deficit irrigation strategies refer to the application of water below the crop evapotranspiration (ET: the combination between the evaporation losses from the soil and transpiration losses from the crop) requirements [4]. Regulated deficit irrigation (RDI) was developed to supervise vegetative vigor and consists of applying limited water during certain stages (in which plant is less sensitive to water stress) of the growing season. In the almond crop, the most recommended and less sensitive phenological period to apply water stress is the stage IV, which is contemporaneous with kernel filling and happens during the summer months of highest evaporative demand [5,6]. On the other hand, sustained deficit irrigation (SDI) is a strategy in which a uniform and reduced amount of water is applied to crops during all growing cycles, creating a progressive stress in plants throughout the season [6]. In this strategy, stress is produced by not entirely refilling the root zone when irrigated [7].

Sensory analysis techniques are essential to establish the quality of a product and to understand consumer preferences. Descriptive sensory analysis consists of detection and description, not only of quantitative but also qualitative sensory attributes of products. These attributes are of utmost importance to define a product, including its appearance, aroma, flavor, and texture [15]. On the other hand, affective tests are used to evaluate consumer preferences or acceptance responses to a product [15].

Society now expects the incorporation of an environmental sustainability plan [16]. In this context, consumers play an essential role because they demand and choose food products with specific characteristics, and nowadays they are concerned, not only about healthy diets but about environmental protection [3]. This has led to the development of the "environmentalism" phenomenon, which mostly relies on government agencies and non-governmental organizations caring about ecological issues [17]. These phenomena have made the consumer more conscious and interested in healthy, safe, and environmentally friendly food; consequently, the consumer has a greater willingness to pay for eco-friendly and hydroSOStainable (hydroSOS) products [17,18].

Under these circumstances, the aim of this study was (i) to determine the descriptive sensory profiles of four different almond types grown using different irrigation strategies (including hydroSOS samples) and (ii) to study their acceptance and consumers' willingness to pay in a cross-cultural study in Romania and Spain. Understanding consumers' preferences and their willingness to pay for hydroSOS almonds is vital for almond growers.

2. Materials and Methods

2.1. Irrigation Treatments

The almond cultivar used in the present study was "Vairo" and was grown on the commercial farm "La Florida" located in Dos Hermanas (Seville, Spain). The following four irrigation treatments were evaluated:

- **T1** was full irrigation treatment using 433 ± 26 mm of applied water throughout the season with a stress integral of SI = 54.2. Trees were irrigated to assure the estimated crop ET, and thus represented the control.
- **T2** were trees under regulated deficit irrigation (RDI) at optimum level (148 ± 24 mm; SI = 91.7). For irrigation scheduling, midday stem water potential (SWP) and maximum daily shrinkage (MDS) measurements were done. Then, in stage IV (kernel filling) of the almond growing cycle, the trees were irrigated when SWP was lower than −1.5 MPa or when MDS signal was above 1.75. The rest of the stages were irrigated to the SWP proposed by McCutchan and Shackel (1992) or MDS equal 1 [19].
- **T3** trees were also irrigated under regulated deficit irrigation but in more severe conditions (103 ± 13 mm; SI = 94.9). Thus, the stage IV trees were irrigated when SWP was lower than −2 MPa or MSD signal above 2.75, and similar conditions as previously described for T2 were applied for the rest of the period.
- **T4** trees were irrigated under sustained deficit irrigation (SDI) conditions (114 ± 13 mm; SI = 74.7). Water was applied gradually throughout the growing season.

In order to determine the accumulative effect of water deficit, water stress integral was calculated by using the following equation:

$$\mathrm{SI} = \left| \sum (\min \Psi_{\mathrm{stem}} - (-0.2)) \times n \right| \quad (1)$$

In this expression, SI was the stress integral, min Ψ_{stem} was the average of minimum SWP, and n represented the day numbers interval.

The field study was conducted during 2017; in August, almonds were harvested, dried (below 5% moisture content), and delivered to University Miguel Hernández of Elche (Spain) facilities, where descriptive and affective studies were carried out. Almonds were also sent to University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (Romania) to perform affective studies with Romanian consumers. Around 1.5 kg of almond kernels was needed for each treatment.

2.2. Descriptive Sensory Analysis

A trained panel with 10 highly trained panelists from the Food Quality and Safety Group (Miguel Hernández University of Elche, Orihuela, Alicante, Spain) conducted the descriptive analysis. Each panelist had more than 600 hours of experience with different types of food products. Although the panel had a vast experience in tasting almond and turrón (traditional Spanish dessert made basically of toasted almonds and honey), they had four orientation sessions for the almond tasting, where the panelists decided the final list of descriptors and reference products for each attribute. The reference and modified lexicon were the ones developed by Vázquez-Araújo et al. [20]. Table 1 shows the reference products used by the panelists for flavor and texture characterization. The almond color scale was developed using instrumental color measurements carried out with a Minolta Colorimeter CR-300 (Minolta, Osaka, Japan) in 400 almonds. The minimum, mean, and maximum values from instrumental color intensities were later converted into pantones with an online program Nix Color Sensor [21] and presented to the panelists as references. The ΔE shows the degree of total color change [22] and was calculated as,

$$\Delta E = [(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2]^{0.5} \quad (2)$$

Table 1. Sensory attributes, reference materials, and their corresponding intensities, used for the descriptive analysis of almonds.

Descriptor	Definition	Reference ‡	Intensity
	Appearance		
Color	The intensity of color from light to dark	L^* = 51.3; a^* = 20.6; b^* = 38.8	1.0
		L^* = 51.3; a^* = 20.6; b^* = 38.8	5.0
		L^* = 51.3; a^* = 20.6; b^* = 38.8	10.0
Size	The visual width of the of the almond from side to side	8–9 mm	1.0
		13–14 mm	5.0
		17–18 mm	9.0
Roughness	The number of hills and valleys perceived by the human eye on the almond surface (visual measured)	0%	1.0
		50%	5.0
		100%	10.0
	Basic Taste and Flavor		
Saltiness	The basic taste associated with a sodium chloride solution	0.15% NaCl	1.0
		0.25% NaCl	3.0
Sweetness	The basic taste associated with a sucrose solution	1% sucrose	3.0
		2% sucrose	5.0
Bitterness	The basic taste associated with a caffeine solution	0.01% caffeine	2.0
		0.02% caffeine	3.0
Astringency	A drying and puckering sensation on the mouth surface	0.03% alum	1.0
		Unripe dates	10.0
Overall nuts	Aromatics related to nuts in general	Mix of grinded Hacendado Nutget:hazelnut, 1:1	5.5
Almond ID	Aromatics reminiscent of almond	Marcona almonds	6.5
Benzaldehyde like	Artificial almond or cherry aromatics	Aroma: almond extract Dr. Oetker	10.0
		Flavor: bitter almond	10.0
Woody	The sweet, musty, dark, and dry aromatics associated with the tree bark	Hacendado walnuts	3.0
Aftertaste	Longevity of key attributes intensity after swallow the sample	30 s	1.0
		1 min	3.0
		1.5 min	6.0
	Texture		
Hardness	The force required to bite completely through the sample with molar teeth. Evaluated on the first bite down with the molars	Baby Bell light cheese	3.0
		Sugus chewy candy	6.0
		Hacendado almond	7.5
		Solano candy	10.0
Cohesiveness	The degree to which the sample deforms prior to breaking apart when compressed between molars	Hochland cheese slices	3.5
		Hacendado raisins	6.5
		Sugus chewy candy	8.0
Crispiness	The intensity of audible noise at first chew with molars	Nestlé cheerios	5.5
		Nestlé fitness	7.0
Fracturability	The force needed to break the almond. The evaluation was done with the molars after first chew	Nestlé cheerios	2.5
		Nestlé fitness	5.0
Adhesiveness	The effort needed to completely remove the sample from the teeth; measured after 5 chews	Kraft Miracle whip light dressing	4.5
		Marshmallow fluff	6.5
		Jif creamy peanut butter	8.5

‡ Intensities are based on a 10-point numerical scale with 0.5 increments, where 0 means "none" and 10 means "extremely strong".

Almond roughness was visually measured and refers to the number of hills and valleys perceived by the human eye on the almond surface [23]. Almond size scale was prepared from the one used by Regulating Council of the Protected Geographical Indications of *Jijona and Turrón de Alicante* (RCPGIJTA) [24].

The texture attributes (Table 1) products were also analyzed using instrumental texture measurements. A texture analyzer (Stable Micro Systems, model TA-XT2i, Godalming, UK) was employed using a 30 kg load cell and a Volodkevich Bite Jaw HDP/VB probe (trigger was set at 15 g, test speed was 1 mm s^{-1} over a specified distance of 3 mm).

After the orientation session, each panelist received four samples corresponding to the different irrigation treatments, and three evaluations per sample were done. The samples were served in odor-free 30 mL covered plastic cup and randomly coded with three digits. Water and unsalted crackers were also provided in order to clean the palates among samples. The descriptive test was carried out in a special tasting room with individual booths (controlled temperature of 21 ± 1 °C and combined natural/artificial light), and ballot charts were used to collect panelists' evaluations. The samples were presented according to a randomized block design to avoid biases. A 0 to 10 numerical scale was used by the panelists to quantify the intensity of the almond attributes, where 0 represents none/no intensity and 10 extremely strong with a 0.5 increment.

2.3. Affective Sensory Analysis

Affective sensory analysis was carried out with 100 recruited consumers from Spain (S) and 100 from Romania (R), with a gender ratio of 50:50 in Spain and 60:50 women:men in Romania. The consumers' age range was 18–25 (S = 33%; R = 45%), 26–35 (S = 29%; R = 30%), 36–45 (S = 10%; R = 15%), and 45–60 (S = 29%; R = 10%). The recruitment process was conducted via e-mail and fliers. Demographic questions regarding gender, age, nut consumption frequency, allergies, intolerances, or diet restriction were also included in the questionnaire. Spanish to Romanian back-translation procedure was conducted to avoid major misunderstandings during the evaluation. All samples were served, and labeled with three digit codes, in the same manner as with the recipients as described above. Consumers were asked for global satisfaction degree using a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely) for scoring and about attributes intensity using Just About Right (JAR) questions. Consumers were also asked to rank samples according to their preference and to check the reasons why they choose that sample as the best (due to the color, flavor sweetness, crunchiness, etc.) by using a question type Check All That Apply (CATA). Consumer interest in the label information (sustainable, bio, healthy, natural, product of Spain/Romania, etc.) using CATA question type was also analyzed. As described in the descriptive section, the affective tests were also carried out in special tasting rooms with individual booths and according to a randomized block design.

2.4. Consumer Willingness to Pay

The willingness to pay was carried out with 100 consumers form Spain and 100 consumers from Romania. Both Spanish and Romanian consumers were first given information about what the hydroSOStainable concept means, and later they were asked for their willingness to pay for hydroSOS almonds compared to the conventional ones. It was decided to inform consumers about hydrosustainability, because it was a relatively new concept and because previous studies have demonstrated that consumers need enough knowledge and access to precise information to prevent the receiving of fake feedback [3]. Without this previous basic information about the hydroSOStainable concept, consumers' responses and resulting conclusions with regard to hydroSOS almonds would be deeply speculative [25]. Later, they were given a price for conventional almonds of 2.60 €/200 g (the normal price for the Mercadona almonds; Mercadona is one of the most popular food supermarkets in the Mediterranean area of Spain) and the options: ≤€2.60, €3.10, €3.60, and >€3.60.

2.5. Statistical Analysis

Statistical analyses were performed by subjecting the data to two or three-way analysis of variance (ANOVA) and then to Tukey's multiple range test. A three-way ANOVA (factor 1: irrigation treatment; factor 2: session; and, factor 3: panelist) was carried out to demonstrate the panel consistency in the descriptive sensory analysis data, while two-way ANOVA (factor 1: irrigation treatment, and factor 2: country) was used for the affective sensory data [26]. Statistically significant differences were considered when $p < 0.05$, and were performed using XLSTAT Premium 2016 (Addinsoft, New York, NY, USA) and Statgraphics Plus (Version 3.1, Statistical Graphics Corp., Rockville, MA, USA).

Penalty analysis was also carried out to supply information about the possible improvement of samples, and for these analyses, JAR data were used [26]. Mean drops (penalties) versus the percentage of the consumers (providing each response in the mean drop plot) were graphically represented.

3. Results and Discussion

3.1. Descriptive Sensory Analysis

The descriptive sensory analysis was performed to evaluate whether significant differences among treatments were found. The descriptive results showed no statistically significant differences for session and panelist, and their two-way interaction demonstrated proper performance of the panel and the lack of effects of the parameters panelist and replication. Thus, only the effect of the parameter "irrigation treatment" is presented and discussed in the manuscript.

Table 2 shows the effect of the studied irrigation treatments on the main sensory descriptors of control and hydroSOS almonds. No significant differences were observed for 12 out of the 17 attributes used to describe the quality of almonds, while statistically significant differences were found for color, size, roughness, sweetness, and hardness.

Panelists found T2 samples having more intense color. This was supported by the instrumental color data, which showed significantly higher values for the a^* coordinate (T1 = 16.7 b; T2 = 17.4 a; T3 = 17.1 ab; T4 = 17.3 ab). Although the differences for AE color were below two units, and differences are difficult to perceive with the human eye [27], the highest values in the a^* (green-red coordinate) indicated that T2 almonds were more reddish. However, other authors obtained no statistically significant differences for this parameter in pistachio and olives [8,10,28]. However, other authors showed that total color increased for apricots and peaches under RDI [29,30].

With respect to the size attribute (Table 2), no significant differences were observed in instrumental size (mm) among treatments (T1 = 16.3; T2 = 16.2; T3 = 16.2; T4 = 16.2), but slightly significant differences were observed in descriptive analysis for T1 and T2, compared to a second group, T3 and T4. The results were partially similar to previous studies about hydroSOS pistachios, in which it was observed that there were no significant differences either for sensory or for instrumental size [10]. It is a general working hypothesis that deficit irrigation can lead to reduced yield, but the fruits produced will be of higher size. The problem of working with fruits, such as almonds, is that the heterogeneity of the fruits is so high that in some cases and parameters/attributes it can mask real differences due to the applied treatments. This is one reason, among others, for this hypothesis not being confirmed by real data. However, current field experiments are being repeated during three years to have more realistic and reliable data, but preliminary data is needed to be able to implement improvements in the experiment design and reach partial goals and objectives.

Although the roughness (Table 2) of T2 and T4 recorded the highest values, they were within the optimal values, which meant that water stress was correctly applied. Applying water stress at the wrong growing stages, for instance, stage III, will lead to very rough kernels, which are indicative that the water stress also reached the fruit and its turgor and moisture content were drastically limited [31].

An important finding was that T2 and T3 were the sweetest samples. An increment in sweetness was demonstrated in "Mollar de Elche" pomegranate cultivar growth under deficit irrigation conditions [32]. Sweetness is a key attribute in the sensory quality of almonds, and it is expected that

increased sweetness intensity will be favorable for consumer satisfaction [33]. Table 2 shows texture results, and only significant differences were found for hardness; T1 almonds were slightly softer than those from the rest of treatments. However, no differences were found for the instrumental hardness (T1 = 73.8 N; T = 73.8 N; T3 = 72.8 N; T4 = 72.2 N). Other authors also showed higher values for both sensory and instrumental texture for DI samples in studies about pistachios and olives samples [8,10].

3.2. Affective Sensory Analysis

Table 3 showed that the overall and attribute specific satisfaction degree of both Spanish and Romanian consumers were not statistically affected by the irrigation strategies under evaluation, with the exception of T4 almonds causing a slightly higher satisfaction. In general, Romanians tend to score higher than Spanish consumers because of the fact that they are less used to consuming this nut. Other authors, in studies about olives under deficit irrigation conditions, also reported no significant differences among the treatments for affective sensory evaluation [9]. On the contrary, there are also plenty of works on olives, pistachio, peaches, and grapes, in which higher consumer acceptance for samples produced by deficit irrigation strategies, such as RDI (moderate level), were observed [8,10,34,35].

Table 2. Descriptive sensory analysis of raw almonds as affected by deficit irrigation. Scale used ranged from 0 = no intensity to 10 = extremely strong intensity.

Irrigation Treatments	Color	Size	Roughness	Saltiness	Sweetness	Bitterness	Astringency	Overall Nuts	Almonds ID	Benzaldehyde-like	Woody	Aftertaste	Hardness	Cohesiveness	Crispiness	Fracturability	Adhesiveness
ANOVA Test †																	
	***	*	***	NS	*	NS	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS
Tukey Multiple Range Test ‡																	
T1	4.0 c	8.0 a	5.3 b	0.5	3.3 ab	0.6	0.5	5.6	5.9	0.4	1.5	5.4	4.5 b	3.0	3.3	2.1	6.7
T2	5.3 a	8.0 a	6.7 a	0.5	3.5 a	0.6	0.6	5.8	5.9	0.3	1.0	5.4	5.1 a	2.7	3.7	2.5	6.6
T3	4.2 c	7.7 b	5.3 b	0.5	3.5 a	0.6	0.6	5.9	6.2	0.3	1.8	6.1	5.6 a	3.3	4.0	2.1	6.5
T4	4.7 b	7.6 b	6.2 a	0.4	2.7 b	0.5	0.7	5.4	5.9	0.3	1.9	6.1	5.6 a	3.3	4.2	2.5	6.4

† NS = not significant at $p < 0.05$; *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (mean of 10 trained panelists) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

Table 3. Affective sensory analysis of raw almonds as affected by deficit irrigation and tested in two countries of Union Europe (Spain and Romania).

	Color	Size	Almond ID	Sweetness	Bitterness	Astringency	Firmness	Crispiness	Teeth Adhesion	Aftertaste	Overall
ANOVA †											
Irrigation	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
Country	***	*	***	***	NS	NS	***	***	**	***	***
Irrigation × Country	NS	NS	***	*	NS	NS	**	***	**	***	***
Tukey Multiple Range Test ‡											
Irrigation											
T1	7.0	7.0	6.7	6.6	6.4	6.5	6.7	6.9	6.3	6.5 ab	6.6
T2	7.0	7.2	6.8	6.6	6.5	6.5	6.5	6.9	6.2	6.2 b	6.5
T3	7.2	7.1	7.0	6.7	6.5	6.6	6.9	7.1	6.5	6.6 ab	6.9
T4	7.0	7.2	7.0	6.8	6.4	6.5	6.7	7.0	6.7	6.8 a	7.0
Country											
Spain	6.8 b	6.9 b	6.3 b	6.3 b	6.5	6.6	6.3 b	6.4 b	6.1 b	6.0 b	6.3 b
Romania	7.2 a	7.3 a	7.1 a	6.9 a	6.5	6.5	6.9 a	7.3 a	6.6 a	6.7 a	7.0 a
Irrigation × Country											
Spain											
T1	6.7	6.9	6.2 b	6.2 c	6.6	6.6	6.2 ab	6.3 d	6.2 ab	6.1 bc	6.2 bc
T2	6.6	6.8	6.1 b	6.2 c	6.4	6.3	6.0 b	6.3 cd	5.9 b	5.6 c	6.0 c
T3	7.0	7.2	6.4 ab	6.4 b	6.4	6.8	6.5 ab	6.6 abcd	6.2 ab	6.2 abc	6.5 abc
T4	6.8	6.9	6.5 ab	6.3 bc	6.4	6.6	6.4 ab	6.4 bcd	6.3 ab	6.3 abc	6.4 bc
Romania											
T1	7.2	7.1	7.0 ab	6.8 ab	6.3	6.5	6.9 ab	7.3 ab	6.4 ab	6.7 ab	6.8 abc
T2	7.1	7.5	7.1 a	6.7 ab	6.5	6.6	6.7 ab	7.2 abc	6.4 ab	6.4 abc	6.8 abc
T3	7.3	7.1	7.2 a	6.8 ab	6.5	6.5	7.1 a	7.4 a	6.7 ab	6.8 ab	7.0 ab
T4	7.1	7.4	7.3 a	7.0 a	6.5	6.5	6.9 ab	7.4 a	6.9 a	7.1 a	7.3 a

† NS = not significant at $p < 0.05$; *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (mean of 100 consumers) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

Figure 1 shows the sample preference order of Spanish (S) and Romanian consumers (R), and the main attributes controlling their preference. Sample T2 was chosen by consumers from both countries as the most liked sample, while T1 almonds were the least like ones (Figure 1a). Spanish consumers scored T4 higher than T3, while the contrary was observed from the Romanian consumers. T2 almonds were chosen as the best ones mainly due to their almond flavor, sweetness, and crispiness (Figure 1b), showing that sweetness was important in consumers' satisfaction, as hypothesized before in this study.

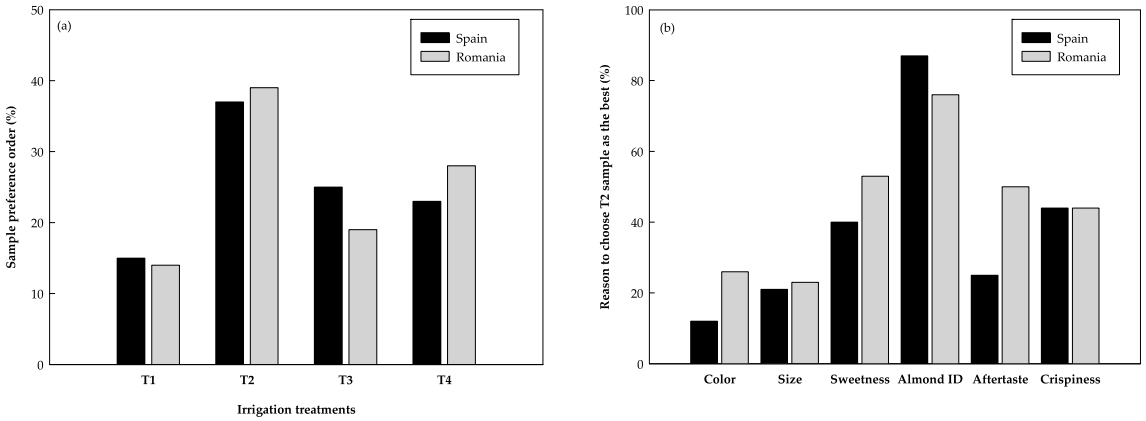

Figure 1. Purchase intent of Spanish and Romanian consumers regarding the studied almonds (**a**), and their reason to choose T2 almonds as the favorite ones (**b**).

Besides, sweetness (S = 29%; R = 64%), flavor (S = 77%; R = 65%), texture (S = 44%; R = 16%), and price (S = 44%; R = 60%) were the most checked parameters in the CATA questionnaire used when consumers were asked about their buying drivers. The most important word in a product label for 63% of the Spanish consumers was "product of Spain", followed by "healthy" (52%) and "natural" (48%), while Romanian consumers were more interested in "natural" (70%), "healthy" (67%), and "ecological" (31%). For both nationalities, the words "natural" and "healthy" seemed to play a key role in their buy decisions. Noguera et al. [18] also found the word "product of Spain" as the most important attribute for Spanish consumers concerning hydroSOS pistachio along with other expressions such as "rich in antioxidants" and "crunchy". The findings were associated with the consumer recognition of national products, health concerns, and composition. Regarding the word "sustainable", 44% of Spanish consumers and 29% of Romanians were interested in this word when purchasing a product. Although it is a relatively new concept [36], other authors also showed great interest of Spanish consumers for this word and concept but when studying other products, pistachios [18].

Penalty analysis, a very popular method in the food industry sector to help one interpret data from JAR questions, was conducted to understand the relationship between consumers' overall liking and the attribute intensity scores of the JAR questions [37,38]. The attributes with a large penalty and a high percentage of consumers, which were placed in the upper right quadrant of the plots, provided information about the most critical diagnostic issues of the product. On the other hand, the preferred attributes were usually located at the lower left quadrant of the plots [26]. The proportion of consumer's opinion plots and the mean penalty is shown in Figure 2 for all four treatments under study. All attributes having a negative impact on the sample liking, for at least 20% of the consumers and producing a drop of at least 1 point for liking, are the ones that might need to be improved. Bitterness was the only parameter susceptible to be improved, and consumers from both Spain and Romania agreed that this was especially true for T1 and T4 almonds.

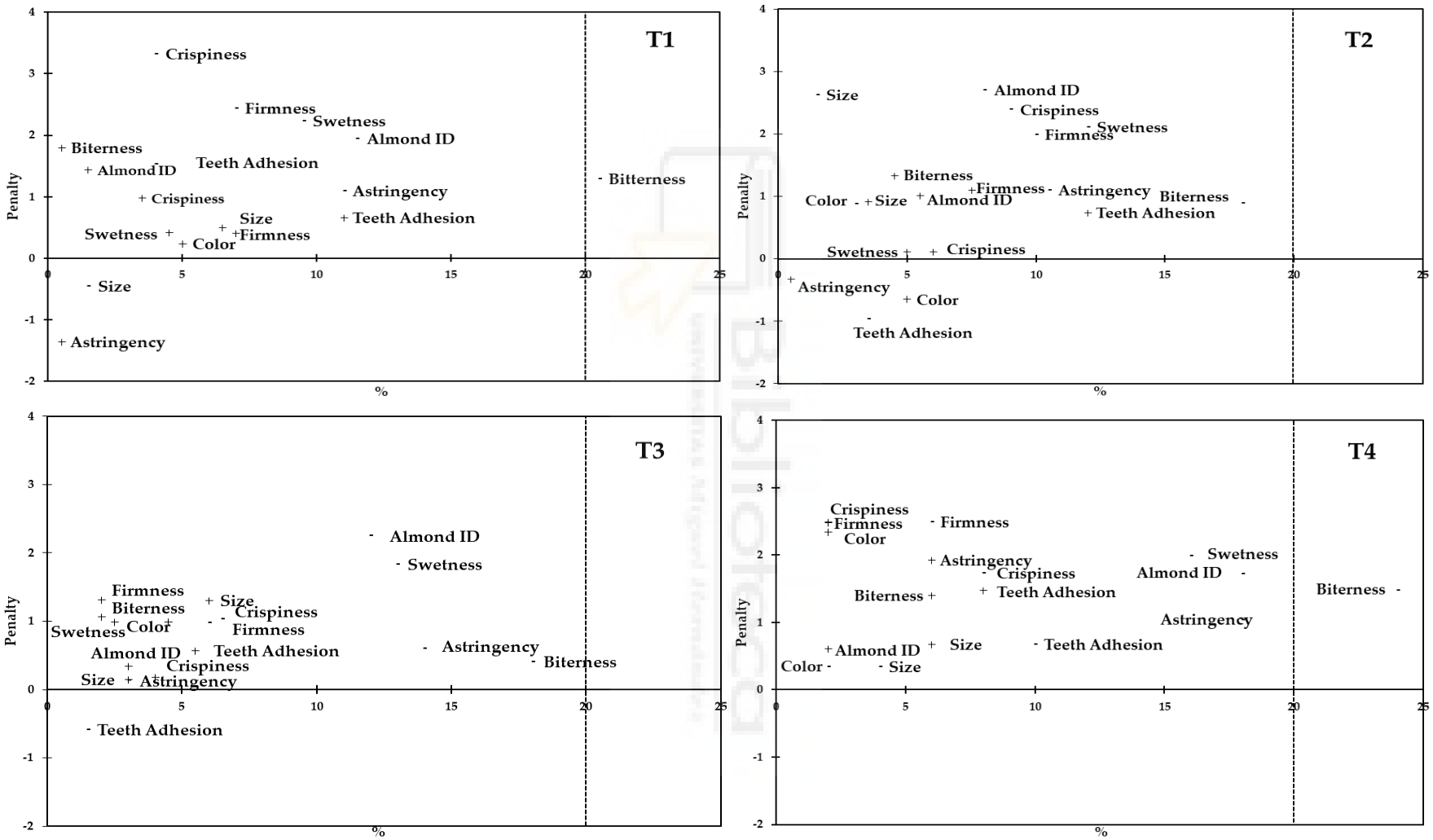

Figure 2. Penalty analysis of attributes intensities assessed by consumers (sample code indicated on the top right of each figure; "too low intensity" is indicated by the symbol "−", and "too high intensity" is indicated by the symbol "+").

3.3. Consumer Willingness to Pay

The Spanish and Romanian consumers were classified according to their willingness to pay for a bag of hydroSOS almonds compared to a bag of conventional almonds: (i) S = 23% and R = 31% were willing to pay less or the same price; (ii) S = 60% and R = 16% wanted to pay 0.50 € more; (iii) S = 13% and R = 24% wanted to pay 1.00 € more; and, finally, (iv) S = 4% and R = 29% wanted to pay more than 1.00 €. These findings agreed with Noguera et al. [18], who reported that Spanish consumers were also willing to pay an extra amount of money for hydroSOS pistachios [18].

Considering the almond Spanish production of ~ 190.000 t, the price received by the farmers for conventional shelled almonds was ~ 4.85 € kg^{-1} [39], and according to the previous data, an extra value of ~2 € kg^{-1} hydroSOS almonds can be expected. The possible economic increase by using deficit irrigation strategies could be ~40% with respect to conventional almonds. These gains might encourage farmers to invest in these novel sustainability tools, contributing to environmentally friendly agriculture.

4. Conclusions

The present study was the first to analyze the sensory properties of hidroSOStaninable almonds and consumers' (Romania and Spain) acceptance and willingness to pay. Although the consumer panels showed similar global and attribute-specific satisfaction degrees, the trained panelists were able to establish slight but significant differences in some key attributes, with T2 almonds showing intense red color, high size, and high intensity of both sweetness and hardness attributes. The penalty analysis also showed that bitterness, which was susceptible to be improved in other treatments, was correct in T2. Consumers are now aware of the importance of the environment and the need to optimize key resources, such as water. This awareness may explain consumers' willingness to pay a higher price for hydroSOS almonds, which will lead to higher incomes and benefits for farmers. These results lead us to conclude that controlling stress in almond trees with deficit irrigation strategies can increase water productivity and farmers' profits from producing of environmentally friendly products without significantly changing the sensory profile and the consumers' satisfaction.

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Long-Term Correlation between Water Deficit and Quality Markers in HydroSOStainable Almonds

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Abstract: Global warming enhances the rainfall and temperature irregularity, producing a collapse in water resources and generating an urgent need for hydro-sustainable thinking in agriculture. The aim of this study was to evaluate the correlation between the water stress of almond trees and quality parameters of fruits, after 3 years of experiments, with the objective of establishing quality markers necessary in the certification process of hydroSOStainable almonds. The results showed positive correlations among the stress integral (SI) and dry weight, color coordinates (L^*, a^* and b^*), minerals (K, Fe, and Zn), organic acids (citric acid), sugars (sucrose, fructose, and total sugars), antioxidant activity, and fatty acids [linoleic acid, polyunsaturated (PUFA)/monounsaturated (MUFA) ratio, PUFA and SFA, among others]. As well as negative correlations of SI with water activity, weight (almond, kernel, and shell), kernel size, minerals (Ca and Mg), fatty acids (oleic acid, oleic/linoleic ratio, MUFA, and PUFA/SFA ratio), and sensory attributes (size, bitterness, astringency, benzaldehyde, and woody). Finally, this research helped to prove key quality parameters that can be used as makers of hydroSOStainable almonds. In addition, it was demonstrated that controlling water stress in almond trees by using deficit irrigation strategies can lead to appropriate yields, improve the product quality, and consequently, lead to a final added value.

Keywords: *Prunus dulcis*; Vairo; water stress; regulated deficit irrigation; sustained deficit irrigation; quality markers

1. Introduction

Almonds [*Prunus dulcis* (Mill.) D.A. Webb] are an economically and nutritionally important agricultural good, widely consumed in the Mediterranean diet either as a snack or as an ingredient for confectionery (*turrón)* and baking [1]. Almond consumption increased by 1.9% at the end of 2018

in Spain, indicating that consumer appreciation for this nut is high and constantly increasing due to its nutrition values, pleasant flavor, and healthy properties [2,3]. Moreover, raising the number of health-conscious consumers, together with environmental and animals care, lactose intolerance, and hypercholesterolemia in consumers, plant-based milk, yogurt, and cheese has grown over the last decade [4]. For instance, the global almond milk market is predicted to expand 14.3% by 2025; this product being considered a dairy alternative rich in vitamin E and omega 3 and 6 fatty acids. After all, this might be also an important reason that led to almond consumption growth [4–6].

Almond is the third largest crop in terms of surface and the most cultivated tree nut in Spain [7]. Besides, Spain is the main European almond producer and the second-largest in the world (339,033 t in-shell almonds), after the United States of America (1,872,500 t) [8]; Andalusia (111,877 t), Aragon (63,235 t), Castilla La-Mancha (53,201 t), and Valencian Community (40,875 t) are the main producing regions [9]. However, almond production in Spain is relatively low because this crop has been mainly grown in marginal areas where it has traditionally cultivated under restrictive conditions [7]. The almond tree is a drought-tolerant species, but due to the low yield in rainfed conditions (380 kg ha^{-1}), irrigation water is necessary to increase its productivity (1842 kg ha^{-1}) [9].

The Mediterranean regions are the most affected areas by water stress due to the scarcity and irregularity of rainfall. Moreover, the highest crop water needs are found in areas that are hot, dry, windy, and sunny due to the growth needs of the plant (foliage expansion, vegetative growth, and fruit yield) [10]. The water scarcity crisis is considered the biggest global risk for the world economy and it is affecting every continent [11]. Regarding agricultural sector, there is a consensus about the inadequate management of water resources and the need of achieving an equilibrium between rural development, food security, and environment protection [12]. The population growth leads to an expansion in intensive food production that alters the environment due to greenhouse gas emissions, soil deterioration, and water stress [13]. The main impact produced by climate change includes significant alteration in the average temperature and the rainfall irregularity [14], which leads to a substantially increase in irrigation water demand. In almond farming, climate change can provoke phenological variations on fruit, which may affect the final yield, quality, and marketability [15]. Consequently, all these changes might lead to a reduction in the productivity of agro-ecosystems, a progressive decline of rural areas, and even, land abandonment [16].

The implementation of sustainable irrigation strategies is an important tool to attenuate these negative aspects. However, these strategies must fulfill two important requirements: (i) causing minimal production losses and (ii) ensuring the final quality of the fruits. Regulated deficit irrigation (RDI) is one of these strategies meant to increase the water productivity with minimal yield losses and consists of reducing the amount of water during the kernel-filling stage in almond orchards [17]. Sustained deficit irrigation (SDI) is another strategy, which consists of applying a uniform and reduced amount of water during the whole growing cycle, creating a progressive stress in plants throughout the season [18].

Recently, new research lines focused on water resources sustainability have been developed for different crops (almonds, pistachios, olives, etc.), and the foodstuffs produced under controlled water stress conditions are called hydroSOStainable foods [19–21]. However, a variability in crop responses to water stress was reported for these products with the quality parameters being cultivar-, crop-, and year-dependent. Therefore, long-term research to decide which quality parameters are really affected by the waters stress conditions is needed.

Consequently, the aim of this study was to correlate water deficit response with quality parameters after 3 years of experiments (2017, 2018, 2019) to identify those parameters that behave in the same way throughout the trials. These results are essential to establish the future hydroSOStainable markers.

2. Materials and Methods

2.1. Plant and Experimental Conditions

The experiment was performed during 3 growing cycles (2017, 2018, and 2019) in a commercial orchard "La Florida" (37.23° N, −5.91 W, Dos Hermanas, Seville, Spain). The almond [*P. dulcis* (Mill.) D.A. Webb cv. Vairo] orchard was 7 years old at the beginning of the experiment. The tree spacing was an 8 m × 6 m square pattern, while the irrigation system used a drip irrigation line (3.8 L h^{-1}) with drippers separated at 0.4 m distance.

The weather data for each season were obtained from the "Instituto de Investigación y Formación Agraria (IFAPA) Los Palacios" station in the Andalusian weather stations network (Figure 1) located about 6 km away from the experimental orchard.

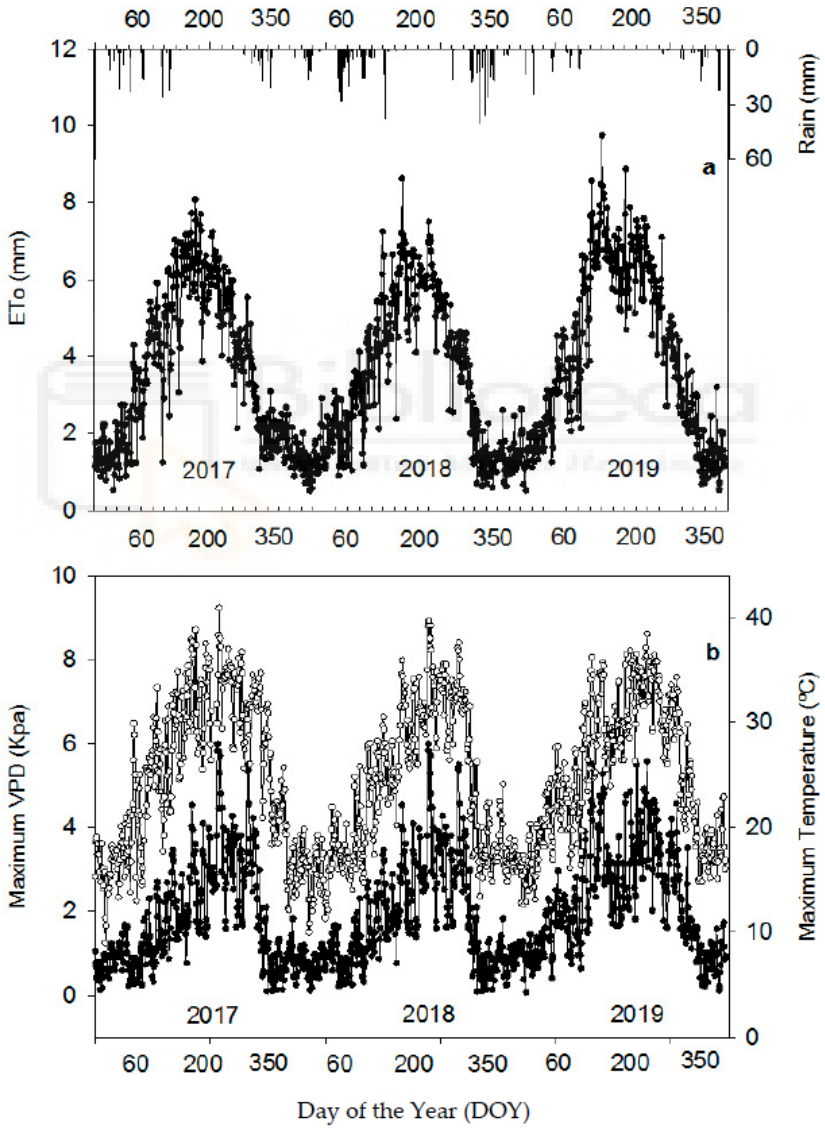

Figure 1. Climatic conditions during the three experimental seasons (2017–2019). (**a**) Seasonal daily reference evapotranspiration (circles) and rain (bars). (**b**) Seasonal daily maximum air temperature (white circles) and maximum vapor pressure deficit (VPD) (black circles). Vertical dots lines indicated from right to left each season, the beginning of pit hardening, early recovery, and regular recovery. DOY: day of the year.

The data for all 3 seasons were typical of Mediterranean zones, with null rainfall during the summer period and warm winters. The threshold values of midday stem water potential (SWP) were measured weekly, most of the dates, or every ten days using a pressure chamber (PMS Instrument Company, Albany, OR, USA). These values were used for the irrigation schedule by evaluating the stress level in the plant with the methodology proposed by Myers [22] according to the following expression Equation (1):

$$SI = \left| \sum \left(\psi_{\text{stem}} - (-0.2) \right) \times n \right. \quad (1)$$

where SI was the stress integral, ψ_{stem} is the average midday stem water potential for any interval, and n is the number of days in the interval. Most of the measurements were weekly.

2.2. Irrigation Treatments

Four irrigation treatments were applied to the experimental plots. Each treatment represents different strategies of farmers in conditions of water scarcity. Moderate RDI is a controlled deficit irrigation in which applied water is lower than full irrigation but restricted considering an accurate water management. Severe RDI (was considered due to the low water availability) represents concentrated irrigation mainly during postharvest. Finally, SDI, is a strategy that was not considered in the phenological stages, and then, postharvest irrigation was very limited. These treatments are described in detail below:

- Full irrigation (T1): irrigated to assure the crop needs. Irrigation was daily and irrigation scheduling was performed every week. Water needs were estimated with the crop evapotranspiration (ETc) approach according to Steduto et al. [23] using reduction coefficients (Kr) around 0.6. In addition, water status was evaluated using midday stem water potential and compared to the McCutchan and Shackel [24] baseline. When water status was more negative than expected, irrigation was increased by 150% ETc.
- Moderate RDI (T2): the water stress was imposed during the kernel-filling period; almond trees were irrigated when SWP was below −1.5 MPa, and for the rest of the time, trees were irrigated to keep an SWP as the baseline proposed by McCutchan and Shackel [24]. Equation (2) estimated optimum midday stem water potential in relation with vapor pressure deficit (VPD):

$$SWP = (-0.41) \times (-0.12VPD) \quad (2)$$

  where: SWP is optimum midday stem water potential (MPa) and VPD is vapor pressure deficit (KPa).
- Severe RDI (T3): the same as T2, except that trees were irrigated when SWP was below −2.0 MPa during kernel filling and maximum seasonal water was considered (120 mm, around 20% ETc). Therefore, after harvest, when total applied water was reached, irrigation stopped.
- SDI (T4): the same as T3, but tree water status was not considered. Irrigation was applied in a constant daily rate around 1–2 mm per day. The main differences between both strategies (T3 and T4) was that T4 limited postharvest irrigation more than T3.

Harvesting was done with a self-propelled trunk shaker with collector in the mid of August (28 weeks after blossom). The treatments were separately harvested , and almonds were sun-dried until a moisture content lower than 5% was achieved . Later , in-shell almonds were delivered to Miguel Hernández University (Orihuela, Alicante, Spain) for analysis.

2.3. Physical Parameters

2.3.1. Kernel Ratio

The ratio between the mass of in-shell almonds and kernel was calculated from 12 kg of whole fruit per treatment and year.

2.3.2. Dry Weight and Water Activity

For the dry weight content (%) analysis, 2 g of ground almonds (Moulinex grinder AR110830, Alençon, France) were added to an aluminum tray and dried in an oven at 60 °C until a constant weight was reached, while water activity (a_w) was measured by placing the cups with almond (2 g) into an a_w meter (Novasina aw-Sprint TH500; Pfaffikon, Zurich, Switzerland) and reading the value. The experiments were done in quadruplicate.

2.3.3. Weight and Size

For the morphological parameters, 100 almonds per treatment (25 almonds × 4 trees × treatment × year) were randomly selected and measured in terms of weight and size (length, width, thickness) of both in-shell almond and kernel using a digital caliper (Mitutoyo 500-197-20, Kawasaki, Japan) and a precision scale (Mettler Toledo model AG204, Barcelona, Spain), respectively.

2.3.4. Instrumental Color

Color measurements were performed at 25 ± 1 °C using a Minolta Colorimeter CR-300 (Osaka, Japan). Outside color was directly measured on the skin of 100 individual almond kernels per treatment each year. Results were presented as international commission on illumination (CIE) L^*, a^* and b^* color coordinates describing the color in a three-dimensional space as following: L^* for the lightness ($L^* = 0$ black; $L^* = 100$ white), a^* for the green-red (a^* = red; $-a^*$ = green), and b^* for the blue-yellow components (b^* = yellow; $-b^*$ = blue).

2.3.5. Instrumental Texture

The texture of 100 almonds per treatment and year was measured using a texture analyzer (Stable Micro Systems, model TA-XT2i, Godalming, UK) with a 30 kg load cell and a probe Volodkevich Bite Jaw (HDP/VB) as following: trigger was placed at 15 g, test speed was 1 mm s^{-1} over a specific distance of 3 mm. Fracturability (mm), hardness (N), work done to shear (Ns), average force (N), and number of fractures (peaks count) were the parameters analyzed.

2.4. Chemical and Functional Analysis/Parameters

2.4.1. Mineral Content Determination

The digestion of 0.5 g of sample with 8 mL of concentrated HNO_3 and 2 mL H_2O_2 (30%) using a START D Medium Microwave Digestion (SK-10) was first carried out [25]. Followed by the determination of macro-nutrients (Ca, Mg, and K) and micro-nutrients (Fe, Cu, Mn and Zn) with a Unicam Solaar 969 atomic absorption–emission spectrometer (Unicam Ltd., Cambridge, UK). Calcium, Mg, Fe, Cu, Mn, and Zn was determined by atomic absorption and K by atomic emission.

2.4.2. Organic Acids and Sugars

High-performance liquid chromatography (HPLC) was used for organic acids and sugars identification and quantification, as previously described [26]. For this, 1 g of ground almond was homogenized (Ultra Turrax T18 Basic, IKA®-Werke GmbH & Co. KG Janke & Kunkel, Staufen, Germany) with 5 mL of 50 mM phosphate buffer (pH = 7.8) for 2 min at 11,300 rpm, centrifuged (Sigma 3−18 K; Sigma Laborzentrifugen, Osterode and Harz, Germany) at 4 °C and 15,000 rpm for 20 min and filtered (0.45 µm Millipore membrane filter, Billerica, MA, USA). The supernatant was injected (10 µL) into a Hewlett Packard (Wilmington, DE, USA) series 1100 (HPLC) using as mobile phase 0.1% orthophosphoric acid elution buffer. Sugars were analyzed using a Supelcogel TM C-610H column (30 cm × 7.8 mm) with a precolumn (Supelguard 5 cm × 4.6 mm; 219 Supelco, Bellefonte, PA, USA) and detected with a refractive index detector (RID). Organic acids were separated as sugars using a diode-array detector (DAD) at 210 nm for the absorbance measurements. Analyses were run in quadruplicate, and results were expressed as g kg^{-1} dry weight (dw).

2.4.3. Antioxidant Activity and Total Phenolic Content

The antioxidant activity and total phenolic content was carried out both for whole kernel and its blanched skin. For the extraction 0.5 g of finely ground almond were sonicated with 10 mL of extractant [MeOH/H_2O_2 (80:20, *v*/*v*) + 1% HCl at 20 °C] for 15 min and stored at 4 °C overnight. The mixture was sonicated again under the same conditions and centrifuged at 10,000 rpm for 10 min. The antioxidant activity of the obtained extract was measured using 3 methods: $ABTS^{\bullet+}$ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power), as previously described by Brand-Williams et al. [27]. The results were calculated according to the Trolox calibration curve and were expressed as mmol Trolox kg^{-1}.

For total phenolic content (TPC) 100 µL of supernatant was mixed with 200 µL Folin-Ciocâlteu reagent and 2 mL of H_2O_2 and was stored at 22 °C for 3 min. Then, 1 mL of 20% Na_2CO_3 was added, followed by 1 h of incubation at room temperature. The results were calculated with the gallic acid calibration curve and expressed as gallic acid equivalents (GAE), g GAE kg^{-1}. All measurements were performed in an ultraviolet-visible (UV-vis) spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK).

2.4.4. Fatty Acids

Ground almond (40 mg) was saponified with 100 µL of dichloromethane (Cl_2CH_2) and 1 mL of sodium methoxide solution and refluxed for 10 min at 90 °C. Later, 1 mL of BF_3 methanolic was added followed by 30 min rest in dark for reaction [25]. The fatty acids methyl esters (FAMEs) were separated in a Shimadzu GC17A gas chromatography coupled with a flame ionization detector and a DB-23 capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) J&W Scientific, Agilent Technologies using the same conditions, as previously described by Lipan et al. [25]. The identification of FAMEs peaks was done by comparing the retention times of the FAME Supelco MIX-37 standards. Analysis were carried out in quadruplicate, and the results were expressed as g kg^{-1} concentration, using methyl nonadecanoate (C19:0) as internal standard.

2.5. Descriptive Sensory Analysis

Descriptive sensory evaluation was performed following the steps previously published in literature using a trained panel [19]. Ten highly trained panelists from the Food Quality and Safety Group (Miguel Hernández University of Elche, Orihuela, Alicante, Spain) with ages between 25–62 years (5 women and 5 men) conducted the descriptive analysis. Once the orientation sessions were finished (4), the panel was asked to evaluate the 4 samples corresponding to the irrigation treatments in terms of appearance, basic tastes, and flavor intensities of almond. For this, a structured scale from 0 to 10 (0.5 increments) was used to quantify the intensity of the almond attributes, where 0 represents no intensity and 10 extremely strong. The samples were presented using a randomized block design to avoid biases in individual tasting booths (controlled temperature of 21 ± 2 °C and combined natural/artificial light) equipped with water and unsalted crackers for palate cleaning among samples. The analysis was run in triplicate.

2.6. Statistical Analysis

Two-way analysis of variance (ANOVA), using "irrigation treatment" and "year" as factors, followed by Tukey's multiple range test were carried out in order to decide the parameters to be used for the correlations. Two supplementary tables were added with the mean values of 3 years (Tables S1 and S2). Only those parameters significantly different among treatments were considered for Pearson's correlations. All statistical analyses were performed using XLSTAT Premium 2016, while Sigma Plot 11 software was used for figures preparation. Statistically differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Agronomic Parameters

Tables S1 and S2 contains supplementary information about the mean values of 3 years study for all the parameters. As observed, a lower amount of irrigation water was received by the trees' growth under deficit irrigation strategies, being T3 and T4 the treatments, which received the less amount of irrigation water; this rebound in the plant status can be observed from the stress integral values. Almond trees from T3 and T4 were the most stressed, although the latter (T4) was statistically significant to T2. During the first season, T2 and T3 were the most stressed treatments followed by T4, which was statistically similar to T2. In 2018, lower values of SI were shown for all treatments, which means that the stress in plant was less severe than the other seasons. T3 and T4 were the most stressed treatments, followed by T2, which was statistically correlated with the control and to the other deficit irrigation treatments. Finally, 2019 was the season in which almonds trees met the highest values of water stress, with T3 and T4 having the highest values, followed by T2. As observed, the SI behaved different yearly, however in the last two seasons, T3 and T4 were similar in terms of water stress in plant. The difference between these treatments is that in the former (T3) the stress was applied in the kernel-filling period, while in the latter (T4), the stress was imposed throughout the whole growing cycle, creating a progressively stress in plant rather than in a single phenological phase (kernel filling). Supplementary data also showed the mean values of kernel yield of all 3 seasons to check how the previous parameters (SI and applied water) influenced the fruit yield. A reduction of this parameter was observed in all the treatments growth under DI conditions with no significant differences among them. If each year production is analyzed, no differences among control and DI treatment was registered in the first season. However, a decrease of 2.4-fold was found for these treatments (T2, T3, T4) regarding the control in the second season (with no significant differences among them) and in the third season. A reduction in kernel yield in deficit irrigation treatments was also observed in 2019 season (1.2-fold in T2 and 1.5-fold in T3 and T4), although this time was lower than in 2018 and T2 was significantly similar to the control. Is important to highlight that in 2018, even though T3 and T4 received a lower amount of water than T2, the kernel yield was similar among them, and that in 2019, although T2 received lower amount of water than the control (T1), the kernel yield was significantly similar between them.

Pearson's correlation coefficients (R) among SWP and SI with agronomical and physical parameters is shown in Figure 2. A negative and significant correlation was found between SI and (i) SWP ($R = -0.67$; $p < 0.001$) and (ii) water activity ($R = -0.39$; $p < 0.01$); this means that at higher waters stress values, lower SWP and a_w values are obtained. On the other hand, a positive and significant correlation was observed between SI and dry weight ($R = 0.53$; $p < 0.001$) as well as between kernel ratio and applied water ($R = 0.36$; $p < 0.05$). Regarding the water stress effect on kernel yield, the results showed that in the first year it was not affected ($R = 0.11$; $p > 0.05$); however, kernel yield was reduced with water stress during 2018 and 2019 seasons demonstrated by the negative correlations of $R = -0.50$; $p < 0.05$ * and $R = -0.79$; $p < 0.001$ ***, respectively.

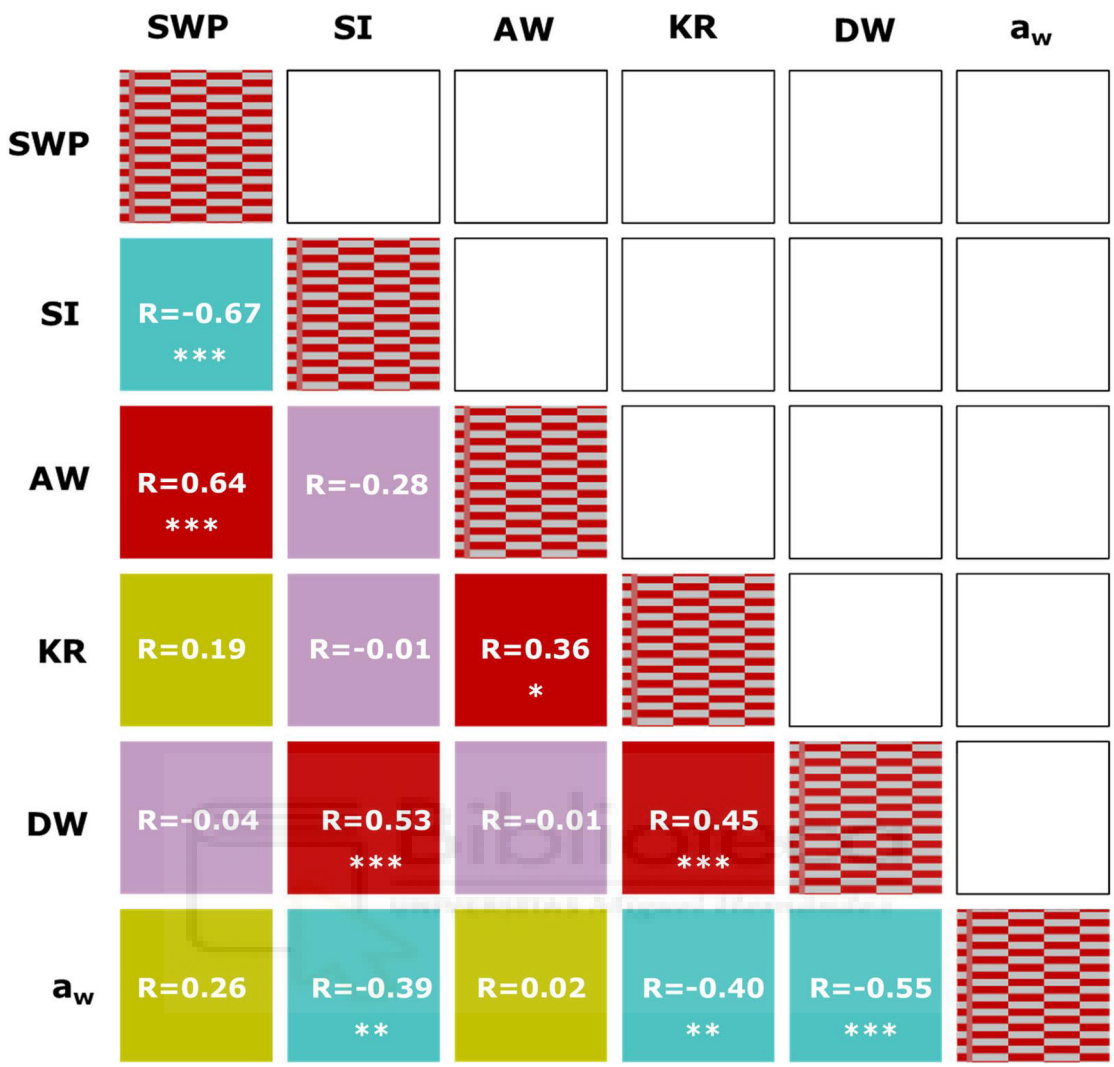

Figure 2. Heat map of correlation matrix of agronomical parameters. Each square indicates Pearson's correlation coefficient for a pair of data and the color represents the positive or negative correlation as: R = 1.00; significant ($p < 0.05$) positive correlation; significant ($p < 0.05$) negative correlation; positive but not correlated; negative but not correlated. *, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. SWP = minimum stem water potential; SI = stress integral; AW = applied water; KR = kernel ratio; DW = dry weight; aw = water activity.

Overall, these results showed that after long-term experiment (3 years), the water stress in almond trees negatively affected the yield but enhanced dry weight. These results suggest that yields differences were related to the number of nuts. Such response could be associated with a postharvest water stress in deficit treatments [18]. However, it was observed that, depending on the treatment, it can lead to yields statistically similar to the control, which might be a good alternative when water restrictions are below the crop needs. In this way, Moderate RDI (T2) could balance water stress effects, because this treatment could secure enough crown volume, which is very important in the tree yield capacity [10] and postharvest recovery, which is according to the current data, the most important effect. On the contrary, T3 and T4 results suggest that water status in postharvest would be better. Then, in conditions of very low water availability, irrigation in this period should be preferential and greater than the ones of T3. Besides, the reduction in the moisture content and water activity with water stress are also important outcomes for food industry, because lower values of these parameters help to maintain at minimum the biological reactions, which essential to increase the almonds shelf life [28]. The obtained

results indicate that the use of deficit irrigation in almond trees water management can improve yield and reduce water use. Thus, it contributes to reduce water consumption for irrigation purposes.

3.2. Morphological Parameters

Table 1 shows the Pearson's correlation coefficients (R) between SWP and SI with morphological parameters. The SI was negatively correlated with almond, kernel, and shell weight, with kernel length and width, and with almond thickness. This means that weight and size were reduced with the water stress in plant. The conclusions regarding the effect of deficit irrigation on the morphological parameters are widely spread throughout the literature. For instance, similar results were obtained in almond cultivar (cv.) Nonpareil [18], and no differences were reported for almond cultivars Marta, Guara, Lauranne, Ferragnes, and Texas [29,30]. Additionally, no differences on the morphological parameters were also reported for other crops such as pistachio cv. Kerman and olives cv. Manzanilla if the stress was applied during shell and pit hardening, respectively [21,31]. Finally, an increase in weight and equatorial diameter but a decrease in longitudinal diameter were observed for olives cv. Manzanilla growth under the following RDI conditions: (i) stage I, trees irrigated under non-limited conditions; (ii) stage II, trees under moderate water deficit conditions, they were no irrigated during this period; and (iii) stage III, water applied in order to provide a water status similar to a full irrigated treatment [32].

A significant positive correlation was observed between the SI and color parameters, showing that a higher stress level leads to higher values of L^*, a^*, and b^* coordinates. This means that hydroSOStainable almonds have a lighter color with reddish and yellowish notes (more intense brown color). As the almond color skin is given by the polyphenol profile, which is unique for each cultivar [33], the increase in color coordinates under water stress conditions might be related to a potential increase in polyphenols. For instance, almond flavonoids have been extensively studied in different plants, and it was concluded that they are decisive pigment in color plants [34]. The brown almond skin pigment is largely concentrated in the high-molecular weight fraction such as proanthocyanidins, which are the main polyphenols found in almonds that can impart color formation [35]. A positive correlation between SI and proanthocyanidins ($R = 0.73$; $p = 0.001$) was previously reported in almonds cv. Vairo after one season of experiment. Besides, a^* values were also reported to be higher in almonds cv. Vairo, Marta, Guara, Lauranne growth under deficit irrigation conditions after one season [25,29]. Finally, a^* was reported to be positively correlated with the contents of nine individual flavonols, total kaempferols, and total flavonols in a study about the relationship between rose petals' (*Rosa* spp.) color and polyphenols content [36].

Table 1. Pearson's correlation coefficients (R) among stem water potential and stress integral with morphological parameters.

	SWP	**SI**	**AWe**	**KWe**	**SHWe**	**AL**	**KL**	**AWi**	**KWi**	**ATi**	**KTi**	***L****	***a****	***b****	**Hue**	**C**	**H**
SWP	1.00																
SI	−0.67 ***	1.00															
AWe	0.17	−0.39 **	1.00														
KWe	0.35 *	−0.56 ***	0.85 ***	1.00													
SHWe	0.11	−0.32 *	0.99 ***	0.75 ***	1.00												
AL	0.05	0.08	0.75 ***	0.43 **	0.80 ***	1.00											
KL	0.26	−0.57 ***	0.84 ***	0.91 ***	0.77 ***	0.40 **	1.00										
AWi	0.08	−0.17	0.90 ***	0.64 ***	0.92 ***	0.89 ***	0.60 ***	1.00									
KWi	0.18	−0.51 ***	0.92 ***	0.88 ***	0.87 ***	0.55 ***	0.88 ***	0.79 ***	1.00								
ATi	0.33 *	−0.41 **	0.80 ***	0.63 ***	0.80 ***	0.68 ***	0.61 ***	0.81 ***	0.70 ***	1.00							
KTi	0.32 *	0.07	−0.36 *	−0.04	−0.44 **	−0.30 *	−0.16	−0.41 **	−0.30 *	−0.15	1.00						
*L**	−0.20	0.61 ***	−0.65 ***	−0.55 ***	−0.64 ***	−0.38 **	−0.61 ***	−0.58 ***	−0.64 ***	−0.64 ***	0.47 ***	1.00					
*a**	−0.30 *	0.80 ***	−0.41 **	−0.57 ***	−0.34 *	0.14	−0.69 ***	−0.13	−0.55 ***	−0.29 *	0.19	0.65 ***	1.00				
*b**	−0.21	0.72 ***	−0.53 ***	−0.55 ***	−0.50 ***	−0.10	−0.67 ***	−0.36 *	−0.61 ***	−0.49 ***	0.37 *	0.87 ***	0.90 ***	1.00			
Hue	0.07	0.02	0.32 ***	−0.04	0.40 **	0.59 ***	−0.06	0.55 ***	0.16	0.48 ***	−0.50 ***	−0.42 **	0.27	−0.05	1.00		
C	−0.09	0.10	−0.40 *	−0.06	−0.48 ***	−0.59 ***	−0.06	−0.60 ***	−0.26	−0.56 ***	0.55 ***	0.56 ***	−0.12	0.23	−0.98 ***	1.00	
H	0.06	−0.40	0.61 ***	0.60 ***	0.58 ***	0.31 *	0.67 ***	0.48 ***	0.63 ***	0.52 ***	−0.32 *	−0.56 ***	−0.56 ***	−0.63 ***	−0.04	−0.08	1.00

*, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. SWP = minimum stem water potential; SI = stress integral; AWe = almond weight; KWe = kernel weight; SHWe = shell weight; AL = almond length; KL = kernel length; AWi = almond width; KWi = kernel width; ATi = almond thickness; KTi = kernel thickness; *L**, *a**, *b** = color coordinates; C = Chroma; H = hardness.

3.3. Mineral, Organic Acids, and Sugars Content

The minerals contained in plant tissue are taken by plants from soil and from the water received in production [34]. For this reason, environmental factors, agronomical practices (location, soil composition, water source, irrigation, and fertilizer) and cultivar are responsible for the final mineral content in kernel. Potassium, Ca, Mg, Fe, P, S, and N are the main elements found in plants mainly accumulated during fruit growing and ripening [37]. It was reported that drought conditions reduces the mineral content transport from root to shoot; however, there are plants with a better water use efficiency (WUE) and consequently with greater drought tolerance [38].

In order to analyze the relationship between SWP and SI with minerals, organic acids, and sugars, Pearson's correlation coefficients (R) were calculated and are displayed in Table 2. Calcium ($R = -0.60$; $p < 0.001$) and Mg ($R = -0.35$; $p < 0.01$) showed significant negative correlations with the SI, and the latter was also positively correlated with SWP ($R = 0.71$; $p < 0.01$). However, if each year is considered both minerals presented significant difference in only one season. Magnesium is a macro element essential component of the chlorophyll molecule, which is necessary in the photosynthesis process [38]. Besides, Mg plays a role in energy preservation and protein synthesis being a cofactor for many enzymes associated with de-phosphorylation, hydrolysis, and in stabilizing the structure of nucleotides and sugar accumulation.

Potassium ($R = 0.60$; $p < 0.001$), Fe ($R = 0.64$; $p < 0.001$), and Zn ($R = 0.44$; $p < 0.01$) were the elements positively correlated with the water stress. Similar results were also reported by other researchers in almonds in which a higher amount of K was reported in moderate RDI attributed to the relationship between water availability and minerals absorption [25]. The authors explain that the excess of water might be the responsible for mineral leaching and also that drought stress could contribute to the saturation of minerals in the rootzone. Potassium is the most important element, after N and P, helping to maintain the plant water status being involved in physiological and molecular mechanisms needed to increase the plant tolerance to stress [38]. Potassium has been reported to be the major mineral cation in almonds kernels (717 mg/100 g in cv. Vairo); in this way, almonds are considered a food high/rich in K because its content is above the minimum threshold (600 mg K/100 g) established in the Regulation (EU) No 1169/2011 of the European Parliament and of the Council [25,39].

Iron and Zn also presented a positive correlation with water stress and these results agreed with other authors reporting that this microelement helps to improve the WUE and the crop yield [38]. Usually, drought induces Fe deficiency with a negative effect on plant tree, causing chlorosis due to low levels of chlorophyll. This microelement is also necessary for an effective function of the antioxidant enzymes because a Fe deficiency reduces the enzymes activity, enhances the ROS production, and reduces the bioactive compounds biosynthesis [38]. The present results might reveal that this controlled stress was below the limit needed to reduce the microelements production; in fact, an opposite phenomenon was observed. Studies in wheat growth in fields under water stress conditions also reported a higher Zn content in grains growth under water stress conditions but not that grown in greenhouses [40].

Positive correlation was shown for citric acid and SI ($R = 0.65$; $p < 0.001$), which was confirmed by studies in almonds of cv. Marta, Guara, and Lauranne growth under RDI versus full irrigated and over irrigated conditions [29] and other crops such as thyme [41]. However, in studies of cv. Guara under non-irrigated almonds versus drip-irrigated, the citric acid was higher in almonds growth in drip-irrigated conditions [42]. In addition, no differences were reported in cv. Vairo [25] and cv. Marta [43]. These differences could be attributed to the irrigation strategies and the levels of stress created in each experiment. The increase in citric acid in response to drought may result from the larger inhibition of the citrate degrading system relative to citrate synthesis as previously reported in CAM plant (*Aptenia cordifolia*), although the capacity for citric acid oxidation and the citrate synthetase activity decreased during drought [44].

Table 2. Pearson's correlation coefficients (R) among stem water potential and stress integral with minerals, organic acids, and sugars.

	SWP	SI	Ca	Mg	K	Fe	Mn	Zn	Cit	Tar	Mal	ΣOA	Suc	Glu	Fru	ΣS
SWP	1.00															
SI	−0.67 ***	1.00														
Ca	0.14	−0.60 ***	1.00													
Mg	0.13	−0.35 **	−0.12	1.00												
K	−0.06	0.60 ***	−0.79 ***	0.39 **	1.00											
Fe	−0.18	0.64 ***	0.79 ***	−0.20	−0.65 ***	1.00										
Mn	0.13	−0.05	−0.51 ***	−0.46 ***	0.44 **	−0.40 **	1.00									
Zn	−0.41 **	0.44 **	−0.26	−0.15	0.40 **	0.11	0.29 *	1.00								
Cit	−0.19	0.65 ***	−0.61 ***	0.34 *	0.58 ***	−0.58 ***	0.10	0.02	1.00							
Tar	−0.07	0.35	0.07	0.75 ***	0.04	−0.05	−0.80	−0.24	0.41 **	1.00						
Mal	−0.01	−0.17	−0.23	−0.56 ***	0.07	−0.01	0.68 ***	0.22	−0.22	−0.74 ***	1.00					
ΣOA	−0.04	−0.09	−0.29*	−0.49 ***	0.13	−0.07	0.67 ***	0.21	−0.10	−0.66 ***	0.99 ***	1.00				
Suc	−0.42 **	0.71 ***	−0.62 ***	0.06	0.52 ***	−0.43 **	0.17 ***	0.19	0.71 ***	0.20	−0.05	0.02	1.00			
Glu	−0.02	0.26	0.03	0.57 ***	0.12	−0.07	−0.51 ***	−0.02	0.13	0.63 ***	−0.53 ***	−0.50 ***	0.03	1.00		
Fru	−0.36 *	0.30 *	0.21	0.28 *	−0.24	0.28	−0.55 ***	−0.14	0.16	0.51 ***	−0.38 **	−0.34 *	0.19	0.24	1.00	
ΣS	−0.39 **	0.70 ***	−0.36 *	0.44 **	0.37 *	−0.27	−0.31 *	0.09	0.60 ***	0.62 ***	−0.43 **	−0.35 *	0.75 ***	0.64 ***	0.50 ***	1.00

*, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. SWP = minimum stem water potential; SI = stress integral; Cit = citric; Tar = tartaric; Mal = malic; ΣOA = total organic acids; Suc = sucrose; Glu = glucose; Fru = fructose; ΣS = total sugars.

Finally, sucrose, fructose, and total sugars were also positively correlated with the water SI sucrose (R = 0.71; $p < 0.001$), fructose (R = 0.30; $p < 0.05$), and total sugars (R = 0.70; $p < 0.001$), and these results agreed with those of other authors in almonds cv. Marta, Guara, and Lauranne [29], with almonds being grown under RDI circumstances. Authors working with cv. Vairo under RDI and SDI conditions reported no differences for total sugars and sucrose in the first year of water deficit; however, a reduction in glucose was reported for the most stressed treatments [25,26]. Lower amounts of sucrose and glucose were reported in cv. Guara growth under non-irrigated conditions when compared to drip-irrigated, while fructose was not affected [42]. Lower values of sucrose with no differences in glucose, fructose, and the total sugars were also reported for cv. Marta under RDI and partial rootzone drying (PRD) in different levels [43]. Although, sucrose started to increase for the most severe treatment of PRD. Moreover, sucrose was reported to increase in non-irrigated conditions for cv. Ferragnes in early harvest, and the opposite was observed for the same cv. in late harvest, while non-irrigation decreased this sugar in cv. Texas in both situations, with the total sugars not affected [30]. If other crops are considered, sugars were also increased in tomatoes under water stress conditions [17], thyme (*Thymus vulgaris* as drought-tolerant and *T. kotschyanus* as drought-tolerant species) under drought stress [41], and peaches in which experiment it was demonstrated that deficit irrigation can enhance both total and individual sugars, if proper water stress is established for each cultivar [45]. A different behavior was reported for each peach cultivar; therefore, it is essential to establish specific conditions not only for each plant species but for each cultivar.

The sugars' enhancement under stress conditions was related to the osmotic adjustment, activated by accumulation of solutes rich in hydroxyl ($^{-}$OH) groups (sugars, proline, etc.) in the cytoplasm [26]. Osmotic adjustment is a biochemical mechanism that helps plants to adapt to dry and saline conditions by protecting the cellular membrane, protein, and enzymes against dehydration [26]; thus, it enhances the capacity to maintain positive turgor, increasing the sugars and organic acid. Another reason of the sugars accumulation during stress might be the induction of the growth inhibitor abscisic acid (ABA) by plants under stress conditions, which activates the sugar accumulation as an adaptation to stress [46]. Drought increases the biosynthesis and accumulation of ABA, which is considered the main regulator of drought stress response inducing the accumulation of osmotically active compounds, which protect cells from damage [38]. Under stress conditions, this phyto-hormone reduces plant growth and enhances desiccation tolerance by inducing de accumulation of stress-associated transcripts such as low-molecular-weight soluble sugars(sucrose) [47].

In summary, K, Fe, Zn, sucrose, fructose, and total sugars can be considered as good quality markers for hydroSOStainable almonds.

3.4. Antioxidant Activity (AA) and Total Phenolic Compounds (TPC)

The antioxidants are important compounds necessary to inhibit the process of oxidation acting like radical scavengers and converting these pro-oxidants to less reactive species. Antioxidants have attracted considerable consumer interest due to their potential preserving, nutritional, and therapeutic effects. For these reasons, the correlations among SWP and SI with antioxidant activity of almond kernel and kernel skin are important and were evaluated within this study along 3 seasons (Table 3). $ABTS^{\bullet+}$ (R = 0.79; $p < 0.001$ and R = 0.44; $p < 0.01$) and FRAP (R = 0.34; $p < 0.05$ and R = 0.41; $p < 0.01$) in both whole kernel and kernel skin showed a significant and positive correlation with the SI, and only $ABTS^{\bullet+}$ (R = −0.44; $p < 0.01$ and R = −0.30; $p < 0.05$) in both matrixes was negatively correlated with the SWP. This showed that the induced water stress led to almonds with a higher antioxidant activity.

Table 3. Pearson's correlation coefficients (R) among stem water potential and stress integral with antioxidant activity of almond kernel and kernel skin.

	SWP	**SI**	**ABTS$^{\bullet+}$K**	**DPPH$^{\bullet}$K**	**FRAP K**	**TPC K**	**ABTS$^{\bullet+}$S**	**DPPH$^{\bullet}$S**	**FRAP S**	**TPC S**
SWP	1.00									
SI	−0.67 ***	1.00								
ABTS$^{\bullet+}$K	−0.44 **	0.79 ***	1.00							
DPPH$^{\bullet}$K	−0.09	−0.25	−0.46 ***	1.00						
FRAP K	−0.07	0.34 *	0.57 ***	−0.54 ***	1.00					
TPC K	0.05	−0.14	0.21	−0.75 ***	0.38 **	1.00				
ABTS$^{\bullet+}$S	−0.30 *	0.44 **	0.12	0.50 ***	−0.20	−0.82 ***	1.00			
DPPH$^{\bullet}$S	−0.07	0.10	−0.20	0.80 ***	−0.46 ***	−0.95 ***	0.82 ***	1.00		
FRAP S	−0.19	0.41 **	0.14	0.24	−0.17	−0.63 ***	0.87 ***	0.62 ***	1.00	
TPC S	−0.11	0.16	−0.15	0.72 ***	−0.43**	−0.92 ***	0.88 ***	0.94 ***	0.73 ***	1.00

*, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. SWP = minimum stem water potential; SI = stress integral; ABTS$^{\bullet+}$ =2.2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH$^{\bullet}$ = (2,2-diphenil-1-picrylhydrazyl); FRAP = ferric reducing ability of plasma; TPC = total phenolic content; K = kernel; S = skin.

An increase in AA under water stress conditions was previously reported in many crops, including (i) almonds cv. Vairo, in which the stress was imposed in the kernel-filling phase using RDI and SDI strategies, (ii) olives cv. Manzanilla, when the stress was created just before harvest without re-hydration [48], and (iii) pistachios cv. Kerman, where the stress was imposed at stage II, which corresponds to shell hardening [49]. During the water stress, the turgor pressure is decreased, the ion toxicity is increased, and the photosynthesis is inhibited [50]. This increase in AA during stress conditions can be related to the antioxidant defense system used by plants to cope with reactive oxygen species (ROS) and also to the phytohormones accumulation by plants in water stress conditions. Phytohormones, as above mentioned, are responsible for the initiation of many defense mechanisms, including the increase in antioxidants to enhance plant tolerance to water stress. Jasmonate (JA), which is a phytohormone involved in sensing and signaling during the stress response, helps with the alleviation of plant to drought stress by increasing total carbohydrates, polysaccharides and soluble sugars by activating the enzymatic and non-enzymatic antioxidative system [50].

However, no correlation was found between the TPC and SI after 3 seasons, being in contrast with results previously reported by Lipan et al. (2019) [26] for almonds grown in the same conditions but in the first year of study. Several studies are reporting an increase in TPC values in plants grown under stress conditions, due to their role as plant molecules in response to biotic and abiotic stress, because when the carbohydrates exceed the amount used for growth needs, the excess of CO_2 assimilated in stress conditions is used for the biosynthesis of carbon secondary metabolites. Thus, not finding a correlation between SI and TPC may happen due to the level of stress applied, which perhaps was not strong enough to affect the TPC accumulation.

On the other hand, the positive correlation between SI and AA, and the no correlation or negative correlation of TPC with $ABTS^{\bullet+}$ ($R = -0.15$; $p > 0.05$) and FRAP ($R = -0.43$; $p < 0.01$) shows that other compounds with antioxidant effect might be responsible for the AA increase observed under water stress rather than only polyphenols. For instance, besides polyphenols, vitamins C and E and carotenoids have been thought to be responsible for most of the AA in foods [41]. Authors reported that, almonds are a valuable source of dietary lipids and have been suggested as a potential source of dietary antioxidants [51]. The same authors in their study about the AA and TPC in 100 different products reported that products with high AA tended to have a higher AA/TPC ratio; thus, this increase may result from compounds with AA that are not phenolic, or some phenolic compounds were more effective than others or with a greater reactivity with peroxyl free radicals (the AA method was an Oxygen Radical Absorbance Capacity (ORAC) Assay on a Plate Reader). Almonds are high/rich in vitamin E (25.6 mg/100 g) [52] because its content is above the minimum threshold (3.6 mg/100 g) established by the European Parliament and Council [39]; thus, this might be a compound contributing to the AA enhancement.

For instance, authors working with almonds cv. Nonpareil under water stress conditions reported higher values of tocopherols when RDI and SDI strategies were applied [53], as well as in sunflower seeds (cvs. Gulshan-98 and Suncross), particularly if the water stress was imposed at the reproductive stage [54]. These results led us to the conclusion that after long term study (3 years), antioxidant activity can be considered an important marker in hydroSOStainable almonds detection, while TPC is not a good indicator, presenting no correlation with water stress.

3.5. Fatty Acids

Pearson's correlation coefficients (R) between SWP and SI integral with fatty acids is showed in Table 4. Polyunsaturated/saturated fatty acids ratio (PUFA/SFA), oleic acid, and consequently, oleic/linoleic ratio (O/L) and monounsaturated (MUFA) fatty acids were significantly negatively correlated with the SI. On the other hand, myristic, palmitic, palmitoleic, margaric, *cis*-heptadecenoic, stearic, *cis*-vaccenic, linoleic, saturated (SFA), polyunsaturated (PUFA) fatty acids, and PUFA:MUFA ratio were positively correlated with the SI. Only linoleic, SFA, and PUFA fatty acids were also correlated in a negative way with the SWP, which helped to confirm the statement that these compounds increased with the water stress in almond trees.

Table 4. Pearson's correlation coefficients (R) among stem water potential and stress integral with fatty acids.

	SWP	SI	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1n9	C18:1n7	C18:2	O/L	SFA	MUFA	PUFA	PUFA/SFA	PUFA/MUFA
SWP	1.00																
SI	−0.67 ***	1.00															
C14:0	−0.14	0.73 ***	1.00														
C16:0	−0.20	0.81 ***	0.95 ***	1.00													
C16:1	−0.13	0.75 ***	0.92 ***	0.96 ***	1.00												
C17:0	−0.14	0.69 ***	0.88 ***	0.87 ***	0.86 ***	1.00											
C17:1	−0.06	0.65 ***	0.90 ***	0.90 ***	0.85 ***	0.84 ***	1.00										
C18:0	−0.20	0.80 ***	0.91 ***	0.97 ***	0.95 ***	0.86 ***	0.86 ***	1.00									
C18:1n9	0.22	−0.82 ***	−0.94 ***	−0.99 ***	−0.95 ***	−0.86 ***	−0.90 ***	−0.98 ***	1.00								
C18:1n7	−0.17	0.48 ***	0.51 ***	0.55 ***	0.40**	0.47 ***	0.62 ***	0.57 ***	−0.60 ***	1.00							
C18:2	−0.32 *	0.82 ***	0.86 ***	0.91 ***	0.86 ***	0.76 ***	0.75 ***	0.88 ***	−0.93 ***	0.46 ***	1.00						
O/L	0.24	−0.82 ***	−0.93 ***	−0.97 ***	−0.93 ***	−0.84 ***	−0.84 ***	−0.95 ***	0.98 ***	−0.52 ***	−0.98 ***	1.00					
SFA	−0.21	0.81 ***	0.94 ***	0.99 ***	0.96 ***	0.88 ***	0.89 ***	0.99 ***	−0.99 ***	0.55 ***	0.90 ***	−0.97 ***	1.00				
MUFA	0.27	−0.84 ***	−0.92 ***	−0.97 ***	−0.93 ***	−0.84 ***	−0.83 ***	−0.95 ***	0.98 ***	−0.49 ***	−0.97 ***	0.99 ***	−0.97 ***	1.00			
PUFA	−0.32 *	0.83 ***	0.86 ***	0.91 ***	0.86 ***	0.76 ***	0.75 ***	0.88 ***	−0.93 ***	0.46 ***	1.00 ***	−0.98 ***	0.90 ***	−0.97 ***	1.00		
PUFA/SFA	0.05	−0.65 ***	−0.84 ***	−0.89 ***	−0.90 ***	−0.83 ***	−0.86 ***	−0.91 ***	0.86 ***	−0.46 ***	−0.64 ***	0.78 ***	−0.90 ***	0.79 ***	−0.64 ***	1.00	
PUFA/MUFA	−0.32 *	0.85 ***	0.89 ***	0.94 ***	0.88 ***	0.80 ***	0.79 ***	0.91 ***	−0.96 ***	0.50 ***	0.99 ***	−0.99 ***	0.94 ***	−0.99 **	0.99 ***	−0.70 ***	1.00

*, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. SWP = minimum stem water potential; SI = stress integral; C16:0 (palmitic); C16:1 (palmitoleic); C17:0 (margaric); C17:1 cis (heptadecenoic); C18:0 (stearic); C18:1n9 (oleic); C18:1n7 (cis-vaccenic); C18:2n6 c9,12 (linoleic); O/L (oleic/linoleic); SFA (saturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids).

A reduction in oleic, MUFA, and O/L ratio and an increase in linoleic, PUFA, and PUFA/MUFA ratio was also reported in almond cv. Marta, Guara, Lauranne, Ferragnes, Texas; olives cv. Mazanilla; pistachio cv. Kerman; and sunflower cv. Suncross [29,30,42,49,54,55].

The decrease in oleic and increase in linoleic in drought conditions was reported in many studies in different crops, although sometimes was cultivar dependent [54]. This effect of water stress on these two fatty acids was attributed to the enzyme Δ12 desaturase, which is responsible for the conversion of oleic acid in linoleic under water stress conditions [56].

As observed, PUFA is increased under water stress and similar results were reported in olives cv. Manzanilla [57] after two years of deficit irrigation. The authors reported that the higher the stress applied during stage III in olives, the greater the linoleic acid concentration and, consequently, (PUFA + MUFA)/SFA ratio, with a correlation of $R^2 = 0.71$ and $R^2 = 0.84$, respectively. An increase in linoleic acid may play an important role in the death of cardiac cells and is an essential fatty acid, which cannot be synthesized by human body [29]. It was reported that consuming 50 g of almonds under RDI conditions can cover approximately 33% of the daily intake of linoleic acid recommended by the European Food Safety Authority [29]. The present study showed an increase in PUFA and a decrease in MUFA with water stress, and this led to a low O/L rate. It is well known that a low O/L rate means almonds are more susceptible to oxidation, because this is initiated in the double bonds of PUFA [58]. However, it was also observed that water stress also enhances compounds with antioxidant activity (polyphenols, α-tocopherol, phytoprostanes, phytofurans, jasmonates, abscisic acid, etc., that are also enhanced by water stress) that might help in maintaining the PUFA in a cell's membrane, preserving its bioactivity [59].

Saturated fatty acids were also observed to increase in almonds under water stress conditions, and the American Heart Association (AHA) encourages people to replace SFA with MUFA for a healthy lifestyle and low-density lipoprotein (LDL) cholesterol levels reductions. Thus, controlling the stress in almond trees might help to reduce the SFA content, because other studies reported that moderate deficiency did not negatively affected SFA content in almond [25,29,53]. Moreover, the levels of SFA in almonds are so low that almonds as well as other nuts fits well in AHA guidelines [60]. In fact, Food and Drug Administration (FDA) implemented a healthy claim regarding the almonds and other nuts consumption, stating that diets containing ~42.5 g of almonds per day as part of a diet low in saturated fat, and cholesterol may reduce the risk cardiovascular diseases [61].

To conclude this section, the fatty acids were significantly affected by water stress and are good markers of the hydroSOStainable almonds.

3.6. Descriptive Sensory Analysis

Table 5 shows the Pearson's correlation coefficients (R) between SI and SWP with sensory attributes. These results highlighted strong negative correlations for the size, bitterness, astringency, benzaldehyde, and woody flavors. Previous studies reported that water stress might enhance the sweetness, nutty, almond ID, and crispiness in almonds cv. Lauranne and pistachio cv. Kerman [29,31]. Thus, an increase in sugars and a decrease in bitterness and astringency with water stress conditions as shown in this study might lead to sweeter almonds.

As previously described by Lipan et al. (2019) [19] and Carbonell-Barrachina et al. (2015) [31], the purchase choice of international consumers was based on sweetness, almond ID, pistachio ID, and crispiness. These findings together with those that consumers were willing to pay more for hydroSOStainable almonds [19], pistachios [20], and table olives [62], and the functional properties of the bioactive compounds described here encourage the almond farming sector to bet on deficit irrigation strategy to reduce irrigation water and simultaneously increase the functional and sensorial quality of almonds.

Table 5. Pearson's correlation coefficients (R) among stem water potential and stress integral with sensory analysis parameters.

	SWP	SI	Color	Size	Sweet	Bitter	Astr	Nutty	Al ID	Benz	Woody	Hardness	Crispiness	Aftertaste
SWP	1.00													
SI	−0.69 *	1.00												
Color	0.24	0.04	1.00											
Size	0.35	−0.90 ***	−0.09	1.00										
Sweet	−0.43	0.35	−0.68 *	−0.26	1.00									
Bitter	0.12	−0.62 *	0.27	0.78 **	−0.42	1.00								
Astr	0.32	−0.70 *	0.12	0.77 **	−0.29	0.51	1.00							
Nutty	0.04	−0.39	−0.45	0.83 ***	0.21	0.64 *	0.59 *	1.00						
Al ID	−0.09	−0.34	−0.84 *	0.40	0.64 *	0.03	0.27	0.78 **	1.00					
Benz	0.09	−0.60 *	−0.68 *	0.69 *	0.38	0.26	0.43	0.83 ***	0.83 ***	1.00				
Woody	0.16	−0.71*	−0.67 *	0.76 **	0.20	0.39	0.48	0.89 ***	0.82 ***	0.91 ***	1.00			
Hardness	−0.32	0.03	−0.81 ***	0.05	0.53	−0.34	0.10	0.36	0.73 **	0.57	0.59 *	1.00		
Crispiness	−0.17	−0.12	−0.95 ***	0.17	0.64 *	−0.29	0.05	0.48	0.85 ***	0.71 **	0.71 **	0.91 ***	1.00	
Aftertaste	−0.15	−0.29	−0.83 ***	0.36	0.51	−0.11	0.31	0.65 *	0.91 ***	0.82 ***	0.81 ***	0.86 ***	0.89 ***	1.00

*, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. SWP = minimum stem water potential; SI = stress integral; Sweet = sweetness; Bitter = bitterness; Astr = astringency; Al ID = almond ID; Benz = benzaldehyde like.

4. Conclusions

Globally, data presented here showed that water stress affected the functional and sensorial parameters of hydroSOStainable almonds showing positive correlations with dry weight, color coordinates (L^*, a^*, and b^*), minerals (K, Fe, and Zn), organic acids (citric acid), sugars (sucrose, fructose, and total sugars), antioxidant activity, and fatty acids (linoleic, PUFA, SFA, PUFA/MUFA, among others). On the other hand, the water stress in almonds was negatively correlated with kernel yield, water activity, weight (almond, kernel, and shell), size, minerals (Ca and Mg), fatty acids (oleic acids, oleic/linoleic ratio, MUFA, and PUFA/SFA), and sensory attributes (size, bitterness, astringency, benzaldehyde, and woody). Considering that moderate RDI led to kernel yields similar to the control, agricultural sector can save approximately 45% of the irrigation water obtaining high-quality products. The current long-term research helped to demonstrate which quality parameters are really affected by water stress conditions and to clarify which may be essential markers to distinguish hydroSOStainable almonds from other types of almonds. All these findings help the agro-food sector to understand (i) that is possible to increase the water use efficiency generating products with high functional and sensory quality; (ii) the need of controlling the water stress in plants for the best agronomical and quality responses; and (iii) to set up key agronomic and quality markers to control and establish whether the water stress created at the field/orchard significantly affected the quality and functionality of the final edible nuts.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/10/1470/s1, Table S1: Mean values of morphological and chemical parameters of irrigation treatments (T1, T2, T3, and T4) for 3 years (2017, 2018, and 2019) and Table S2: Mean values of functional and sensorial parameters of irrigation treatments (T1, T2, T3, and T4) for 3 years (2017, 2018, and 2019).

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Table S1. Mean values of morphological and chemical parameters of irrigation treatments (T1, T2, T3 and T4) for 3 years (2017, 2018 and 2019).

	T1	T2	T3	T4
Agronomic parameters				
SWP	-1.50±0.07	-2.02±0.04	-2.13±0.09	-2.03±0.08
SI (MPa × day)	44.3±7.05	87.2±10.9	114±18.2	103±18.2
AW (mm)	691±108	382±58.6	114±3.66	114±5.68
KY (kg ha^{-1})	1807±371	1269±320	1088±263	1175±252
KR (%)	31.9±0.41	31.0±0.46	30.8±0.35	31.1±0.46
DW (%)	96.7±0.20	96.5±0.27	96.6±0.19	96.6±0.21
a_w	0.54±0.01	0.52±0.01	0.52±0.01	0.51±0.01
Morphological parameters				
Awe (g)	4.51±0.13	4.53±0.14	4.47±0.10	4.56±0.15
KWe (g)	1.44±0.03	1.41±0.04	1.38±0.02	1.41±0.03
SHWe (g)	3.07±0.11	3.12±0.11	3.10±0.08	3.14±0.12
AL (mm)	33.4±0.61	33.7±0.59	33.6±0.57	34.0±0.40
KL (mm)	25.7±0.31	25.6±0.42	25.4±0.23	25.6±0.34
AWi (mm)	23.9±0.38	24.0±0.40	24.0±0.39	24.5±0.43
KWi (mm)	16.1±0.21	16.0±0.26	15.8±0.17	16.0±0.26
ATi (mm)	15.6±0.13	15.4±0.14	15.3±0.13	15.4±0.17
KTi (mm)	8.50±0.13	8.27±0.10	8.09±0.08	8.25±0.07
*L**	45.8±0.39	46.1±0.50	45.8±0.45	46.2±0.44
*a**	16.8±0.21	17.0±0.29	17.0±0.33	17.2±0.31
*b**	31.0±0.49	31.4±0.62	30.9±0.47	31.8±0.61
Hue	44.1±3.62	44.1±3.55	43.8±3.64	44.6±3.56
C	52.7±3.88	53.1±3.79	52.7±3.73	53.1±3.72
H (N)	71.7±1.81	72.8±2.01	73.8±2.09	71.9±1.94
Chemical parameters				
Minerals (mg kg^{-1})				
Ca	3463±423	3369±500	3025±513	3392±395
Mg	2128±70.2	1986±50.2	2011±49.0	2007±50.8
K	6869±198	7224±270	7452±262	6555±266
Fe	22.8±1.67	25.0±1.72	28.0±1.97	26.3±1.50
Cu	10.0±039	10.4±0.40	10.5±0.42	11.1±0.61
Mn	20.9±1.75	20.0±1.85	19.9±1.66	20.6±1.91
Zn	39.2±1.87	42.3±0.96	44.5±1.58	40.2±1.31
Organic acids (g kg^{-1})				
Cit	2.23±0.05	2.37±0.06	2.39±0.07	2.34±0.09
Tar	1.32±0.09	1.33±0.10	1.27±0.09	1.25±0.09
Mal	6.97±0.58	6.78±0.67	7.54±0.92	8.58±1.18
ΣOA	10.5±0.52	10.4±0.56	11.1±0.86	12.2±1.08
Sugars (g kg^{-1})				
Suc	30.0±0.49	33.6±0.76	35.2±1.37	34.3±1.50
Glu	13.3±0.46	13.7±0.55	11.9±1.20	10.9±1.15
Fru	4.58±0.28	5.27±0.40	5.85±0.43	6.15±0.26
ΣS	49.9±0.78	52.5±1.14	52.9±2.28	51.3±1.96

T1=full irrigated; T2=moderate RDI; T3=severe RDI; SDI=sustained deficit irrigation; AWe=almond weight; KWe=kernel weight; SHWe=shell weight; AL=almond length; KL=kernel length; AWi=almond width; KWi=kernel width; ATi=almond thickness; KTi=kernel thickness; *L**, *a**, *b**=color coordinates; C=Chroma; H=hardness; Cit=citric; Tar=tartaric; Mal=malic; ΣOA=total organic acids; Suc=sucrose; Glu=glucose; Fru=fructose; ΣS=total sugars.

Table S2. Mean values of functional and sensorial parameters of irrigation treatments (T1, T2, T3 and T4) for 3 years (2017, 2018 and 2019).

	T1	T2	T3	T4
Functional parameters				
Antioxidant activity (mmol Trolox kg^{-1}) and total phenolic content (g GAE kg^{-1})				
$ABTS^{\bullet+}K$	8.27±0.32	9.14±0.37	9.32±0.54	9.73±0.30
$DPPH^{\bullet}K$	36.2±2.22	38.2±2.25	37.9±2.61	37.6±2.69
FRAPK	3.06±0.68	3.38±0.59	3.31±0.29	4.22±0.32
TPC K	2.78±0.50	3.09±0.60	3.14±0.58	3.06±0.52
$ABTS^{\bullet+}S$	55.4±4.80	65.9±6.47	60.0±6.42	58.0±6.43
$DPPH^{\bullet}S$	102±15.1	99.3±14.2	96.7±13.4	102±14.1
FRAP S	70.9±4.85	86.3±5.50	78.8±5.37	73.4±6.58
TPC S	29.3±3.59	30.7±3.90	29.3±3.48	27.4±3.54
Fatty acids (%)				
C14:0	0.039±0.003	0.040±0.003	0.040±0.004	0.040±0.003
C16:0	8.16±0.28	8.31±0.29	8.53±0.30	8.28±0.28
C16:1	0.56±0.04	0.59±0.05	0.58±0.05	0.58±0.04
C17:0	0.10±0.01	0.11±0.01	0.11±0.01	0.11±0.01
C17:1	0.14±0.02	0.15±0.01	0.15±0.01	0.15±0.01
C18:0	2.72±0.18	2.94±0.21	2.88±0.19	2.89±0.20
C18:1n9	62.9±1.67	61.7±1.74	61.3±1.91	61.8±1.80
C18:1n7	3.59±0.53	3.76±0.58	3.65±0.53	3.66±0.54
C18:2	22.2±0.42	23.0±0.47	23.3±0.59	23.0±0.50
O/L	2.85±0.13	2.71±0.13	2.68±0.15	2.72±0.13
SFA	11.2±0.44	11.6±0.48	11.6±0.47	11.5±0.46
MUFA	66.2±0.90	65.1±0.94	64.7±1.12	65.1±1.00
PUFA	22.4±0.42	23.1±0.47	23.4±0.29	23.1±0.50
PUFA/SFA	2.02±0.05	2.02±0.05	2.03±0.04	2.03±0.05
PUFA/MUFA	0.34±0.01	0.36±0.01	0.37±0.02	0.36±0.01
Descriptive sensory analysis				
Color	2.94±0.44	2.97±0.61	2.67±0.40	2.41±0.58
Size	7.88±0.32	8.01±0.25	7.24±0.39	7.23±0.39
Sweetness	3.51±0.07	3.72±0.08	3.92±0.13	3.49±0.22
Bitterness	0.43±0.07	0.41±0.07	0.40±0.05	0.34±0.05
Astringency	0.59±0.04	0.65±0.01	0.53±0.02	0.48±0.07
Nutty	5.79±0.18	5.85±0.16	5.95±0.14	5.56±0.18
Almond ID	6.37±0.15	6.37±0.15	6.57±0.12	6.26±0.14
Benzaldehyde like	0.49±0.04	0.42±0.04	0.40±0.06	0.39±0.07
Woody	2.06±0.34	1.82±0.30	1.89±0.32	1.96±0.29
Hardness	5.57±0.32	5.85±0.20	6.00±0.12	6.13±0.10
Crispiness	5.13±0.82	5.77±0.85	5.37±0.85	5.44±0.87
Aftertaste	6.08±0.21	6.33±0.25	6.44±0.14	6.35±0.15

T1=full irrigated; T2=moderate RDI; T3=severe RDI; SDI=sustained deficit irrigation; $ABTS^{\bullet+}$=2.2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); $DPPH^{\bullet}$=(2,2-diphenil-1-picrylhydrazyl); FRAP=ferric reducing ability of plasma; TPC=Total Phenolic Content; K=kernel; S=skin; C14=(myristic), C16:0 (palmitic), C16:1 (palmitoleic), C17:0 (margaric), C17:1 cis (heptadecenoic), C18:0 (stearic), C18:1n9 (oleic), C18:1n7 (cis-vaccenic), C18:2n6 c9,12 (linoleic), O/L (oleic/linoleic), SFA (Saturated Fatty Acids), MUFA (Monounsaturated Fatty Acids), PUFA (Polyunsaturated Fatty Acids).

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Optimization of roasting conditions in hydroSOStainable almonds using volatile and descriptive sensory profiles and consumer acceptance

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Abstract

HydroSOStainable almonds are harvested from trees cultivated under controlled water stress by using a regulated deficit irrigation (RDI) strategy. The aim of this study was to investigate consumers' perception to select the best roasting temperature for the hydroSOStainable almonds and its correlation with volatile compounds, descriptive sensory attributes, instrumental color and texture. Thirty-five volatile compounds were identified and the key compounds for the roasting process were 2,5-dimethylpyrazine, furfural and trimethyl pyrazine. Pyrazines, furans and, in general, volatiles were higher in hydroSOStainable almonds than in control. Instrumental color and trained panel showed that almonds roasted at 190 ºC presented intense color and burnt notes in both irrigation treatments, while almonds roasted at 150 ºC were under-roasted. Principal component analysis (PCA) grouped together the samples of the same irrigation treatment, but separated samples roasted at different temperature. Partial least square regression (PLS) results indicated that consumers overall liking was positively linked to specific volatiles (alkanes, alcohols, aldehydes and furans) and sensory attributes (sweetness, roasted, almond ID, nutty, hardness and crispiness), but, negatively correlated with pyrazines, bitterness, astringency, woody and burnt flavor notes. Penalty analysis showed that almonds roasted at 150 ºC and 190 ºC were penalized due to low roasted aroma and soft almonds, and over-roasted samples with too intense color and burn notes, respectively. While no penalization being found for almonds roasted at 170 ºC. Overall, roasting at 170 ºC for 10 min in a convective oven were the optimum conditions for roasting *Vairo* almonds.

Key words: *Prunus dulcis*, liking drivers, volatile compounds, pyrazines, regulated deficit irrigation, water stress.

Practical application

This research describes the link between physicochemical and sensory analysis of roasted almonds giving evidence about possible sensory quality markers. Besides, it provides valuable information for the food industry to produce roasted almonds that meet consumer demands and for the agricultural sector by encouraging reduction of irrigation water consumption by almond trees.

1. Introduction

Many areas of the world are currently experiencing an unprecedented drought which has extreme consequences in all sectors, including farming. Water limitations

for irrigation in southeastern Spain (provinces of Alicante and Murcia) reach worrying dimensions and seriously jeopardize biodiversity (e.g. loss of traditional minor crops) (García-Tejero et al., 2014). In this context, agriculture needs to implement deficit irrigation strategies, select drought-tolerant and less-water-demanding species leading to sustainable fruits and vegetables (García-Tejero et al., 2014).

Almond (*Prunus dulcis*) is the third most cultivated tree in Spain after olive trees and grape vines, and although it is a drought resistant crop, for a profitable production, irrigation water is needed (FAOSTAT, 2018; Egea et al., 2013). The regions of southeastern Spain have low or no rainfall and high evaporative demands during most of the phenological growth of the almond tree (Egea et al., 2013). Cutting off irrigation water during kernel filling (when the plant is less sensitive to water stress) is a regulated deficit irrigation (RDI) strategy aimed to reduce water consumption with minimal production losses (Girona et al., 2005). The fruits obtained under RDI conditions are called hydroSOStainable (*hydroSOS*) products and are characterized by higher contents of C-secondary metabolites in plant and of bioactive compounds in edible fruits (Noguera-Artiaga et al., 2016; Lipan, Cano-Lamadrid, et al., 2019; Lipan, Martín-Palomo, et al., 2019; Lipan, Moriana, et al., 2019; Sánchez-Rodríguez, Lipan, et al., 2019).

Nuts are widely used in the Mediterranean cuisine and can be consumed as fresh products (raw or roasted) but also can be used as ingredient for confectionery, bakery, etc., due to their desirable and characteristic flavor, and high nutritional values (Xiao et al., 2014). Almond roasting is the key unit operation in the processing of this nut; for instance, in the production of the most popular Spanish Christmas confection "*turron*" (a typical Spanish sweet made from roasted almonds and honey)(Vázquez-Araújo, Verdú, Navarro, Martínez-Sánchez, & Carbonell-Barrachina, 2009). In the food industry, roasting is used to enhance sensory attributes (leading to product acceptance) but also to extend product shelf life (Youn & Chung, 2012).

Volatile compounds are key markers in evaluating the effectiveness of the roasting process and the quality of the roasted products, as they determine the characteristic flavor of the roasted almonds. No information on the aroma profile of hydroSOStainable roasted almonds is available in literature. However, volatile aldehydes, ketones, alcohols, alkanes, and terpenes have been reported in "raw" almonds, and aldehydes such as hexanal, nonanal and benzaldehyde were the main aromatics (Erten & Cadwallader, 2017; Lipan, Moriana, et al., 2019).The authors have previously reported 26 volatile compounds in raw almonds (cultivar *Vairo*), with alcohols being the main chemical family (Lipan, Moriana, et al., 2019). With regard to dry "roasted" almonds, a previous study reported that aldehydes, ketones, alcohols, aromatic hydrocarbons, terpenes, and linear hydrocarbons were the main

volatile compounds (Erten & Cadwallader, 2017). However, other authors reported that pyrazines, pyrroles, furans and aldehydes were the main groups in this same matrix (Takei, Shimada, Watanabe, & Yamanishi, 1974; Takei & Yamanishi, 1974; Valdés et al., 2015; Vázquez-Araújo, Chambers, & Carbonell-Barrachina, 2012; Vázquez-Araújo, Enguix, Verdú, García-García, & Carbonell-Barrachina, 2008; Yang et al., 2013).

The moisture content is essential in establishing proper roasting conditions, with drier almonds reaching their optimal quality before than wet ones (Vázquez-Araújo et al., 2009). Almond cultivar and growing conditions (especially irrigation strategies and water stress) can influence the almond moisture and, therefore, the optimal roasting conditions (Vázquez-Araújo et al., 2009).

In this context, the aim of this study was to determine the best roasting conditions (time and temperature) for almonds grown under different irrigation strategies. To reach the decision on which roasting parameters were the best ones, data on volatile compounds, descriptive sensory profiles, and consumers acceptance were considered.

2. Materials and methods

2.1 Samples

Almonds were harvested from hydroSOStainable fields during 2019 season, in a commercial orchard "La Florida", located in Dos Hermanas (Seville, Spain). The irrigation treatments were those previously described by Lipan, Moriana, et al. (2019). Briefly, T1 treatment consisted of irrigation being provided to assure crop needs, and T2 consists of moderate regulated deficit irrigation, in which trees during the kernel filling period were only irrigated when the stem water potential was below -1.5 MPa. The stem water potential was measured using a pressure chamber (PMS Instrument Company, Albany, OR, USA). The monitored trees were harvested (28 weeks after blossom) with a self-propelled trunk shaker with collector. Samples were exposed horizontally to sun light until a moisture content below 5 %. This operation (either by natural or artificial drying) is essential to avoid the damage produced by mold and insects (when a higher moisture content is presented by almonds), as well as to increase the oil stability and overall edible quality (Schirra & Agabbio, 1989). Later, in-shell almonds were sent to Miguel Hernández University for quality analysis and affective tests.

2.2 Roasting of almonds

T1 and T2 almonds with similar size (length=25.0 ± 1.37 mm; width=15.5 ± 1.00 mm; thickness=8.98 ± 0.57 mm) and moisture content (2.34 ± 0.07 %) were selected to have a uniform material for the roasting process. Roasting conditions were chosen based on published literature (Lukac et al., 2007; Vázquez-Araújo et al., 2008; Lin et al., 2016) and after preliminary experiments, the time (10 min) was considered as constant variable. The moisture content was determined from 2 g of ground almonds dried to constant mass in a stove at 60 °C. Roasting experiments were carried out using a hot-air circulation drying oven Distform My Chef (Lleida, Spain), equipped with temperatures probes to measure the air temperature inside the roasting chamber. After the heating period, roasted almonds were immediately cooled until reaching a temperature of 50 ºC. Batches of 200 g were roasted in one layer for a constant time (10 min), at 3 temperatures: 150, 170 and 190 ºC at an air velocity of 3 m s^{-1}. Once the cooling temperature was achieved, almonds were removed from the oven and kept in a stainless-steel tray at 24.4 ± 1.0 ºC and 47.4 ± 0.5 % relative humidity until 25 ºC was achieved.

2.3 Determination of color

Color determination was carried out at 25±1 °C using a Minolta Colorimeter CR-300 (Osaka, Japan). Outside color was directly measured on the skin of 10 individual almond kernels, which afterwards were half cut, and the inside color kernel was measured for each of them. The color data was presented as CIE*L*a*b** coordinates explaining the color in a three-dimensional space. The degree of color difference (ΔE) was calculated as previously described (Cano-Lamadrid et al., 2017).

2.4 Determination of texture

The texture of 10 almonds per roasting treatment was measured at 20 ºC ± 1ºC using a texture analyzer (Stable Micro Systems, model TA-XT2i, Godalming, UK) with a 30 kg load cell and a probe Volodkevich Bite Jaw (HDP/VB) using the following conditions: trigger was placed at 15 g, test speed was 1 mm s^{-1} over a specific distance of 3 mm. The almonds were oriented in a way that the probe perpendicularly cut the almond and all almonds were positioned with the same orientation (Figure 1). Fracturability (mm), hardness (N), work done to shear (Ns), average force (N) and number of fractures (peaks count) were the parameters analyzed.

2.5 Descriptive sensory analysis

Sensory evaluation with a trained panel was used to describe and quantify the roasted almonds appearance, basic tastes, and flavor intensities of both irrigation

treatments. Ten highly trained panelists (5 females and 5 males) from the Food Quality and Safety Group (Miguel Hernández University of Elche, Orihuela, Alicante, Spain) with ages between 25-62 years (median age 32) conducted the descriptive analysis. The reference products and lexicon used were based on those previously reported by other authors working with almonds and *turrón* (Vázquez-Araújo et al., 2012; Lipan, Cano-Lamadrid, et al., 2019). Once finished the orientation sessions (4), the panel was asked to evaluate the 6 samples corresponding to the different irrigation and roasting treatments (2 × 3); the analysis was run in triplicate. The samples were presented in 30 mL covered plastic cups using a randomized block design to avoid biases. To cleanse the palate between samples, water and unsalted crackers were available. A tasting room with individual booths (controlled temperature of 21±2 ºC and combined natural/artificial light) was used for the descriptive tests. A structured scale from 0 to 10 and 0.5 increments was used to quantify the intensity of the almond attributes, where 0 represents *no intensity* and 10 *extremely strong.*

2.6 Affective sensory analysis

Affective sensory analysis was carried out with 100 recruited consumers from the SensoFood Solutions consumer database (UMH). Demographic questions concerning gender, age, nuts consumption frequency, allergies, intolerances, and diet restrictions were also included in the questionnaire. The consumers profile was: 41 % male and 59 % female, 25 % belonging to the 18-25 years old, 35 % to 26-35 years old, 22 % to 36-45 years old, and 18 % to > 45 years old group. Also, nuts frequency consumption was asked, and the answer was as following: 74 % daily, 6 % several times a week, 10 % weekly, 6 % several times a month, 4 % once per month or less.

The samples were distributed, labeled with 3-digit codes, and served in the same recipients as described in the descriptive section. A 9-point hedonic scale was used to rate consumer liking (1 meaning "dislike extremely" and 9 meaning "like extremely"). Also, Just About Right (JAR) scales, where 1 corresponded to "not enough at all", 5 to "Just About Right" and 9 to "too much", were used to assess the attributes intensity appropriateness. Consumers were also asked to rank samples according to their preference and to check all reasons for them to choose that sample as the best (due to the color, flavor sweetness, crispiness, etc.) by using a Check All That Apply (CATA) question. The tests were also carried out in special tasting rooms with individual portable booths and using a randomized block design.

2.7 Volatile compounds

Headspace solid phase microextraction (HS-SPME) was used to determine the volatile composition of the roasted almonds. Approximately 1 g of ground sample in a Moulinex grinder, model AR110830 (Alençon, France) for 20 s was placed in a hermetic vial with polypropylene cap and PTFE (polytetrafluoroethylene)/silicone septa, together with 500 μL of 12.5 % aqueous NaCl and 2.5 μL of 2-acethylthiazole (1000 mg L^{-1}) used as internal standard. This internal standard was used for the semi-quantification of the volatile compounds because no calibration curve was done for each one of the single compounds reported in this study. The vial was heated to 40 °C simulating the mouth temperature when chewing almonds as previously described by Lipan, Moriana et al. (2019). After a stabilization period of 40 min, a 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber was introduced in the headspace of the vial for 35 min. The fiber was specially chosen for its high capacity of trapping volatile compounds from fruits and nuts (Xiao et al., 2014). The isolation, identification and semi-quantification of the volatile compounds was performed in a gas chromatograph Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) coupled with a mass spectrometer (MS) detector Shimadzu GC-MS QP-5050A (identification) and a flame ionization detector, FID (semi-quantification). The fiber was desorbed for 3 min in the injector port of the GC-MS. The GC was equipped with a SLB-5ms Fused Silica Capillary Column of 30 m × 0.25 mm × 0.25 μm film thickness, 5% diphenyl, and 95% dimethyl siloxane (Supelco Analytical). Helium was used as gas carrier at a flow rate of 0.9 mL min^{-1} in a split ratio of 1:5. The oven program was: (a) initial temperature 50 °C, (b) rate of 4.0 °C min^{-1} to 130 °C, (c) rate of 10 °C min^{-1} from 130 ºC to 180 °C, (d) rate of 20 ºC from 180 ºC to 280 ºC. The injector and the detector were kept at 250 °C. The identification of the volatile compounds was performed using 3 methods: (a) retention indices, (b) retention times of standards, and (c) mass spectra (authentic chemicals and NIST69 spectral library collection) (NIST, 2018).

2.8 Statistical analysis

Data was analyzed using two-way analysis of variance (ANOVA), using "irrigation treatment" and "roasting temperature" as factors, followed by Tukey's multiple range test. Statistically significant differences were considered when $p<0.05$. Partial least square regression (PLS) analysis was done to study the relationship of the descriptive sensory parameters and volatile compounds (x: independent variables) with the consumers overall liking data (y: dependent variable). Principal component analysis (PCA regression map) was conducted to project the samples depending on the instrumental parameters, sensory descriptors and chemical families of the volatile compounds. Penalty analysis was also conducted,

using the JAR data, to provide information about the attributes which penalized the liking of the samples under analyses and can be improved by optimizing the roasting conditions (Lawless & Heymann, 2010). Statistical analysis was performed using XLSTAT Premium 2016 and Statgraphics Plus (Version 3.1).

3. Results and Discussion

3.1 Instrumental color

Almonds outside and inside instrumental color was evaluated (**Table 1**). Values of almonds lightness (L^*) of the outside and inside color significantly decreased ($p<0.01$) by increasing the roasting temperature, indicating that samples became darker due to browning reactions. The characteristic golden brown color occurs due to the Maillard reaction, caramelization of sugars and dextrins to furfural and hydroxymethyl furfural and carbonization of sugars fats and protein (Skovgaard, 2004). Simultaneously, a^*, b^* and C^* increased inside the kernel, implying a more intense and brownish (mix of red and yellow color coordinates) color as result of increasing roasting temperature. The decrease in L^* values and increase in a^* values were previously also reported in roasted peanuts and the phenomenon was related to the higher melanoidin production from Maillard reactions and with a lower finished moisture content (Lykomitros, Fogliano, & Capuano, 2018). However, the outside color was characterized by lower values of a^*, b^* and C^* which meant that red and yellow notes decreased in the almond skin exposed to the highest temperature, 190 ºC. Other authors have reported different results, maybe because they have measured color in ground almonds (Vázquez-Araújo et al., 2009), while in the present study it was measured directly on the almonds skin. The skin polyphenols might have had also an impact, because each almond cultivar has a unique polyphenol profile that can affect the almond color (Bolling, 2017); they also contribute to the degree of browning developed during roasting, as polyphenols may appear to affect Maillard browning (Bolling, 2017). For instance, a negative correlation has been previously reported between polyphenols and the browning reaction precursors (sugars and amino acids) (Noor-Soffalina, Jinap, Nazamid, & Nazimah, 2009). Because the polyphenols have a high tendency to form complexes with protein, polysaccharide and alkaloid; and consequently, to influence color and to reduce the flavor due to binding of polyphenol on aroma precursors (free amino acids and sugars) and aroma compounds formed during roasting (Misnawi, Jinap, Jamilah, & Nazamid, 2004).

Regarding the irrigation treatment, lower L^* value was observed in almonds irrigated at the optimum plant needs (T1). Almonds of T1 became darker before those of T2 at 190 ºC roasting temperature, while the other two roasting temperatures showed similar results between the irrigation treatments. This might be related to

the differences in chemical composition of hydroSOStainable almonds (T2), which had been reported to have a higher sugar content etc., (Lipan, Moriana, et al., 2019; Lipan et al., 2020).

High ΔE (which represents the total color difference) values indicated a higher color change with respect to the raw almonds (**Table 1**). In general, the outside color was not changed did not significantly change when the roasting temperature was increased from 150 to 170 ºC, but significantly increased at 190 ºC. While the inside color increased with each roasting temperature. Conventional and hydroSOStainable almonds exhibit statistically similar behavior in inside color parameter in each roasting temperature. However, the hydroSOStainable almonds recorded a higher inside color change for the lowest roasting temperature, and no changes between conventional and hydroSOStainable almonds were observed at 170 and 190 ºC.

3.2 Instrumental texture

Fruit texture is important as it is a primary determinant of consumer acceptance and the rejection of roasted almonds is mainly due to inappropriate textural attributes (e.g. not crunchy products) (Cheely et al., 2018). Hardness, work done to shear, and average force were significantly reduced with increasing roasting temperatures, while no significant differences were observed for fracturability and number of fractures (**Table 1**). Authors working with raw *Vairo* almonds reported higher values of facturability (1.87 mm), hardness (73.2 N), and lower numbers of fractures (9) (Lipan, Martín-Palomo, et al., 2019) than those found in roasted almonds from the same cultivar (F=1.35 mm, H=52 N, and NF=16, respectively); as roasted almonds are less hard but more fracturable and crispy than the raw ones (Lipan, Martín-Palomo, et al., 2019). Crunchiness is a textural characteristic important for consumer acceptability (Cheely et al., 2018), and an increment in crunchiness mainly occurs due to dehydration, browning, lipid oxidation and other structural changes (Varela, Chen, Fiszman, & Povey, 2006). The degradation of the structure during roasting cause changes in textural attributes such as crispiness, grittiness, porosity and fracturability (Varela et al., 2006).

Significant differences (p<0.001) were detected between the irrigation treatments (T1 and T2) and also among the roasting temperatures for the hardness attribute. HydroSOStainable almonds (T2) had the hardest texture, which decreased when the roasting temperature increased. Some authors have reported that water deficit can impact the texture of the nut due to the water stress effect on cell size, cell turgor, solute transport, and the accumulation of osmotically active solutes at the cell level (Ripoll et al., 2014). Thus, a reduction in turgor and moisture content which is directly affected by the roasting process, because this involved dehydration,

with effect on color, flavor and texture (Huang, 2014; Perren & Escher, 2013). Moreover, water deficit has also been shown to cause alteration in the chemical composition and the physical properties of the cell wall (Peleman et al., 1989; Lipan, Martín-Palomo, et al., 2019; Lipan, Moriana, et al., 2019;). It has also been reported that the accumulation on antioxidants might prevent oxidative damage with a positive effect on the tomatoes texture (Dumville & Fry, 2003). In this way, the nuts texture was reported to be correlated to the fat content in walnuts, showing that when the fat content is reduced, the hardness is also reduced due to the cell wall collapse (Crowe & White, 2003).

3.3 Volatile composition

Many volatile compounds are generated through Maillard reaction and lipid oxidation during the roasting process (Vázquez-Araújo et al., 2008; Vázquez-Araújo et al., 2009). A total of 35 volatile compounds were identified in roasted almonds: aldehydes (n=9), pyrazines (9), alkanes (9), alcohols (5), furans (2) and ketone (1). **Table 2** shows the retention time, the retention indexes used for the identification of the aroma compounds and their odor descriptors. Similar results were previously reported by others with pyrazines and aldehydes being the chemical families in roasted almonds (Takei et al., 1974; Takei & Yamanishi, 1974; Vázquez-Araújo et al., 2008; Vázquez-Araújo et al., 2012; Yang et al., 2013; Valdés et al., 2015), and also linear hydrocarbons (Erten & Cadwallader, 2017). In this way, the previously identified chemical families in nuts aroma include (Alasalvar, Shahidi, & Cadwallader, 2003): (i) alcohols (heptanol, 1-octanol, (ii) ketones (2-heptanone), (iii) aldehydes (heptanal), (iv) aromatic hydrocarbons (benzaldehyde), (v) furans (furfural), (vi) pyrazines (2-methyl pyrazine), and (vii) linear hydrocarbons (nonane, dodecane, etc.).

The total content of volatile compounds was significantly different among samples (**Table 3**). Almonds roasted at 150 ºC showed the lowest total content of volatile compounds (4.77 mg kg^{-1}), followed by those roasted at 170 ºC (7.32 mg kg^{-1}) and at 190 ºC (11.9 mg kg^{-1}). Almonds roasted at 150 ºC showed similar volatile content to those reported in raw almond for *Vairo* (4.39 mg kg^{-1}) (Lipan, Moriana, et al., 2019), and *Bute* and *Padre* cultivars (4.36 mg kg^{-1}), which meant that almonds roasted at 150 ºC temperature showed very similar volatile profile to the raw almonds and were under-roasted. Generally, the aroma of raw almonds is weak and a low total content of volatile compounds is expected (Lipan, Moriana, et al., 2019); however, as observed, the roasting process increases the generation of volatile organic compounds in almonds if a threshold temperature is reached; 150 ºC is below this threshold. This might happen because Maillard reaction, which is responsible for the increasing in volatile compounds, generally begins at temperatures above 140 ºC

(Ghaderi & Monajjemzadeh, 2020). The total volatile content in almonds roasted at 170 ºC and 190 ºC were similar to those reported by other authors, who showed similar results (in the range between 6.17 and 16.0 mg kg^{-1}) in *Marcona* and *Comuna* cultivars roasted at 200 ºC for 12, 15, 17, 20, 23 min (Vázquez-Araújo et al., 2009) and *Bute*/*Padre* cultivars roasted at 138 ºC for 28, 33 and 38 min (Xiao et al., 2014), using convection ovens in both studies.

In general, the contents of aldehydes, pyrazines, furans, ketones, alkanes, alcohols, and consequently the total volatile content of hydroSOStainable almonds (T2) were significantly higher than in T1 samples. T2 samples were characterized by a higher content of volatiles having sensory descriptors such as almond, nutty, bready, and chocolate notes (**Table 2**). An increase in volatile compounds in hydroSOStainable almonds may occur due to the alteration in the chemical composition under water stress conditions (Ju et al., 2018).

Aldehydes were one of the main chemical family found, with hexanal (V2), benzaldehyde (V10), benzeneacetaldehyde (V17), and nonanal (V24) being the predominant aldehydes; all three compounds increased with the roasting temperature. Aldehydes, as well as ketones may be formed by degradation and auto-oxidation of fatty acids during roasting process and storage, because 48-67 % of kernel is oil, composed by 63-78 % oleic acid and 12-27 % linoleic acid among others with roasting temperature and storage (Xiao et al., 2014; Erten & Cadwallader, 2017; Lipan, Martín-Palomo, et al., 2019). The unsaturated fatty acids are precursors of nonanal and hexanal aldehydes, which in high concentrations (more than 2.1 and 6.0 ug kg^{-1} hexanal) are responsible for the fat oxidation and consequently for the off-flavors and inedible almonds (Perren & Escher, 2013; Yang et al., 2013). Thus, the present values of hexanal, even those obtained in almonds roasted at the highest roasting temperature 190 ºC (0.33 mg kg^{-1}) were way below than those previous reported in roasted almonds at the end of shelf life (Yang et al., 2013), demonstrating the freshness of the samples.

Benzaldehyde (V10), is a breakdown product of amygdalin (cyanogenic glycoside naturally generated in almond) and so the predominant volatile compound mainly in raw bitter almond (Kwak et al., 2015). Although it was reported that roasting process might reduce the benzaldehyde level about 90 % in sweet and bitter almonds (Hojjati, Lipan, & Carbonell-Barrachina, 2016; Xiao et al., 2014), the opposite was observed in the present study, in which benzaldehyde increased with the heating treatment being higher in T2 samples. Other authors also reported an increase in V10 with the roasting conditions (time and temperature) in roasted sunflower seeds. This phenomenon might occur as a consequence of lipid oxidation in which a carbonyl-amine reaction (Strecker degradation) is required to produce

compounds that would be later degraded by the free radicals produced in the decomposition of lipid hydroperoxides to benzaldehyde and other compounds (Hidalgo & Zamora, 2019). In addition, "Vario" cultivar is characterized by very low levels of benzaldehyde in raw almonds (Lipan, Moriana, et al., 2019).

Pyrazines were also one of the main volatiles found in these roasted nuts. Pyrazines such as 2,5-dimethylpyrazine (V8), 2-ethyl-3-methylpyrazine (V14), trimethylpyrazine (V15), 2,5-dimethyl-3-ethylpyrazine (V19), 2,3-diethyl-5-methylpyrazine (V23) and 2,6-diethyl-3-methylpyrazine (V26) were already present in samples roasted at 150 ºC; however, different pyrazines appeared in the aroma profile of roasted almonds only after reaching temperatures of 170 and 190 ºC; these were 2-methyl pyrazine (V3), 2,3-dimethyl-5-ethylpyrazine (V20), and 2,6-dimethyl-3-ethylpyrazine (V21). The most abundant pyrazine found in this study was V8 (1.22 mg kg^{-1} mean value for all samples), followed by V15 (0.47 mg kg^{-1}) and V19 (0.29 mg kg^{-1}) and their contents increased with increasing roasting temperatures. Similar results were also reported by other authors working with roasted almonds from *Marcona* and *Comuna* cultivars roasted at 200 ºC for 12, 15, 17, 20 and 23 min (Vázquez-Araújo et al., 2009). These compounds are formed during heating *via* Maillard sugar-amine reactions and Strecker degradation and contribute to the nutty and roasted notes of the roasted-almond aroma (Alasalvar et al., 2003). Pyrazines might be considered key compounds in the roasted almonds aroma with a positive correlation between pyrazines and roasted-almond odor (Vázquez-Araújo et al., 2009), when the sample does not present burn notes (Vázquez-Araújo, Verdú, Navarro, Martínez-Sánchez, & Carbonell-Barrachina, 2009). However, as shown in **Table 3**, 2,6-dimethyl-3-ethylpyrazine (V21), which is a pyrazine with "burn", almond and coffee notes (The Good Scent Company, 2018), was not found at 150 ºC, appeared at 170 ºC and doubled its concentration at 190 ºC. This could be expected to be described as burn notes by the trained and consumer panels that will evaluate the samples later on. Regarding irrigation treatments, in general pyrazine tend to increase in hydroSOStainable almonds and similar results were also observed in pistachios when a moderate RDI was applied (Carbonell-Barrachina et al., 2015). However, the pyrazines were reduced in pistachios when the stressed was increased. This increase in pyrazine of fruits growth under moderate water stress, might be related to the sugars and amino pyrazine precursors) acids which were reported to increase in deficit irrigation conditions (Lipan et al., 2020; Ju et al., 2018). Moreover, authors found a close relationship between the accumulation of the volatile compounds and amino acids concentration in grapes (Ju et al., 2018).

Other compounds such as furans (e.g. furfural and furaneol), resulting from sugars degradation (glucose and fructose) (Vázquez-Araújo et al., 2008; Xiao et al.,

2014), were also found as the roasting process was more intense (higher temperatures). Furfural was not detected at 150 ºC for the T1 almonds but appeared at 170 ºC (0.48 mg kg^{-1}) and increased at 190 ºC (1.22 mg kg^{-1}). This phenomenon was expected because furfural is a compound of Maillard reaction and is a marker of the severity of heat treatments (Agila & Barringer, 2012). This compound was not reported in raw *Vairo* (Lipan, Moriana, et al., 2019) cultivar, neither in *Nonpareil* (Kwak et al., 2015), *Bute* or *Padre* cultivars in previous studies, and is a by-product of Maillard reactions initiated with the roasting process (Xiao et al., 2014). In raw fruits, furfural was reported in wild almonds (Hojjati, Lipan, & Carbonell-Barrachina, 2016) and sunflower seeds (Guo, Na Jom, & Ge, 2019). It was also reported that increased with the roasting time, temperature and microwave power due to the acid hydrolysis or heating of fruits polysaccharides containing hexoses or pentoses (Skovgaard, 2004; Petisca, Pérez-Palacios, Farah, Pinho, & Ferreira, 2013). Regarding the irrigation treatment, in T2 treatment appeared even from the lowest heating temperature (150 ºC) being always higher than in T1 samples (4.1-fold, 1.8-fold and 1.5-fold at 150, 170 and 190 ºC respectively). This might be related to the sugars content which was reported to be raised in fruits grown under water stress conditions due to the osmotic adjustments (Lipan, Moriana, et al., 2019).

3.4 Descriptive sensory analysis

Descriptive sensory analysis was performed to establish the sensory profile of the roasted almonds. Fourteen flavor and texture attributes were evaluated in roasted almonds and their intensities are summarized in **Table 4**. As shown, all the assessed attributes, except benzaldehyde and cohesiveness, were significantly affected by the roasting temperatures and irrigation treatment. The higher the oven temperature, the higher the intensity of the following attributes: bitterness, astringency, roasted, burnt, woody, and aftertaste. The opposite was shown for sweetness, which significantly decreased with increasing temperature; the same trend was also observed for the overall nut and almond-ID flavor attributes. Hardness, crispiness, and adhesiveness were the texture attributes evaluated; hardness and crispiness had similar intensities in samples roasted at 150 ºC and 170 ºC and lower in those roasted at 190 ºC. Finally, adhesiveness was slightly reduced from 4.1 to 3.0 when the roasting temperature increased.

Sweetness, overall nuts, almond-ID, hardness, and crispiness intensities were higher in hydroSOStainable samples (T2) than in full irrigated almonds (T1). This sensory finding agreed with previously discussed findings showing that volatile compounds with nutty (pentanal and 2,6-dimethyl-3-ethylpyrazine) and almond (benzaldehyde, furfural, etc.) descriptors having also higher contents in hydroSOStainable almonds. Roasted notes, however, were higher in fully irrigated

samples, as found by the trained panel and the analytical analysis (2-ethyl-3-methylpyrazine).

3.5 Principal component analysis

For an easy visualization of the relationships among all variables of the roasted almonds at different temperature, a principal component analysis (PCA) was run for all 6 samples, including only significantly different variables: color coordinates, volatile compounds and descriptive sensory attributes. **Figure 2** shows the 2 principal components which explained 84.96 % of the samples variation. As observed, samples roasted at different temperatures were grouped separately, but the irrigation treatments roasted at the same temperature were grouped together. Samples roasted at 150 ºC were mainly described by ketones, alcohols, alkanes, adhesiveness, sweetness and almond ID; these chemical families were those very close to raw almonds (fresh, fruity, herbal, sweet and green notes) (Lipan, Moriana, et al., 2019). Sensory attributes also demonstrated the proximity between 150 ºC almonds with the raw ones, being high intensities of adherence to teeth, sweetness and fresh almond-ID. Almonds roasted at 170 ºC were described as hard, with nutty flavor and aldehydes aromatics, and with light inside color. Aldehydes was the chemical family closer to the 170 ºC almonds, with descriptors such as nutty, chocolate, bready and almond notes (The Good Scent Company, 2018; Lu Xiao et al., 2014). Finally, 190 ºC roasted almonds were characterized by burnt, woody, benzaldehyde, and roasted notes, long aftertaste, due to their astringency and bitterness and volatiles such as pyrazines and furans. As shown, these samples presented burnt notes which were clearly identified by the sensory panel, and also detected using analytical technics such as GC-MS. Some pyrazines (2,5-dimethyl-3-ethylpyrazine, 2,3-dimethyl-5-ethylpyrazine) and volatile compounds derived from furans (e.g. furaneol) have been reported to contribute to flavor notes such as burnt, roasted, coffee and burnt brown (The Good Scent Company, 2018). Burnt notes in roasted almonds are not desirable and roasting conditions must be adjusted to avoid their generation (Hojjati et al., 2016). In this context, "the higher the volatile compounds, the better the odor and aroma of roasted almonds" statement (Hojjati et al., 2016) is not valid due to the burnt notes generated by the excessive heat treatment at 190 ºC for 10 min.

3.6 Consumers acceptability and driving sensory attributes

A consumer study was carried out to determine the drivers of liking for roasted almonds, and to offer industries processing almonds relevant information regarding the temperature guidelines. For the samples preference, most consumers chose T1 (27 %) and T2 (24 %) samples roasted at 170 ºC as the best almonds due to their

roasted almond flavor (62 %), aftertaste (43 %), texture (38 %) and sweetness (29 %). Regarding the purchase intention:

i) 61 % (T1) and 59 % (T2) of consumers were willing to buy almonds roasted at 150 ºC;
ii) 78 % (T1) and 82 % (T2) of consumers were willing to buy almonds roasted at 170 ºC; and,
iii) Only 25 % (T1) and 42 % (T2) of consumers were willing to buy almonds roasted at 190 ºC;

Partial least square regression (PLS) analysis was conducted to determine the drivers of liking of the samples and was explained by 93 % of the variation in Y variables (volatiles and sensory descriptive results) and 87 % of the variation in X variables (consumers) (Vázquez-Araújo, Chambers, Adhikari, & Carbonell-Barrachina, 2010; Vázquez-Araújo, Koppel, Chambers IV, Adhikari, & Carbonell-Barrachina, 2011; Calín-Sánchez et al., 2011; Cano-Lamadrid, Vázquez-Araújo, Sánchez-Rodríguez, Wodyło, & Carbonell-Barrachina, 2018). Data are represented using a map to provide information of consumers overall liking with the 35 detected volatile compounds and descriptive sensory parameters (**Figure 3**); only statistically significant parameters ($p<0.05$) were included in this analysis. **Figure 3** shows how almost all consumers were close to volatiles corresponding to alkane (V6, V12, V13, V22, V27, V29, V31, V33 and V34), alcohol (V11, V16, V18, V28 and V35), aldehyde (V1, V2 and V7) and ketone (V25) families. These compounds are characterized by fruity, creamy, fresh, fatty, bready, nutty, caramel-like and cocoa chocolate notes; aromas mainly related to a mild roasting. On the other side, there were those compounds belonging to the pyrazines family. Pyrazines together with furans and pyrroles are considered key compounds of roasted almonds formed during heating via Mallard sugar-amine reactions (Hojjati et al., 2016); they contribute to desirable nutty and toasty odors in roasted hazelnuts and almonds, if they are in a proper concentration (Alasalvar et al., 2003; Vázquez-Araújo et al., 2009). However, a high content of pyrazines is not associated to high quality roasted almonds, if they smell and taste burnt (Vázquez-Araújo et al., 2009). Regarding the consumer overall liking and the relationship with different descriptive sensory attributes the main group of consumers was located close to the attributes: sweetness, almond-ID, overall nut, hardness and crispiness showing that these descriptors are good drivers of consumer liking. While, roasted, burnt, woody, astringency and bitterness were shown to be the less liked attributes.

In general, consumers overall liking was positively linked to specific volatiles (alkanes, alcohols, aldehydes and furans) and sensory attributes (sweetness, roasted, almond ID, overall nut flavors together with a hard and crispy texture). On

the contrary, a negative correlation was observed between consumers overall liking and pyrazines, bitterness, astringency and woody and burnt flavor notes. Similar results were also reported for roasted peanuts revealing that the consumers drivers of liking were even similar across different countries (Spain, Netherlands and Turkey), with color, sweetness, roasted peanut parameters increasing the liking and the contrary for bitterness (Lykomitros et al., 2018).

3.7 Penalty analysis

Additionally, to overall liking, JAR questions were also asked during the consumer study to see which of the attributes penalized liking. To understand the relationship between JAR scores and consumer liking, penalty analysis was conducted (Narayanan, Chinnasamy, Jin, & Clark, 2014; Cano-Lamadrid et al., 2018; Lipan, Cano-Lamadrid, et al., 2019). **Figure 4** shows the proportion of consumers opinion plots against the mean drops (penalty). The aspects susceptible of improvement were those that had the greatest negative impact on the sample liking for at least 20 % of consumers and caused a drop of at least 1 unit for liking. There was an apparent need to improve T1 samples roasted at 150 ºC (**Figure 4A**), which were characterized by low roasted almond flavor, aftertaste, and sweetness. In the same way, samples T2 (**Figure 4B**) roasted at the same temperature needed to increase the intensity of color, roasted and almond flavor, sweetness, and hardness. The 170 ºC roasting temperature was associated with high consumer acceptance of roasted almonds from both irrigation treatments (T1 and T2), and results of penalty analysis (**Figure 4C** and **4D**) suggested that this was the optimum temperature. Finally, samples roasted at 190 ºC were penalized due to their too high bitterness, aftertaste, color, and roasted notes of almonds from T1 and T2 irrigation treatments (**Figure 4E** and **4F**).

4. Conclusions

This is the first study reporting volatile composition, sensory profile and consumer acceptance of roasted almonds grown under deficit irrigation conditions. Results indicated that the heat treatment of 170 ºC was the optimum roasting temperature from an aromatic, descriptive and affective point of view for the *Vairo* almonds. These samples were characterized by (i) a proper total content of volatile compounds (7.22 mg kg^{-1}), with 2.5-dimethyl pyrazine and furfural being the main predominant compounds and contributing to the almond, baked bread, roasted nuts notes; and, (ii) also by having hard and crispy texture, and intense almond and nutty flavor. Penalty analysis showed that almonds roasted at 150 ºC were penalized because of their low roasted aroma and soft hardness, and almonds roasted at 190 ºC were perceived as over-roasted, with too intense color and burn notes. Almonds roasted at 170 ºC were not penalized and, therefore, did not need to be optimized. Regarding the irrigation treatments, hydroSOStainable almonds (T2) were characterized by a higher total content of volatile compounds and for being harder, sweeter, and by having a higher intensity of almond and roasted notes at the optimum roasting temperature (170 ºC).

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Author Contributions

Lipan run the physico-chemical, sensory data, conducted part of the statistical analysis and writing the manuscript; Cano-Lamadrid helped with statistical analysis; Vázquez-Araújo helped with the design of the work, interpretation of data, and the manuscript revision; Łyczko helped with the physico-chemical analysis; Moriana and Hernández prepared the samples and were the experts in irrigation strategies; García-García revised and approved the final manuscript; Carbonell-Barrachina coordinated and assisted with acquiring founding for the study.

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Figure 1- Texture analysis of the roasted almonds

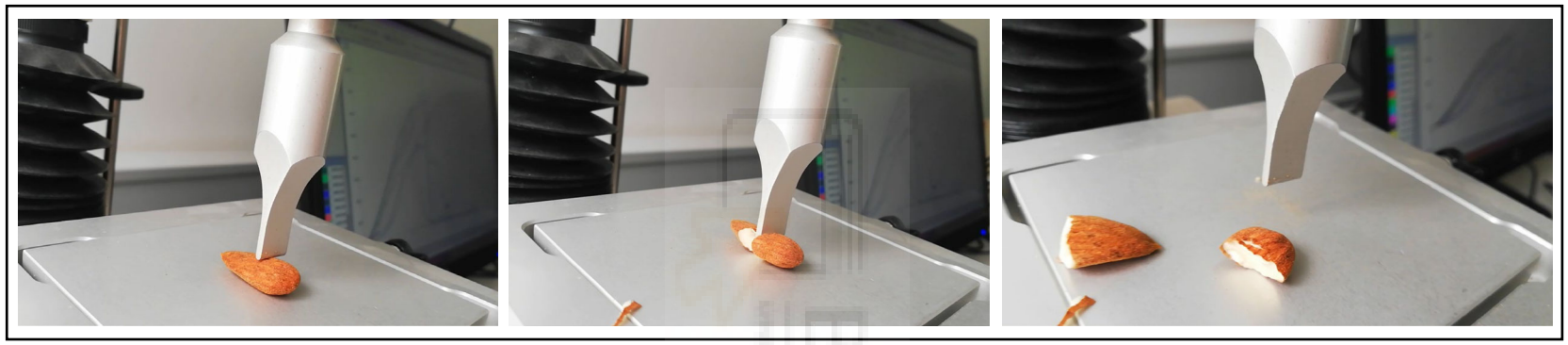


Figure 2-Principal Components Analysis (PCA) scores biplot showing the relationship among instrumental color, texture, volatile chemical family and descriptive sensory attributes.

<u>Legend</u>: ■ roasting treatments; Δ color coordinates outside (L^*_o, a^*_o, b^*_o) and inside (L^*_i, a^*_i, b^*_i) kernel; ○ volatile compounds; ◇ sensory attributes.

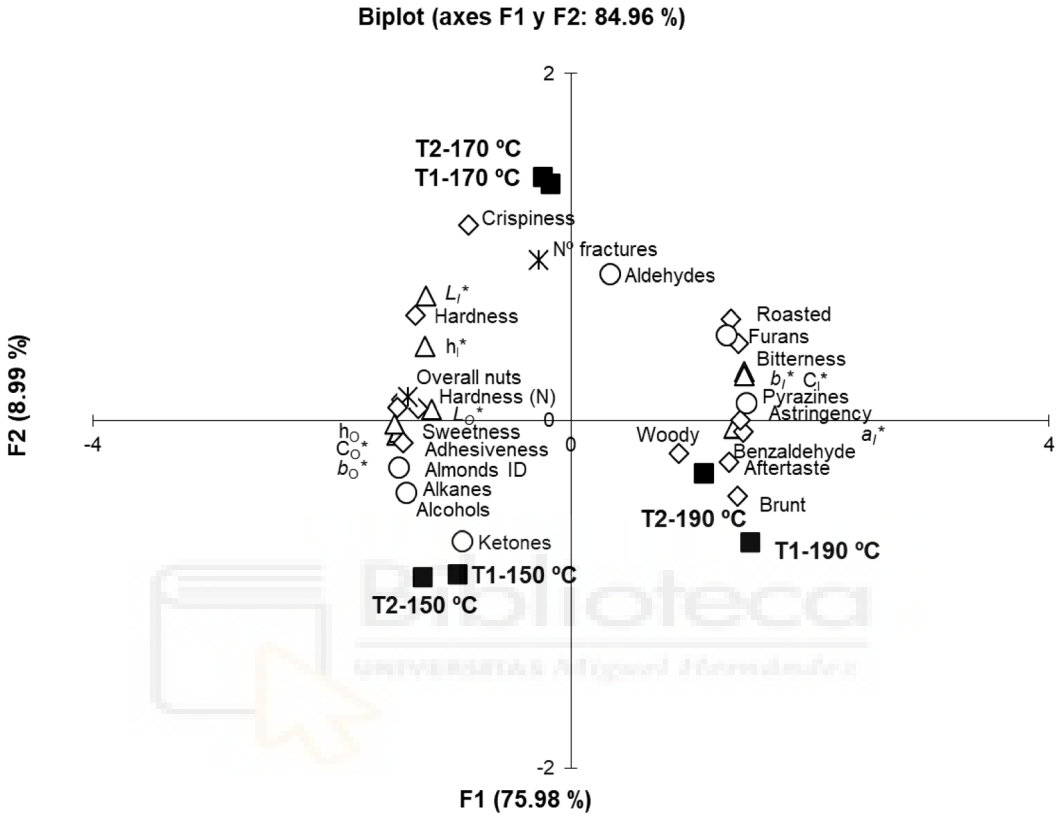


Figure 3-Partial Least Squares (PLS) regression of volatile compounds and descriptive sensory attributes (Y) and consumers overall liking (X) of roasted almonds.

<u>Legend</u>: □ roasting treatments; Δ volatile compounds, × sensory attributes, ○ consumers' overall liking.

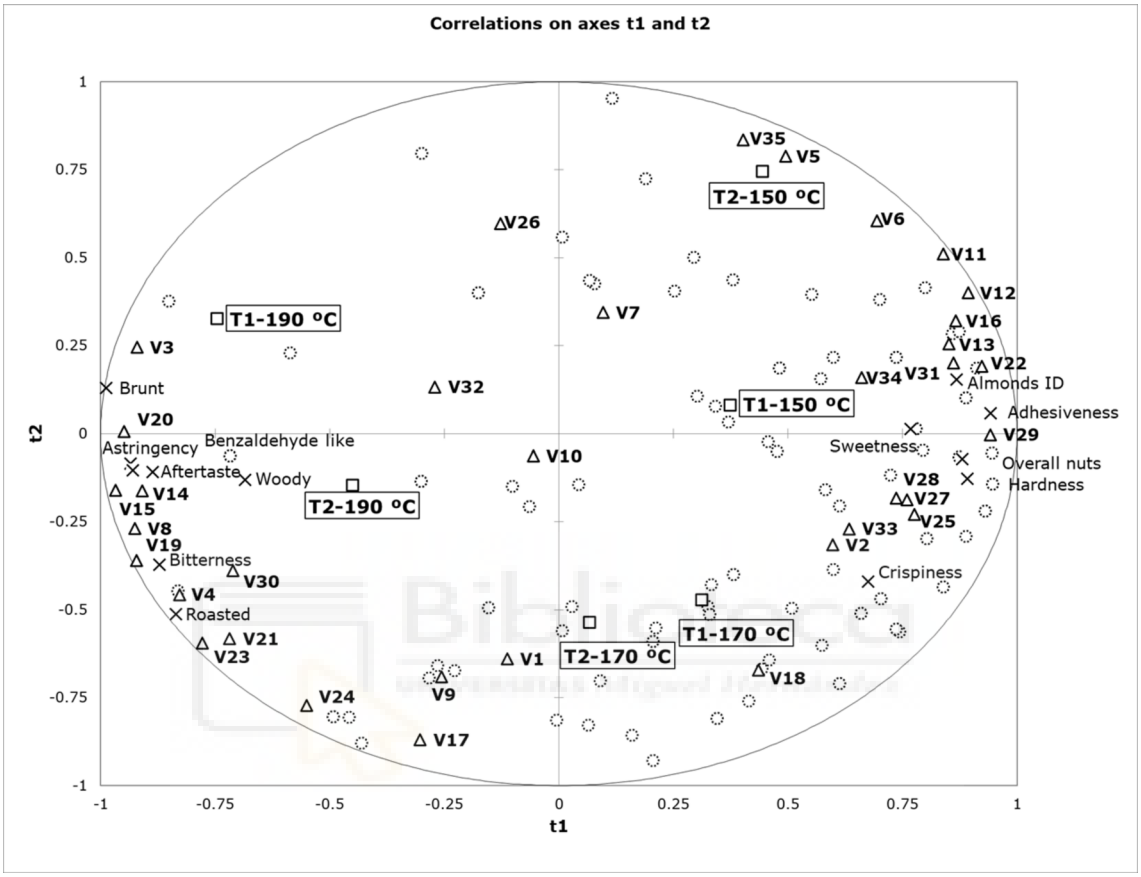


Figure 4-Penalty analysis of roasted almonds attributes intensities assessed by consumers (sample code indicated on the top right of each figure; "too low intensity" is indicated by the symbol "-" and "too high intensity" is indicated by the symbol "+").

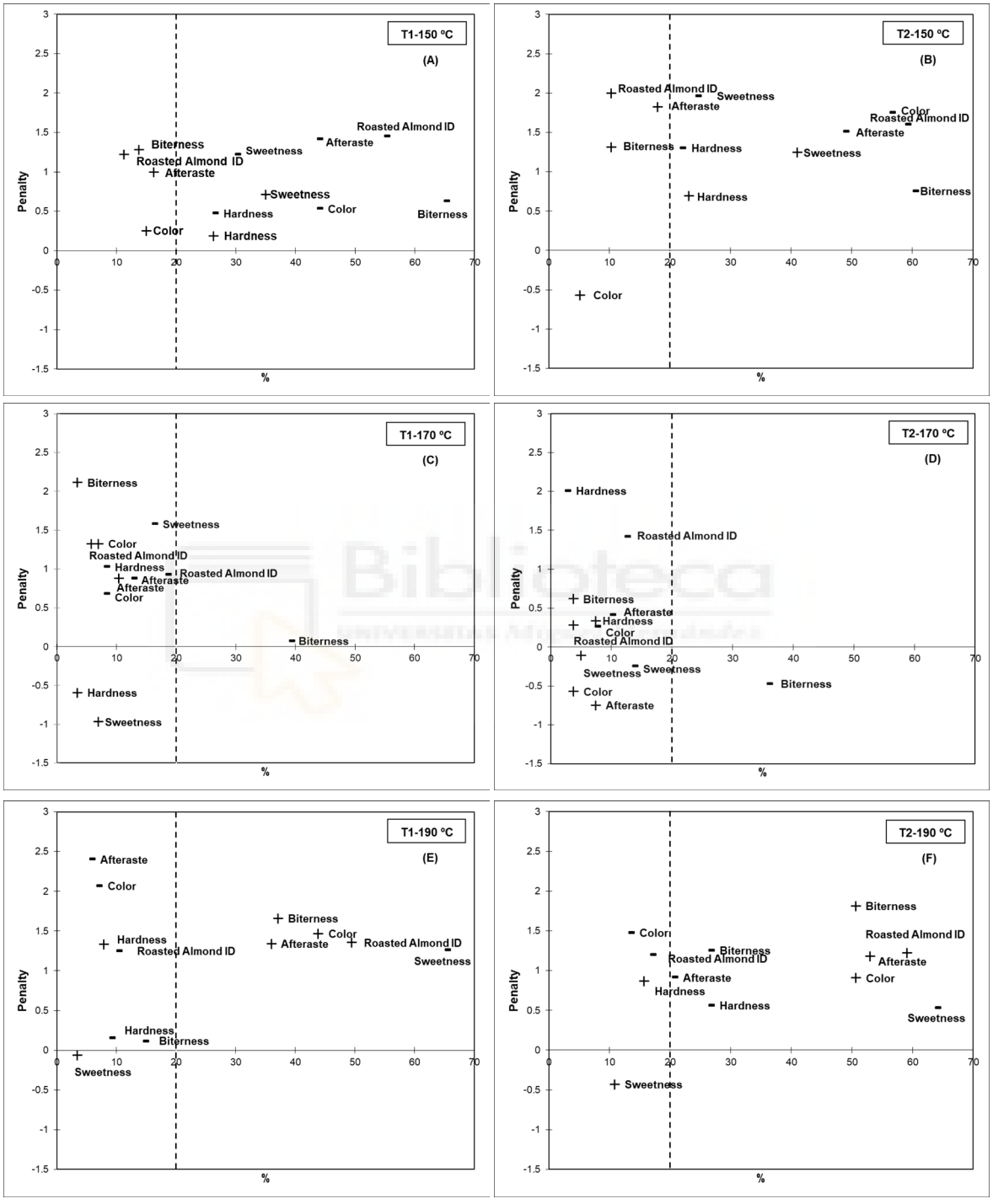


Table 1-Instrumental color and texture of roasted almonds as affected by roasting temperature and irrigation treatments.

	Outside color						Inside color						Texture				
	*L**	*a**	*b**	*C**	*h*	**ΔE**	*L**	*a**	*b**	*C**	*h*	**ΔE**	F (mm)	H (N)	WS (Ns)	AF (N)	NF
ANOVA[†]																	
Irrigation	**	***	***	***	***	NS	NS	***	***	***	*	***	NS	***	**	NS	NS
Roasting	**	***	***	***	***	***	*	***	***	***	*	***	*	***	**	***	NS
Irrigation × Roasting	**	***	***	***	***	***	*	***	***	***	**	***	*	***	**	***	NS
Tukey Multiple Range Test[‡]																	
Irrigation × Roasting																	
T1-150ºC	43.3a	18.1a	28.3ab	33.6a	57.4ab	4.94b	79.5a	1.53b	13.3c	13.4c	83.3a	6.59c	1.40a	59.6ab	42.1ab	29.8ab	16.8
T2-150ºC	46.0a	18.0a	31.7a	36.5a	60.4a	6.01b	76.9a	1.76b	11.7c	11.9c	80.9ab	9.21bc	1.41a	67.8a	47.1a	32.8a	18.5
T1-170ºC	41.9ab	18.1a	26.9ab	32.4a	56.0b	5.56b	79.0a	3.03b	21.5b	21.7b	82.5a	11.20b	1.38b	53.2abc	34.4ab	24.3abc	12.9
T2-170ºC	41.1ab	16.6a	25.1b	30.6a	54.9b	6.67b	80.1a	3.53b	22.9b	23.2b	81.5ab	11.81b	1.50a	46.1bc	29.2ab	19.1c	15.2
T1-190ºC	36.1b	13.3b	15.8c	20.6b	50.0c	15.0a	71.3b	6.75a	31.9a	32.6a	78.0ab	24.60a	1.18b	41.0c	24.1b	19.9c	14.6
T2-190ºC	41.0ab	12.6b	15.3c	19.8b	50.3c	12.9a	71.6b	6.95a	29.2a	30.0a	76.8b	22.44a	1.20b	43.4bc	25.6ab	20.8bc	15.9

*[†]NS = not significant at p<0.05; *, **, and *** significant at p< 0.05, 0.01, and 0.001, respectively. [‡] Values (mean of 10 repetitions per roasting treatment) followed by the same letter, within the same column and factor, were not significantly different (p<0.05), according to Tukey's least significant difference test. L*=0-black and L*=100-white, a*=reddish and -a*=greenish, b*=yellowish and -b/*=blueish, C=Chroma, h=Hue,* ΔE=the degree of color difference*; F=Fracturability, H=Hardness, WS=Work to Shear, AF=Average Force, NF=Number of Fractures.*

Table 2-Volatile compounds profile in roasted almonds of *Vairo* cultivar, retention index, and main odor and aroma descriptors (The Good Scent Company, 2018; National Center for Biotechnology Information, 2018).

Compound	Chemical Family	Code	RT (min)	Retention Index †		Odor Descriptor
				Experimental	Literature‡	
Pentanal	Aldehyde	V1	2.194	723	715	Bready, fruity, nutty berry, cocoa chocolate notes, coffee
Hexanal	Aldehyde	V2	3.901	804	804	Fresh green fatty aldehydic grassy leafy fruity sweaty
2-Methyl pyrazine	Pyrazine	V3	4.608	832	833	Nutty, brown, nut skin, roasted
Furfural	Furan	V4	4.679	842	845	Almond, caramel, sweet, woody, baked bread
2-Heptanone	Ketone	V5	5.800	895	892	Cheese, banana like, fruity odor
Nonane	Alkane	V6	5.907	900	900	Gasoline
Heptanal	Aldehyde	V7	6.083	906	906	Fresh, fatty green herbal, citrus
2,5-Dimethylpyrazine	Pyrazine	V8	6.595	923	922	Cocoa, roasted nuts, woody
2-Heptenal	Aldehyde	V9	7.716	961	959	Green, fatty
Benzaldehyde	Aldehyde	V10	7.965	970	967	Almond, fruity, powdery, nutty, cherry, sweet, bitter
Heptanol	Alcohol	V11	8.197	978	970	Musty, leafy green, fruity, apple, banana and nutty and fatty notes
2,2,4,6,6-Pentamethylheptane ¥	Alkane	V12	8.557	990	997	
Decane	Alkane	V13	8.860	1000	1000	
2-Ethyl-3-Methylpyrazine	Pyrazine	V14	9.094	1007	1001	Roasted, nutty, potato, corn, peanut, raw
Trimethylpyrazine	Pyrazine	V15	9.244	1011	1018	Cocoa, earthy, musty, nutty, roasted peanut hazelnut
2-Ethylhexanol	Alcohol	V16	10.032	1034	1030	Citrus, fresh, floral oily sweet
Benzeneacetaldehyde	Aldehyde	V17	10.649	1052	1048	Green, sweet, floral, hyacinth, rose, chocolate
1-Octanol	Alcohol	V18	11.597	1079	1074	Waxy, green, orange, rose, mushroom, sweet fatty, coconut
2,5-Dimethyl-3-Ethylpyrazine	Pyrazine	V19	11.770	1085	1079	Potato, cocoa, roasted, nutty
2,3-Dimethyl-5-Ethylpyrazine	Pyrazine	V20	12.018	1091	1090	Burnt, popcorn, roasted, cocoa
2,6-Dimethyl-3-Ethylpyrazine	Pyrazine	V21	12.115	1094	1094	Burnt, almond, roasted nuts, coffee, caramel, peanut
Undecane	Alkane	V22	12.336	1100	1100	Waxy, fruity, creamy, fatty, floral, pineapple
2,3-Diethyl-5-methylpyrazine	Pyrazine	V23	12.530	1104	1094	Musty, nutty, meaty, vegetable, roasted hazelnut, nut skin
Nonanal	Aldehyde	V24	12.653	1109	1107	Waxy, aldehydic, citrus, green lemon peel, orange peel
Furaneol	Furan	V25	14.311	1155	1159	Sweet cotton candy, burnt brown caramel, strawberry, sugar
2,6-Diethyl-3-Methylpyrazine	Pyrazine	V26	11.551	1161	1163	Green, nutty, meaty, vegetable
3-Methylundecane	Alkane	V27	14.885	1170	1169	Mild aliphatic hydrocarbon odor
1-Nonanol	Alcohol	V28	15.409	1184	1174	Fresh, clean, fatty, floral, rose, orange, dusty, wet, oily
Dodecane	Alkane	V29	15.969	1200	1200	
2-Decenal	Aldehyde	V30	18.417	1269	1266	Fatty, orange, rose, floral, green
Tridecane	Alkane	V31	19.552	1300	1300	
2,4-Decadienal	Aldehyde	V32	20.444	1331	1323	Fatty, oily, citrus, green, chicken skin-like
Tetradecane	Alkane	V33	22.493	1400	1400	Mild waxy
Hexadecane	Alkane	V34	26.187	1600	1600	
1-Tetradecanol	Alcohol	V35	26.867	1664	1671	Fruity, waxy, coconut

¥ tentatively identified (identification only based on spectral database); † RT = retention time; ‡ = NIST (National Institute of Standards and Technology) (NIST, 2018).

Table 3-Volatile compounds (mg kg^{-1}) found in roasted almonds as affected by water stress and roasting process. The quantification of these volatile compounds is based on the use of 2-acethylthiazole as internal standard.

Code	ANOVA†			Irrigation x Roasting					
	Irrigation	Roasting	Irrigation × Roasting	T1 150 ºC	T2 150ºC	T1 170 ºC	T2 170ºC	T1 190 ºC	T2 190º C
				mg kg^{-1}					
V1	***	***	***	0.04de	0.02e	0.15b	0.06cd	0.08c	0.25a
V2	NS	***	***	0.22b	0.10c	0.27b	0.15c	0.30a	0.35a
V3	***	***	***	nd	nd	0.02b	nd^{c}	0.40a	0.19b
V4	***	***	***	nd	0.02e	0.34d	0.62c	0.97b	1.47a
V5	***	***	***	0.01c	0.04a	0.02c	0.01c	0.03b	0.02b
V6	***	***	***	0.03b	0.04a	0.02c	0.01d	0.01d	0.04a
V7	***	***	***	0.01d	0.02bc	0.02bc	0.02cd	0.03b	0.06a
V8	***	***	***	0.04c	0.02c	0.87b	0.84b	2.65a	2.93a
V9	***	***	***	0.01c	0.01c	0.03b	0.03b	0.03b	0.07a
V10	***	***	***	0.13c	0.12c	0.16c	0.18c	0.24b	0.41a
V11	***	***	***	0.02c	0.02b	0.02b	0.02b	0.03b	0.03a
V12	*	*	*	2.70b	3.54a	3.00ab	3.22ab	2.74b	2.87ab
V13	NS	NS	NS	0.12	0.11	0.13	0.11	0.12	0.11
V14	***	***	***	0.01d	nd	0.05d	0.11c	0.28a	0.16b
V15	***	***	***	0.02d	0.02d	0.19cd	0.33c	1.03b	1.23a
V16	***	***	**	0.16ab	0.16ab	0.16ab	0.13bc	0.12c	0.19a
V17	***	***	***	0.04d	0.03d	0.24c	0.30ab	0.26bc	0.34a
V18	NS	***	***	0.02cd	0.01d	0.03bc	0.03b	0.03b	0.04a
V19	***	***	***	0.07d	0.05d	0.21c	0.25c	0.54b	0.64a
V20	***	***	***	nd	nd	0.01c	0.01c	0.04a	0.03b
V21	***	***	***	nd	nd	0.04c	0.04c	0.07b	0.09a
V22	**	**	**	0.13c	0.16bc	0.21a	0.16abc	0.17ab	0.16bc
V23	***	***	***	0.01d	0.01d	0.04c	0.06c	0.08b	0.11a
V24	***	***	***	0.11d	0.07d	0.20c	0.22c	0.29b	0.44a
V25	***	***	***	0.01c	0.01c	0.01c	0.02a	0.01bc	0.02ab
V26	***	***	***	0.03c	0.02c	0.01d	0.01d	0.05b	0.08a
V27	NS	***	***	0.03cd	0.02d	0.03bcd	0.04a	0.03abc	0.04ab
V28	***	***	***	0.05bc	0.04c	0.05bc	0.07a	0.06ab	0.08a
V29	***	***	**	0.13c	0.16bc	0.19ab	0.22a	0.16bc	0.16bc
V30	NS	***	***	nd	0.01c	0.01c	0.02b	0.03a	0.02b
V31	***	***	***	0.09c	0.14b	0.12bc	0.17a	0.10c	0.11c
V32	***	***	***	0.01d	0.02c	0.01cd	0.02b	0.02b	0.05a
V33	***	***	***	0.03c	0.03c	0.03c	0.06a	0.04bc	0.05b
V34	NS	NS	***	0.01bc	0.01b	0.01c	0.02a	0.01b	0.01b
V35	NS	NS	***	0.01cd	0.03a	0.01d	0.02c	0.03b	0.01cd
Σ	***	***	***	**4.31d**	**5.06cd**	**6.90b**	**7.55b**	**11.1a**	**12.8a**

*†NS = not significant at p<0.05; *, **, ***, significant at p<0.05, 0.01, and 0.001, respectively; ǂ values (mean of 3 replications) followed by the same letter, within the same row and factor, were not significantly different (p<0.05), according to Tukey's least significant difference test; ¥ = tentatively identified.*

Table 4-Descriptive sensory analysis of roasted almonds as affected by roasting temperature and deficit irrigation. The used scale ranged from 0 = no intensity to 10 = extremely strong intensity.

	Sweetness	Bitterness	Astringency	Overall nut	Almond-ID	Roasted	Brunt	Benzaldehyde	Woody	Hardness	Cohesiveness	Crispiness	Adhesiveness	Aftertaste
ANOVA Test[†]														
Irrigation	***	***	***	***	***	***	***	NS	*	**	NS	*	*	***
Roasting	***	***	***	***	***	***	***	NS	*	**	NS	*	*	***
Irrigation × Roasting	***	***	***	***	***	***	***	NS	*	**	NS	*	*	***
Tukey Multiple Range Test[‡]														
Irrigation x Roasting														
T1-150 ºC	4.90a	0.10c	0.40bc	7.00a	7.30a	3.20c	nd^{c}	0.10	2.95ab	5.20ab	1.75	6.80b	4.05a	7.00abc
T2-150 ºC	4.75a	0.15c	0.30c	6.25ab	6.55ab	2.45c	nd^{c}	0.10	2.20b	5.40a	2.15	7.30a	4.05a	6.15c
T1-170 ºC	3.50b	2.10b	1.10bc	5.70b	4.90c	5.90b	nd^{c}	0.15	2.45ab	5.35ab	1.85	7.80a	3.55ab	6.90bc
T2-170 ºC	4.95a	2.00b	0.90bc	6.40ab	5.95b	6.75ab	nd^{c}	0.20	2.90ab	5.45a	2.25	7.70a	3.80ab	6.75bc
T1-190 ºC	2.75b	3.40a	2.45a	4.40c	3.50d	7.85a	2.80^{a}	0.50	3.35a	4.35b	1.95	6.50b	2.75b	8.05a
T2-190 ºC	3.45b	2.85a	1.50ab	4.90c	3.60d	7.40a	2.10^{b}	0.25	2.70ab	4.15b	2.10	6.40b	3.15ab	7.40ab

*[†] NS = not significant at p< 0.05; *, **, and *** significant at p< 0.05, 0.01, and 0.001, respectively. [‡] Values (mean of 10 trained panelists) followed by the same letter, within the same column and factor, were not significantly different (p<0.05), according to Tukey's least significant difference test.*

8. RESULTS AND DISCUSSION

This section includes the main results and discussions of the published articles which are summarized in 4 parts grouped according each specific objective. The detailed results can be consulted in the publications included in the previous section.

Objective 1

To determine the effect of deficit irrigation strategies on agronomic, morphological, and functional parameters of almonds after one season of water stress.

The results of this objective are reflected in the first 4 publications which included the following parameters:

- 1st publication: applied water, min SWP, stress integral, production, kernel ratio, size, weight, instrumental color and texture, moisture content, water activity, ash, protein, fat, carbohydrates, minerals, and fatty acids. Title: *Almond fruit quality can be improved by means of deficit irrigation strategies.*
- 2nd publication: yield, organic acids and sugars, antioxidant activity and total phenolic content, and volatile compounds. Title: *Nutrition quality parameters of almonds as affected by deficit irrigation strategies.*
- 3rd publication: phytoprostanes and phytofurans. Title: *Phytoprostanes and phytofurans–oxidative stress and bioactive compounds–in almonds are affected by deficit irrigation in almond trees.*
- 4th publication: phenolic compounds profile, proanthocyanidin profile, total proanthocyanidins after phloroglucinolysis and polymerization degree of proanthocyanidins. Title: *How does water stress affect the low molecular weight phenolics of hydroSOStainable almonds?*

Applied water, yield, and physiological response to different irrigation strategies

Four irrigation treatments were applied under different DI strategies from which T1 was the control one (almond trees received optimum water requirements), T2 was moderate RDI, T3 was severe RDI and T4 was SDI in which the stress was gradually generated through the whole season. T1 was the treatment with the highest amount of applied water during the year (433 mm), with the highest levels of SWP (statistically similar to T4) and with the lowest values for the SI. On the other hand, the amount of water received by T2, T3 and T4 was similar among DI treatments

(148, 103, and 114 mm, respectively), but T2 and T3 registered the highest values of water stress in plant. This showed that the stress created in plant under RDI was higher than under SDI conditions, because in the SDI strategy the water stress increases progressively as the season advances and combines the uniform application of a reduced amount of water and the soil water reserve drop (Fereres & Soriano, 2007). In this way, the stress is developed slowly and allows the plant to adapt to water deficit, which is opposite to RDI in which the application of stress is concentrated in certain phenological stage (kernel filling). It is worth mentioning that after one year of water stress, the yield was not affected by none of the DI strategies although, the hydroSOStainable almonds (T2, T3 and T4) received a lower amount of irrigation water along the season and registered the highest values of SI (92, 95, and 75 MPa × day, respectively) with regard to the control (T4 = 54 MPa × day).

Morphological and physicochemical parameters

Weight, size, texture, kernel ratio, moisture content, and water activity were not changed in almonds growth under DI conditions, except for *a** color coordinate, which was higher in almonds from moderate RDI, indicating a more reddish skin color. Regarding the chemical composition, the fat content of almonds cultivated under moderate RDI and severe RDI was 7.17% and 1.42% higher than the control, while SDI samples had similar content to that of the control. Previous studies proved an increase in the lipid content of almonds and pistachios under moderate RDI (Carbonell-Barrachina et al., 2015; Egea et al., 2009). Other authors reported constant fat content in almonds under moderate DI, the same content when an excess of irrigation water was applied, and a decrease when water stress reached severe levels (Zhu, Taylor, Sommer, Wilkinson, & Wirthensohn, 2015). Thus, the most severe level of water stress created in this study was intense enough to reduce the lipid content.

Minerals

The contents of Ca and Mn were reduced in the most stressed almonds T3, while Mg, Fe, Cu and Zn were maintained as similar levels as those of the control samples (T1). Potassium was the element positively affected by RDI when applied at moderate levels. An increase in K content was observed for T2 almonds and similar values to those of the control (T1) were observed for T3 and T4 samples, even though T1 received more than double amount of K through irrigation. This K depletion in fully irrigated almonds was associated with the leaching of minerals with the excess of water and minerals absorption. The soluble mineral absorption depends on the water flow in the soil path to the plant roots; this explains that deficit irrigation applied at

correct levels could reduce minerals losses through leaching but also the saturation of minerals and biocides in the root zone (Alikhani-Koupaei, Fatahi, Zamani, & Salimi, 2018).

Organic acids and sugars

The main organic acids found in this study were citric and malic acids, from which the latter was increased 2.4 and 4.2 g kg^{-1} in T3 and T4, respectively. Other authors also reported an increase in fruit quality (tomatoes and grapes) under water stress conditions and was associated to the increase in organic acids content (Nahar & Gretzmacher, 2002). However organic acids may play a limited role in almonds quality.

Sugars on the other hand are key factors in the basic sweet taste (essential for consumer acceptance) and aroma profile of processed almonds, being precursor of aroma compounds formation during thermal processing, especially toasting (Erten & Cadwallader, 2017). Sucrose was the main soluble sugar found in almonds and the present conditions of the applied DI did not affect their contents. However, the glucose content was significantly decreased with severe RDI and SDI treatments and maintained under moderate RDI conditions.

Antioxidant activity and total phenolic content

The results showed that water stress in almonds after one season in the present DI conditions did not significantly affected the antioxidant activity (AA) of raw whole kernels. However, a significant and negative correlation was found between SWP and TPC and the nonlinear (quadratic) equation between these parameters showed that at these stress levels conditions no decrease in TPC was observed, which means that up to -2.2 SWP (107 MPa × day SI) values the plant was still able to produce phenolic compounds (**Figure 19**). Previous studies on olives *cv*. Arbequina growth under RDI conditions, also presented a quadratic relationship between water stress and TPC, reporting that the TPC increased up to -4.0 MPa SWP but started to decrease from that point (Sánchez-Rodríguez, Kranjac, et al., 2019). Which affirmed that water stress in tree generates an increase in phenolic compounds precursors (free phenylalanine) and their synthesis could be more sensitive in moderate water stress conditions (Horner, 1990).

The present results agree with other studies in which this same positive correlation was also observed in tomatoes and maize (Sánchez-Rodríguez et al., 2010). Water stress produces damages in plant due to the reactive oxygen species (ROS) formation and water-plant relationship alteration. The plant resistance degree

to water is determined by the degree in which this can avoid or soften the physiological processes (Sánchez-Rodríguez et al., 2010).

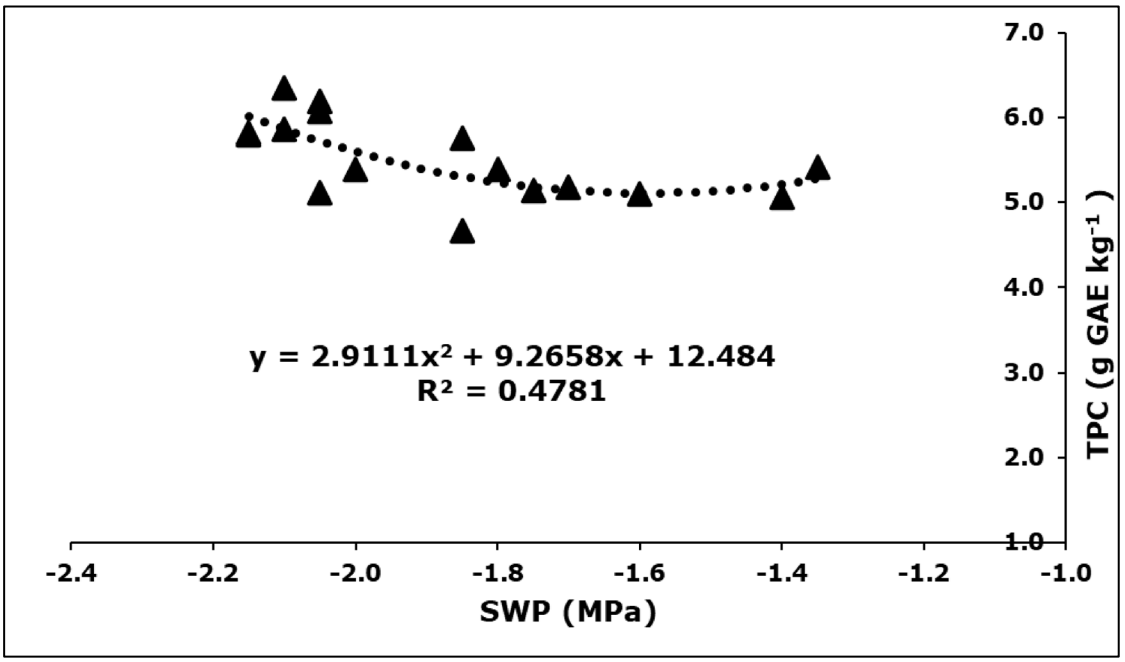


Figure 19. Quadratic correlation between total phenolic content (TPC) and stem water potential (SWP). Four repetitions per treatment were used for the correlation

Finally, it is worth mentioning that skin removal of the almonds considerably reduced the AA and TPC which are important parameters from a functional point of view. For instance, a reduction of 5.6, 1.3, 9.9-fold for the values of the $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP methods, respectively, were observed after skin removal for the AA and a decrease of 10.4-fold for the TPC. In the almond processing, skin has a low economic value representing only ~4.00% of the total almond weight; however, more than 70% of the total phenols are present in the skin (Monagas et al., 2007). Which means that, **removing the skin, for different manufacturing purposes, eliminates almost all the phenolic compounds and reduces their antioxidant effect on the fruit preservation and human health**.

Polyphenolic profile

The phenolic composition of almonds (skin, whole and de-skinned kernels) was measured with a liquid chromatograph (LC-PDA-MS-QTof). The almonds polyphenols found in this study belonged both to flavonoids and non-flavonoids groups. The former was the major group and included compounds belonging to the families: (i) *flavanols* [(-)-epicatechin and procyanidins], (ii) *flavonols* (3-*O*-glucosides, -galactosides, and -rutinosides of kaempferol and isorhamnetin) being kaempferol-3-

O-galactoside the major compound and representing 53% of the total phenols, and *flavanones* (naringenin-7-*O*-glucoside). On the other hand, the non-flavonoids found within this study included vanillic and *p*-hydroxybenzoic acids.

The total phenolics ranged from an average content of 37.3 mg 100 g^{-1} in skin to 1.13 mg 100 g^{-1} in deskinned kernel, with the whole almonds representing an intermediate position of 4.77 mg 100 g^{-1}. Similar results have been previously reported in Spanish almonds, but 1.7-fold lower values for the American varieties (Milbury et al., 2006; Monagas et al., 2007). Only 6 out of the total 15 phenolic compounds were kept in the deskinned almonds: vanillic acid, procyanidin B-type dimer, (-)-epicatechin, procyanidin B type dimer, naringenin-7-*O*-glucoside, and kaempferol-3-*O*-rutinoside, the rest being lost during the skin removal. In general, DI positively affected both the total and individual phenolic compounds (*p*-hydroxy-benzoic acid, vanillic acid, procyanidin B-type dimer, procyanidin B-type dimer, procyanidin B type trimer, procyanidin B-type tetramer, procyanidin B-type trimer, naringenin-7-*O*-glucoside, and kaempferol-3-*O*-rutinoside) in almond skin. Nevertheless, the total phenolics of whole and deskinned kernels was not significantly affected by water stress.

Proanthocyanidins profile and content

Proanthocyanidins (PAs) are the most abundant polyphenol family in almonds and the main flavonoids in our diet, responsible for the astringency and bitterness in foods by their reaction with salivary proteins (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). Nevertheless, their polymeric nature and high structural complexity have led to very few studies exploring them (Bolling, 2017; Deprez, Mila, Huneau, Tome, & Scalbert, 2001). In the present study, 10 oligomeric PAs were identified and quantified using the HPLC–PDA–ESI/MSn and authentic standards. Out of the 10 PAs found in almonds, the 3 most abundant were trimers (mean values of all treatments: trimer 1=10.50 mg 100 g^{-1}, trimer 2=10.00, mg 100 g^{-1} dw and trimer 5= 6.27 mg 100 g^{-1} dw) together with 1 tetramer (7=6.16 mg 100 g^{-1} dw). Most of the identified and quantified PAs have been previously reported in almond blanching water (Pérez-Jiménez & Torres, 2012). The total PAs concentration ranged between 39.5 mg 100 g^{-1} dw (T2) and 49.8 mg 100 g^{-1} dw in (T4), but no statistically significant differences were observed among the irrigation treatments for the total PAs.

Regarding water deficit, 2 specific PAs (one trimer and one tetramer) were significantly different among the treatments. For instance, the trimer was increased under moderate RDI (T2=6.38 mg 100 g^{-1} dw) and SDI T4=8.84 mg 100 g^{-1} dw and decreased when the stress in plant was higher (T3=5.06 mg 100 g^{-1} dw), displaying similar values to the fully irrigated samples T1=4.80 mg 100 g^{-1} dw. The

concentration of the PA tetramer was also raised as a consequence of water stress, and a significant positive correlation (R=0.73; p=0.001) was observed between the content of this tetramer (Rt=34.7 min) and the stress integral. This relationship agreed with authors working on analysis of flavan-3-ols in tea plants growth under water stress conditions (Hernández, Alegre, & Munné-Bosch, 2006); they observed an increase of 10-fold in (-)-epicatechin in tea plants exposed during 19 days to water stress. This compound helps to build PAs blocks; thus, it may finally polymerize and accumulate PAs in vacuoles. It is possible that the oxidation of (-)-epicatechin to its quinone, observed in water stress samples, is a step of an oxidative process leading to the biosynthesis of PAs (Hernández et al., 2006). Proanthocyanidins have a significant protective role linked to biotic stress resistance such as defence against different external factors (Hernández et al., 2006).

Proanthocyanidins content after phloroglucinolysis

In addition to the methods previously described to analyse polyphenols and PAs, *phloroglucinolysis* was also done to corroborate previous results, because this is the only quantification method which allowed to measure the total PAs concentration (extractable and non-extractable PAs) and can better reflect the real concentration of total PAs (Kennedy & Jones, 2001). The *phloroglucinolysis* was carried out in all three parts of the edible almond: skin, deskinned and whole kernel. The results obtained on total PAs in skin, deskinned, and whole kernel showed that the highest PAs content is found in skin (7796 mg 100 g^{-1}, on average), followed by whole kernels (774 mg 100 g^{-1}, on average), while in the deskinned almonds, the PAs were below the limit of quantification. These results are important from a functional and medical point of view and show the importance of the almond skin, which is often removed to make almonds less bitter and astringent. It has been demonstrated the antioxidant defence and hepatoprotection of almond skin procyanidins by regulating the protein expression of phase II detoxifying and antioxidant enzymes in HepG2 cells and acetaminophen treated hepatotoxic mice (Truong et al., 2014).

Besides, the *phloroglucinolysis* showed that both moderate RDI (T2) and severe RDI (T3) increased the PAs content in whole kernels. Lower values of PAs content were reported in pistachios nuts (352-427 mg 100 g^{-1}), although the same tendency was observed regarding the deficit irrigation; the PAs content was increased with the water stress in plant (Noguera-Artiaga et al., 2018).

Degree of polymerization of proanthocyanidins

Phloroglucinolysis method enabled to also determine the average degree of polymerization (DP). The PAs DP is strongly correlated to the health benefits that

these compounds provide (antioxidant, anticarcinogenic, cardio-protective, antimicrobial, and neuroprotective activities) because it influences their bioavailability *in vivo* (Manach et al., 2004; Monagas et al., 2007). Proanthocyanidins are different from other polyphenols particularly due to their polymeric nature and high molecular weight, that reduces the absorption through gastrointestinal tract (Manach et al., 2004). Only dimers and trimers crossed the intestinal epithelium, while oligomers higher than trimers are not absorbed in their natural form (Deprez et al., 2001). Thus, PAs with high DP are retained in the small intestine, which help to protect mucosa against aggression by toxic compounds from food products (Deprez et al., 2001). The present results showed PAs reaching up to tetramers and a DP measured by *phloroglucinolysis* up to 5.1 in whole kernel and up to 4.7 in skin. However, in the literature values on DP above 10 were reported in almonds (Gu et al., 2004) and this difference might be related to the equipment sensibility and the extraction method. Regarding irrigation treatments, a higher DP was found in almond skin under moderate RDI, while no differences were observed for the whole kernels.

Fatty acids

Eighteen fatty acids (FAs) were identified and quantified in all 4 treatments with oleic acid being the most abundant compound, followed by linoleic, palmitic, stearic, *cis*-vaccenic and palmitoleic acids. The FAs composition predominantly contained MUFAs and PUFAs and was mainly not affected by current DI treatments, except for myristic (C14:0), palmitic (C16:0), margaric (C17:0), *cis*-heptadecenoic (C17:1), *cis*-vaccenic (C18:1n7), and arachidic (C20:0) acids, which were higher in moderate RDI. This finding agreed with different authors who concluded that using a moderate deficit irrigation in pistachios or olives increased the contents of FAs and the opposite trend was observed when severe water stress was imposed (Cano-Lamadrid et al., 2015b; Cano-Lamadrid et al., 2017; Carbonell-Barrachina et al., 2015; Zhu et al., 2015). An increase in MUFAs and PUFAs provides a higher functionality to *hydroSOS* almonds due to many studies in which authors stated that products containing these compounds help in cardiovascular and coronary heart diseases as well as obesity, diabetes and cancers (Bitok & Sabaté, 2018). For instance, *cis*-vaccenic, which is an omega-7 fatty acid, was associated with a lower risk of heart failure from ischemic origin (Djoussé et al., 2014). Moreover, DI treatments did not influence in the atherogenic and thrombogenic index (these markers inform whether a diet could promote coronary heart disease), maintaining in this way low values and consequently health properties of almonds.

Phytoprostanes (PhytoPs) and Phytofurans (PhytoFs)

Eight PhytoPs belonging to two series (9 and 16) of the D_{1t}, F_{1t}, L_1, and B_1 classes were found in this study. In addition, two PhytoFs were also observed in hydroSOStainable almonds: *ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF and *ent*-16-(*RS*)-13-*epi*-ST- Δ^{14}-9-PhytoF. It is worth mentioning that both PhytoPs and PhytoFs were below the limit of quantification in T1 almonds (fully irrigated treatment). PhytoPs content increased as a consequence of water deficit with the following values: below the limit of quantification (LOQ), 4551, 7377 and 8151 ng 100 g^{-1} in T1, T2, T3 and T4 almonds, respectively. Lower values of PhytoPs were reported in raw "Manzanilla" green table olive fruits (581-999 ng 100 g^{-1}) (Collado-González et al., 2015), olive oil, sunflower oil (1500-3900 ng 100 g^{-1}) (Collado-González et al., 2015) and algae from several species (6-1381 ng 100 g^{-1}) (Barbosa et al., 2015). While similar values were reported in refined sunflower oil (Collado-González et al., 2015) and table olives processed using the "Spanish style" (Collado-González et al., 2015), which indicate that the oxidative stress suffered by almonds under DI was equivalent to that caused by the industrial processing. Regarding the diverse PhytoP classes, the predominant one was the 9-series of F_{1t}-PhytoP in all treatments except in full irrigated conditions (T1) which means that this could be the first compound involved in plant defense against different types of stress (Durand et al., 2009). Moreover, 16-series of F_{1t}-PhytoP class can also be considered a good marker as previous studies also reported to appear only in almonds growth in rain-fed conditions but not in kernels from irrigated almond trees (Carrasco-Del Amor et al., 2015). Finally, 16-B_1-PhytoPs might be also considered another important marker of water stress in almonds as previously was reported to be the dominant class in refined oils due to the very high temperatures of refining process that can induce a higher ALA oxidation (Collado-González et al., 2015).

Two PhytoFs were found in almonds cultivated under DI conditions, with *ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF being the most abundant compound followed by *ent*-16-(*RS*)-13-*epi*-ST- Δ^{14}-9-PhytoF. PhytoFs were also detected below the limit of quantification in control almonds (T1). The *ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF was significantly higher in T2 (56 ng 100 g^{-1}) than in both T3 (33 ng 100 g^{-1}) and T4 (39 ng 100 g^{-1}) irrigation treatments, suggesting that this compound was mostly produced under moderate water deficiency but its concentration decreased in T3 and T4. Consequently, the total PhytoFs registered the highest content in T2 almonds (56 ng 100 g^{-1}) and the lowest in T3 irrigation conditions displaying the highest and the lowest (33 ng 100 g^{-1}) contents, respectively.

The evaluation of the almond under DI strategies provided valuable information on the relative abundances of the separate PhytoPs and PhytoFs, as well as on their interest as dietary sources of these compounds.

Volatile compounds

Twenty-six compounds were identified and quantified in the volatile profile of *cv*. Vairo almonds. From which 10 were alcohols, 9 alkanes, 3 aldehydes, 1 terpene, 1 ketone and 1 organic acid. Alcohols, the main chemical family (0.82 mg kg^{-1}) found within this study, is released by enzymatic reactions in raw almond and contribute to the characteristic sweet aroma and to the consumer acceptance (Kwak et al., 2015). Hexanol, compound that increases with almond ripening and is linked to the herbal odor (fruity, alcoholic, sweet, green notes) and green flavor (fruity, apple skin, oily) (The Good Scent Company, 2018; García-Esparza et al., 2018) was 1.29 fold higher in almonds growth under moderate RDI conditions (T2) than in control almonds. High levels of hexanol content was also found by other authors in Nonpareil almonds extracted with a similar method (Kwak et al., 2015). Studies in grapes concluded that irrigation during post-veraison (the change of grapes color) at 75% of the crop ET compared to rain fed, decreased the alcohols and increased the aldehydes, compounds that generate herbaceous non-desirable aromas in wines (García-Esparza et al., 2018). Tridecane was also increased under RDI conditions and this alkane is formed by decarboxylation of myristic fatty acid (C14:0) (Bergaentzle, Sanquer, Hasselmann, & Marchioni, 1994), which was also observed to increase under DI conditions. Limonene associated with fresh, citrus, and sweet note, was the only terpene found in this study being also raised by the moderate RDI (T2). These results were similar to those previously reported by Carbonell-Barrachina et al. (2015), who stated that a moderate RDI lead to higher amounts of limonene in pistachios. Consequently, the total content of volatile compounds was significantly higher in the moderate RDI almonds (T2).

A reduced number and content of aldehydes was found within this study which validate the freshness of the studied almonds. Aldehydes levels (e.g. hexanal) usually increase with roasting process and storage time (Lee, Xiao, Zhang, Ebeler, & Mitchell, 2014). Hexanal is a compound generated by the lipid oxidation thus is an indicator of oxidation/rancidity (low degree of freshness) in nuts and nut oils (Beltrán, Ramos, Grané, Martín, & Garrigós, 2011). Thus, the freshness (low level of rancidity) of all 4 almond samples was confirmed by the low content of hexanal and nonanal found (Beltrán et al., 2011; Fullana, Carbonell-Barrachina, & Sidhu, 2004). The Pearson's correlation showed a significant relationship between linoleic acid and hexanal ($R=0.83$; $p<0.05$), and between oleic acid and nonanal ($R=0.68$; $p<0.05$), and none of them were affected by deficit irrigation.

Conclusions of the 1st objective

In summary, water scarcity is affecting many agricultural systems and the implementation of sustainable irrigation strategies is urgent, particularly in semiarid regions. In this sense, the 1st objective of the current PhD thesis evaluated the agronomic, morphological, physicochemical, nutritional, and functional quality of almonds cultivated during one season under different irrigation strategies designed to fight water scarcity. As a general conclusion, it can be highlighted that the morphological parameters and almond production were not affected either by RDI or SDI. However, almonds from moderated RDI (T2) were characterized by a redder color, and higher contents of fat, K, glucose (potentially linked with almond sweetness) and total phenolic content, together with greater contents of individual phenolic compounds (p-hydroxy-benzoic acid, vanillic acid, procyanidin B-type dimer, procyanidin B-type tetramer, procyanidin B-type trimer, and naringenin-7-O-glucoside), individual PAs (one trimer B-type and one tetramer B-type), degree of polymerization, antioxidant activity, unsaturated fatty acid (cis-heptadecenoic and cis-vaccenic) and total volatile content (potentially linked with almond odor/aroma/flavor). Moreover, the TPC was positively correlated to SI, increasing with the water stress in plant. Additionally, it was revealed, for the first time, the profile and content of PhytoFs in almonds and it is important to highlight that PhytoPs and PhytoFs were below the limit of quantification in samples from fully irrigated almond trees. This leads us to conclude that DI strategies can be considered an important approach to reduce the water consumption by enhancing compounds with a potential beneficial effect on human health, such as PhytoPs and PhytoFs, because there are evidences about their effect on the regulation of immune function and anti-inflammatory and apoptosis-inducing activities. However, further research during several growing cycles is needed to check other important quality markers of hydroSOStainable almonds.

Objective 2

To determine the descriptive sensory profile of hydroSOStainable almonds and the international consumers' acceptance, preference, and willingness to pay.

The results of this objective are reflected in the 5th publication, which includes the following parameters:

- 5th publication: descriptive sensory analysis, affective sensory analysis with consumers from Spain and Romania (sample acceptance and preference) and consumers' willingness to pay. Title: S*ensory profile and acceptability of hydroSOStainable almonds.*

Descriptive sensory analysis

The descriptive sensory analysis was carried out to establish the sensory profile of the hydroSOStainable almonds and to evaluate whether the irrigation treatments led to significant differences. Significant differences were observed for 5 out of the 17 attributes used to describe the sensory quality of almonds: color, size, roughness, sweetness, and hardness.

Panelists found that almonds growth under moderate RDI (T2) conditions had a more intense red-brown **color** also supported by the instrumental color data, which showed significantly higher values for the *a** coordinate responsible for reddish notes. Other authors also reported an increase in total color for apricots and peaches under RDI conditions (Pérez-Sarmiento et al., 2016; Sotiropoulos, Kalfountzos, Aleksiou, Kotsopoulos, & Koutinas, 2010).

Although the instrumental size was not significantly different among treatments, the panel observed lower **size** for the severe RDI (T3) and SDI (T4) kernels, while those from moderate RDI were similar to the control.

The **roughness** parameter showed higher values for T2 and T4 kernels, even though they were within the optimal values, due to the controlled water stress. For instance, it was reported that reducing the water irrigation in another phenological stage than kernel filling, water stress will reach the fruit turgor leading to very rough kernels (Doll, 2014).

Sweetness was significantly higher in kernels obtained under RDI conditions (T2 and T3), and similar findings were also reported in pomegranate *cv*. Mollar de Elche produced under DI strategies (Cano-Lamadrid et al., 2018). Sweetness is a significant attribute in the sensory quality of almonds, and an increase in this

parameter intensity might be positive for consumer satisfaction (Verdú, Serrano-Megías, Vázquez-Araujo, Pérez-López, & Carbonell-Barrachina, 2007). The highest intensity of the sweetness perceived by the panelists in RDI almonds was also corroborated by the instrumental analysis of sugars, with sucrose content being also higher in the same samples (although non significantly differences were observed).

The panel scored a significantly higher **hardness** intensity for the almonds cultivated under DI conditions, even though instrumental texture did not support these findings. Other authors working with pistachios and olives cultivated under DI strategies reported higher values for both sensory and instrumental hardness for DI samples (Cano-Lamadrid et al., 2015a; Carbonell-Barrachina et al., 2015).

Affective sensory analysis

The affective studies were carried out both in Spain (S) and Romania (R) with a total of 200 consumers. Both nationalities agreed that the samples were significantly similar in terms of **overall liking** and attribute **specific satisfaction degree**. In general, Romanians tended to score higher than Spanish consumers, which meant that they liked most these almonds. This fact might be related to the fact that Romania is not an almond producer country and the high price of this product make it less consumed by most of the consumers. When the consumers were forced to choose between the 4 samples through **preference test**, both Romanian and Spanish consumers chose almonds from moderate RDI (T2) as the best sample due to their almond flavor, sweetness, and crispiness. Thus, the previous mentioned hypothesis that sweetness could be an important marker in consumer choice has been demonstrated. The most important concept in a product "label" for 63% of the Spanish consumers (S) was "product of Spain", followed by "healthy" (52%) and "natural" (48%). While Romanian consumers (R) were more interested in "natural" (70%), "healthy" (67%) and "ecological" (31%) concepts. For both nationalities, the concepts "natural" and "healthy" seemed to play a key role in their buying decisions. Besides, the buying drivers for consumers were: sweetness (S=29%; R=64%), flavor (S=77%; R=65%), texture (S=44%; R=16%), and price (S=44%; R=60%).

Penalty analysis, a very popular method in the food industry sector, correlates consumers overall liking and the attribute intensity scores of the JAR questions to determine which sensory attributes might be improved. All attributes with a negative impact on the sample liking, for at least 20% of the consumers and producing a drop of at least 1 point for liking, are the ones which might need to be improved. In the present study, only bitterness was susceptible to be improved and both Spanish and Romanian consumers agreed that this was needed for T1 and T4 almonds.

The assessment of **consumers' willingness to pay** for hydroSOStainable almonds compared to conventional almonds showed that most of the consumers from both countries were willing to pay a higher price for the hydroSOStainable almonds. The results were (being S "Spaniards" and R "Romanians"): (i) S=23% and R=31% were willing to pay less or the same price, (ii) S=60% and R=16% wanted to pay 0.50 € more, (iii) S=13% and R=24% wanted to pay 1.00 € more, and finally (iv) S=4% and R=29%, respectively wanted to pay more than 1.00 €. These finding agreed with other authors, who reported that Spanish consumers were also willing to pay an extra amount of money for hydroSOS pistachios (Noguera-Artiaga et al., 2016).

Conclusions of the 2nd objective

The present study was developed to determine the sensory properties of hydroSOStainable almonds and the overall liking, acceptance, and willingness to pay of international consumers. The results showed that the trained panel was able to establish slight but significant differences for several sensory descriptors, with T2 almonds showing an intense red color, and higher intensity of both sweetness and hardness attributes as compared to control samples. On the other hand, both Spanish and Romanian consumers were not able to differentiate among treatments until they were forced to choose the preferred sample. The preference test showed that T2 was the most liked sample by both nationalities due to the highest intensity of sweetness, almond flavor, and crispiness. Besides, penalty analysis also revealed that there were no attributes needed to be improved in almonds obtained from RDI treatments. All these results helped us to understand that consumers are now more aware than ever on the importance of the optimization of key natural resources, such as water. This awareness may explain the consumer willingness to pay a higher price for hydroSOStainable almonds, which means higher incomes and benefits for farmers. In addition, it was demonstrated that controlling the stress in almond trees with DI strategies can increase the water productivity and the farmers profit producing environmentally friendly products without significant changes in the sensory profile and well accepted by the consumers.

Finally considering the almond Spanish production of ~ 190000 t, the price received by the farmers for conventional shelled almonds ~ 4.85 € kg^{-1} and the extra value of ~2 € kg^{-1} of hydroSOStainable almonds paid by consumers, possible economic increase of ~ 40% for hydroSOStainable almonds might be obtained with respect to conventional ones. These gains are essential for farmers to encourage them to adopt sustainable irrigation strategies contributing to an environmentally friendly agriculture.

Objective 3

To correlate water stress response with quality parameters after 3 years of experiments (2017, 2018, 2019) and to identify those parameters that behave in the same way throughout the trials. These results are essential to establish the future hydroSOStainable markers.

The results of this objective are reflected in the 6th publication, which included the following parameters:

- 6th publication: stem water potential, stress integral, kernel yield, kernel ratio, size, weight, instrumental color and texture, moisture content, water activity, minerals, organic acids and sugars, antioxidant activity, total phenolic content, fatty acids, and descriptive sensory analysis. Title: *Long-term correlation between water stress and quality markers in hydroSOStainable almonds.*

After one season of water stress in almond trees, it was observed that many of the almonds quality and functional parameters/characteristics increased and other were maintained as the control. To establish which of these parameters can be considered hydroSOStainable almonds "markers" for the future certification protocols, most of the analyses were carried out during 3 consecutive seasons.

Agronomical parameters

These data have been granted by the research group from Universidad de Sevilla, led by Dr. Alfonso Moriana and are only used to present the agronomical results in this thesis.

Regarding the applied water (**Figure 20 A**) (i) in 2017, a lower amount of irrigation water was received by the DI treatments compared to the control, but no differences among the 3 DI treatments (T2, T3 and T4) were observed, (ii) on the other hand in 2018, T2 was similar to the control, while T3 and T4 presented lower but similar values between them; (iii) finally in 2019 the lowest values of applied water were also registered by T3 and T4, followed very far by T2 and then by T1.

Figure 20 B represents the results of kernel yield as affected by water deficit. As previously mentioned, deficit irrigation strategies did not affect the kernel yield in 2017 season, even though the applied water was lower and consequently the SI was higher in DI treatments. However, in the next season (2018) a reduction of 2.4-fold, for these treatments (T2, T3, T4) with regard to the control was observed (with no significant differences among them, as the SI was also similar among them). This

phenomenon was expected, due to the post-harvest sensitivity to water stress (García Tejero, Moriana, Rodríguez Pleguezuelo, Durán Zuazo, & Egea, 2018).

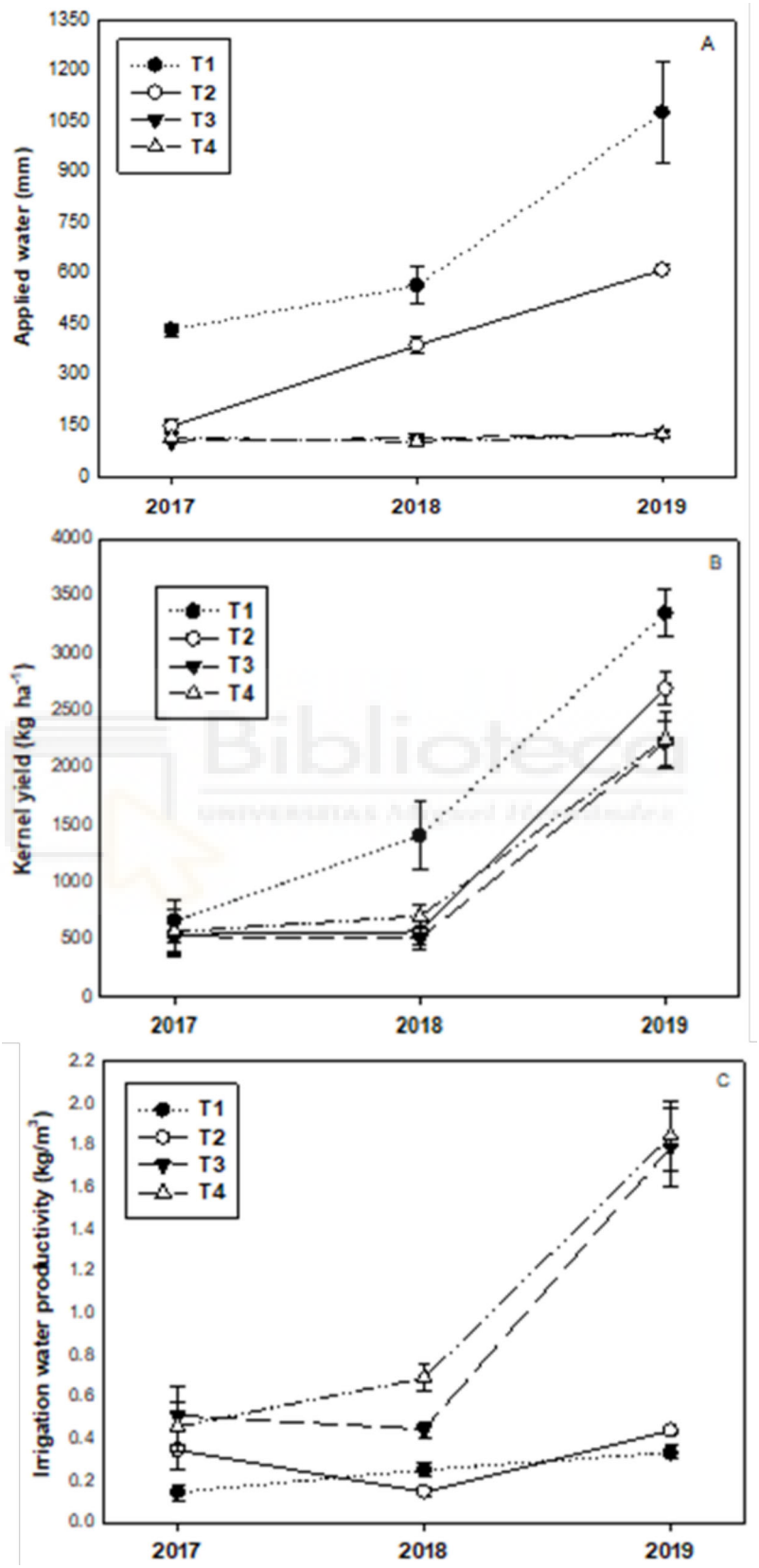


Figure 20. Applied water (A), kernel yield (B), and irrigation water productivity (C) during 3 years under study

A reduction in kernel yield in deficit irrigation treatments was also observed in 2019 season (1.2-fold in T2 and 1.5-fold in T3 and T4), although this time was lower than in 2018 and T2 was significantly similar to the control. Is important to highlight that in 2018, although T3 and T4 received less amount of water than T2, the kernel yield was similar among them, and that in 2019 although T2 received lower amount of water than the control (T1) the kernel yield was significantly similar between them. It is remarkable how the applied water and kernel yield was 3.8-fold higher in 2019 than in the other 2 seasons. This variation is directly related to the increase in canopy volumes and plant density yearly, that leads to a higher transpiration level with more irrigation requirements and consequently significantly higher yields (Gutiérrez-Gordillo et al., 2020).

Irrigation water productivity (IWP) was calculated and presented in **Figure 20 C**. As seen, in 2017 season the values were not significantly different among treatments, while in 2018 and 2019 the greatest values were recorded by T3 and T4 samples. If we compare among growing seasons, the highest values of IWP were obtained in 2019 while no significant differences were found for the other seasons. These results were expected because 2019 was the year in which both the applied water volume and yield were higher.

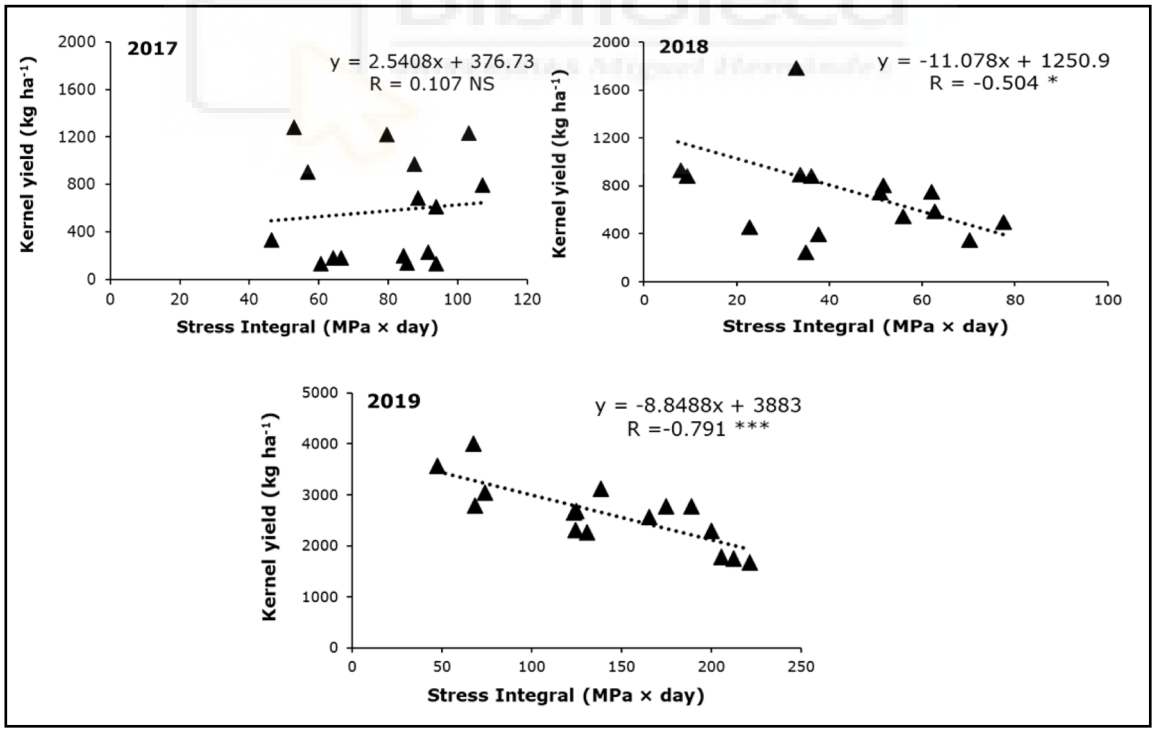


Figure 21. The lineal regression of stress integral on kernel yield

Figure 21, shows the relationship among SI and kernel yield, which emphasized that no relation (R=0.11; p>0.05) was shown after 1st season, a soft

negative and significant relation (R=-0.50; p<0.05*) was found after 2nd season, and a significant negative correlation (R=-0.79; p<0.001***) was shown after the 3rd season of water deficit.

Afterall, water deficit produced a stress in plant which did not influenced the kernel yield in the first year, and reduced it in the second year, but this reduction was lowered in the third year of experiment. This led us to deduct that as well as water deficit, the season is also an important factor in kernel yield and that in time plant might develop the ability to cope with the water stress, as in the last year of experiment the reduction in yield was lower than in the second season. Moreover, different correlation between water stress and yield, explains, why long-term experiments about the effect of DI on agronomical parameters are required.

Morphological and physical parameters

The results on morphological parameters showed that after long term water stress, the weight and size were reduced, which after one season of water stress were not significant. The SI and color parameters were positively correlated, showing that a higher stress level is linked with higher values of *L*, a** and *b** coordinates. Consequently, the hydroSOStainable almonds are lighter but with reddish and yellowish notes (more intense brown color). Similar results were also reported in other almond cultivars such as Marta, Guara, Lauranne after one season of RDI conditions (Lipan et al., 2020). The almond color skin is controlled by the polyphenol profile which is unique for each cultivar (Bolling, 2017); thus, the rise in color coordinates due to water deficit could be related to a potential increase in polyphenols which was previously showed that increased with the water stress. It was reported that almond flavonoids are decisive pigment in color plants (Li, Lu, Tang, & Shi, 2015) and that brown almond skin pigment is mainly given by proanthocyanidins, which were positively correlated with SI after one season of experiment.

Regarding the physical properties of almonds, Pearson's Correlation Coefficients demonstrated that after long-term experiment, the levels of water stress (SI) were negatively correlated with the water activity, and positively correlated with the almond dry weight. The reduction in the moisture content and water activity with water stress contribute to increase the almond shelf life by maintaining the biological reactions at minimum level.

Minerals

Plant tissue minerals are taken by plants from soil solution and irrigation water, which means that environmental factors, agronomical practices (location, soil composition, water source, irrigation, and fertilizer) and cultivar account for the final mineral content in the almond kernel. In general, drought conditions lowers the upwards transport of minerals from root to shoots, but there are plants with a better water use efficiency and drought tolerance (Ahanger, Morad-Talab, Abd-Allah, Ahmad, & Hajiboland, 2016).

The long-term experiment showed that water stress was positively correlated to K, Fe, and Zn contents and negatively to Ca and Mg. **Potassium** is the most important element, after N and P helping to maintain the plant water status and its tolerance to stress (Ahanger et al., 2016). From a nutritional point of view K is the major mineral in almond kernels with 717 mg 100 g^{-1}, meaning that almonds can be considered a food high/rich in K because its content is above the minimum threshold (600 mg 100 g^{-1}) established in the Regulation (EU) No 1169/2011 of the European Parliament and of the Council. **Iron** is the element involved in chlorophyll pigment and is essential for the efficient function of the antioxidant enzymes with a key role in plant protection against oxidative stress produced by water deficit (Ahanger et al., 2016). From a functional point of view, this element is involved in hemoglobin formation and oxygen transport (Abbaspour et al., 2014). Thus, if the total iron intake from foods or its absorption is below the recommended thresholds, an iron deficiency anemia occurs (Shubham et al., 2020). Deficiency of iron has been considered the major form of malnutrition and this is the main responsible for anemia occurrence in industrialized countries (Abbaspour et al., 2014). Considering the present results, eating 43 g of hydroSOS almonds (the amount of nuts per day recommended by FDA), 1.12 mg of Fe can be ingested, which is more than 50% from the iron requirements according to World Health Organization for adults (2 mg day^{-1}) (WHO, 2015). This amount of Fe intake is 9% higher than in the control samples (0.94 mg Fe in 43 g of conventional almonds). **Zinc** also helps to improve the water use efficiency and the crop yield (Ahanger et al., 2016). Studies in wheat growth in field under water stress conditions also reported a higher Zn content in grains (Karim et al., 2012). Zinc is also one of the most essential trace elements in human health with biological functions such as catalytic, structural, and regulatory (Kashian & Fathivand, 2015). It was reported that more than 100 enzymes depend on zinc catalytic action as well as in DNA and RNA synthesis. For this reason, daily intake of these element through food is essential being 8.2 and 9.9 mg day^{-1} the Zn average requirements in women and men, respectively (EFSA, 2015). Thus, consumption of 43 g of hydroSOStainable almonds will help to intake 1.82 mg Zn compared to 1.69 mg Zn

in full irrigated almonds. Finally, it was observed a reduction on **Mg** and **Ca** content in fruit as a consequence of water stress which was supported by other authors who concluded that water stress reduced the uptake of calcium in plant (Nahar & Gretzmacher, 2002).

Organic acids and sugars content

The long-term experiment showed that citric acid, sucrose, fructose, and total sugars were positively correlated with water stress, although this phenomenon was not observed after one season. A rise in **citric acid** and the mentioned sugars were also reported in other almond cultivars Marta, Guara and Lauranne grown under RDI conditions (Lipan et al., 2020) and other crops such as thyme (Ashrafi et al., 2018). The increase in citric acid may result from the larger inhibition of the citrate degrading system related to citrate synthesis, as previously reported in CAM plant (*Aptenia cordifolia*), even though the ability for citric acid oxidation and the citrate synthetase activity decreased during drought (Peckmann & Herppich, 1998). While the sugars boost in stress circumstances might occur due to: (i) the osmotic adjustment, initiated to adapt the plant to dry and/or saline stress by accumulation of solutes rich in hydroxyl (-OH) groups (sugars, proline, etc.) in the cytoplasm (Sanders & Arndt, 2012), and (ii) to the induction of the growth inhibitor abscisic acid (ABA), which activates the sugar accumulation as an adaptation to stress (Kashem et al., 2000). Water stress raises the ABA biosynthesis, inducing the accumulation of osmotically active compounds, which defends cells from damage (Ahanger et al., 2016). Under stressed circumstances, ABA lowers plant growth and increases desiccation tolerance by inducing the accumulation of stress associated transcripts such as low molecular weight soluble sugars, including sucrose (Jahan et al., 2019).

In summary, **K, Fe, Zn, sucrose, fructose, and total sugars** *can be considered as good quality markers for hydroSOStainable almonds*.

Antioxidant activity (AA) and total phenolic compounds (TPC)

$ABTS^{\bullet+}$ and FRAP were positively correlated with water stress in plant, while the TPC was not significantly correlated with SI as was previously showed after first year of water stress. Not finding a correlation between SI and TPC after 3 seasons of water stress may occur due to the low level of stress reached, which possibly was not sufficient to affect the TPC accumulation. An increase in AA under water stress conditions was previously reported in other crops (pistachios *cv*. Kerman) grown under DI irrigation conditions, where the stress was imposed at stage II shell hardening (Noguera-Artiaga, Sánchez-Bravo, Pérez-López, et al., 2020) and olives *cv*. Manzanilla, where the stress was imposed just before harvest without re-

hydration (Sánchez-Rodríguez, Cano-Lamadrid, et al., 2019). During the water stress, the turgor pressure is decreased, the ion toxicity is increased, and the photosynthesis is inhibited (Ali & Baek, 2020); thus, the antioxidant defense system is activated by plants to cope with ROS. Phytohormones are also responsible for the initiation of many defense mechanisms, including the increase in antioxidants to enhance plant tolerance to water stress (Ali & Baek, 2020).

Antioxidant activity *can be considered an important marker in hydroSOStainable almonds detection*.

Fatty acids

Myristic, palmitic, palmitoleic, margaric, *cis*-heptadecenoic, stearic, *cis*-vaccenic, linoleic, SFAs, PUFAs, and PUFAs:MUFAs ratio were positively correlated with SI, while the oleic and consequently MUFAs were negatively correlated with water stress. Similar results were also reported in almond *cv*. Marta, Guara, Lauranne, Ferragnes, Texas, but also in other crops such as olives *cv*. Mazanilla, pistachio *cv*. Kerman, and sunflower *cv*. Suncross (Ali, Ashraf, & Anwar, 2009; Lipan et al., 2020; Nanos, Kazantzis, Kefalas, Petrakis, & Stavroulakis, 2002; Noguera-Artiaga, Sánchez-Bravo, Hernández, et al., 2020; Noguera-Artiaga, Sánchez-Bravo, Pérez-López, et al., 2020; Sanchez-Bel, Egea, Martinez-Madrid, Flores, & Romojaro, 2008). As shown, the negative correlation between oleic and linoleic fatty acid under water stress was cultivar dependent (Ali et al., 2009) and was ascribed to the enzyme Δ12 desaturase, which is responsible for the conversion of oleic acid in linoleic under water stress conditions (Baldini, Giovanardi, Tahmasebi Enferadi, & Vannozzi, 2002). Linoleic acid is an essential fatty acid with a key role in death of cardiac cells but that cannot be synthetized by human body and might be consumed through died. Thus, consuming 50 g of almonds under RDI conditions brings approximately 33% of the daily intake of linoleic acid recommended by the European Food Safety Authority (Lipan et al., 2020).

The **fatty acids** were significantly affected by water stress and *are good markers of the hydroSOStainable almonds*.

Descriptive sensory analysis

The results of the 3 years study showed strong negative correlations between SI and size, bitterness, astringency, benzaldehyde, and woody flavors. Previous studies reported that water stress might enhance the sweetness, nutty, almond ID, and crispiness in almonds *cv*. Lauranne and pistachio *cv*. Kerman (Carbonell-Barrachina et al., 2015; Lipan et al., 2020). Thus, the decrease in bitterness and astringency with water stress and the increase in sugars as previously mentioned

might lead to sweeter almonds which explains the purchase choice of international consumers based on sweetness, almond ID, and crispiness. These results and those of consumer willingness to pay a higher price for hydroSOStainable almonds should encourage the almond farming sector to consider implementing deficit irrigation strategy as a good alternative to increase water use efficiency and simultaneously increase the functional and sensory quality of almonds.

Conclusions of the 3rd objective

*Generally, water stress affected the agronomical and quality characteristics of hydroSOStainable almonds displaying positive relationship with dry weight, color coordinates (L*a*b*), minerals (K, Fe, and Zn), organic acids (citric acid), sugars (sucrose, fructose and total sugars), antioxidant activity and fatty acids (linoleic, PUFAs, SFAs, PUFAs/MUFAs, among others). In contrast, the water stress in almond trees showed a negative correlation with water activity, weight (almond, kernel and shell), size, minerals (Ca and Mg), fatty acids (oleic acids, oleic/linoleic ratio, MUFA and PUFA/SFA) and sensory attributes (size, bitterness, astringency, benzaldehyde and woody). The current long-term research contributed to establish the quality parameters that are really affected by water stress and to clarify the key markers to differentiate between hydroSOStainable and conventional almonds. In summary,* ***K, Fe, Zn, sucrose, fructose, total sugars, antioxidant activity and the fatty acids*** *can be considered important markers for hydroSOStainable almonds detection.*

Objective 4

To determine the best roasting conditions for hydroSOStainable almonds in terms of physicochemical parameters, descriptive sensory profile, and consumers acceptance and to check the effect of deficit irrigation strategy on roasted almond quality.

The results of this objective are reflected in the 7th publication which included the following parameters:

- 7th publication: instrumental color and texture, volatile compounds, descriptive sensory profile, and consumer acceptance (sample acceptance and preference). Title: *Optimization of roasting conditions in hydroSOStainable almonds using volatile and descriptive sensory profiles and consumer acceptance*

Two treatments (T1=conventional vs. T2=hydroSOStainable) were roasted at 150, 170 and 190 ºC. The samples were analyzed in terms of instrumental color and texture, volatile compounds, descriptive and affective sensory analysis. One hundred consumers from Spain were provided with 6 samples of roasted almonds belonging to the conventional (T1) and hydroSOStainable (T2) treatments. Consumers were required to give their informed consent prior tasting and only those who agreed to sign the document were then invited to taste the samples and answer the questionnaire.

Instrumental color and texture

The results showed that almond lightness (L^*) was significantly reduced for both outside and inside almond color with increasing the roasting temperature. The decrease in lightness means a darker color of the kernel, usually produced by the browning reactions. For instance, the typical brown color occurs with the Maillard reactions, caramelization of sugars and dextrins to furfural and hydroxymethyl furfural and carbonization of sugar, fat, and protein (Skovgaard, 2004). On the contrary, a^*, b^* and C^* values were higher for the greater roasting temperatures, leading to brownish kernels due to the mix of red and yellow color notes. Regarding the irrigation treatment, almonds of T1 became darker before those of T2 at 190 ºC roasting temperature, while the other two roasting temperatures showed similar results between the irrigation treatments. However, if the total color difference (ΔE) is considered, conventional and hydroSOStainable almonds of *cv.* Vairo exhibit statistically similar behavior color change in each roasting temperature.

The instrumental texture data showed that as temperature increased the hardness decreased reaching to almonds more fracturable and crispy. The irrigation treatments also influenced on the roasted kernel texture being the hydroSOStainable almonds harder than the control ones at 150 and 190 ºC, respectively, but similar at 170 ºC. Crispness is the most important textural characteristic for the consumer acceptability an occurs with the product dehydration, browning reactions, lipid oxidation and structural changes (Varela et al., 2006). Besides, water stress effect on cell size, cell turgor, solute transport, and chemical composition can also have an impact on the raw almond texture which will also change the kernel behavior within the roasting process (Ripoll et al., 2014).

Volatile composition

The aroma compounds are more or less volatile and can exert their effect even in extremely low concentrations. Their odor threshold (the minimum concentration in which the substance can be perceived by smell) is usually around mg L^{-1} or µg L^{-1}, and even in some substances below these concentrations (Baltes, 2000). In most cases, the aroma of a food originates from the interaction of many compounds, sometimes more than 200 different flavorings, which as individual components transmit totally different aromatic notes (Baltes, 2000). Only some flavorings alone can represent the flavor of a food, such as vanillin with vanilla flavor.

Raw almonds are usually characterized by hexanal, nonanal and benzaldehyde, while the roasted almonds by aldehydes, pyrazines, pyrroles, and furans as the main volatile compounds (Yang et al., 2013; Valdés et al., 2015). In the present research a total of 35 volatile compounds were identified in roasted almonds: aldehydes (n=9), pyrazines (9), alkanes (9), alcohols (5), furans (2) and ketone (1) being the more representative pentamethyl heptane, dimethyl pyrazine, furfural, trimethyl pyrazine, 2,5-dimethyl-3-ethylpyrazine and hexanal. The aroma compounds increased with the roasting temperature 4.77, 7.32 and 11.9 mg kg^{-1} at 150, 170 and 190 ºC, respectively. Meaning that almonds roasted at 150 ºC showed similar volatile content to those reported in raw almond for *Vairo* (4.39 mg kg^{-1}) (Lipan, Moriana, et al., 2019), and *Bute* and *Padre* cultivars (4.36 mg kg^{-1}).

Regarding the irrigation treatments, aldehydes, pyrazines, furans, ketones, alkanes, alcohols, and consequently the total volatile content of hydroSOStainable almonds (T2) were significantly higher than in control (T1) samples. T2 samples were characterized by a higher content of volatiles with odor descriptors such as almond, nutty, bready, and chocolate notes. An increase in volatile compounds in hydroSOStainable almonds may occur due to the alteration in the chemical composition under water stress conditions (Ju et al., 2018).

3.4 Descriptive sensory analysis

Descriptive sensory analysis was performed to establish the sensory profile of the roasted almonds. All the assessed attributes, except benzaldehyde and cohesiveness, were significantly affected by the roasting temperatures and irrigation treatment. The bitterness, astringency, roasted, burnt, woody, and aftertaste intensities raised with the roasting temperature. The opposite was observed for sweetness overall nut and raw almond-ID flavor attributes. Hardness and crispiness were similar in intensities in samples roasted at 150 ºC and 170 ºC and lower in those roasted at 190 ºC.

Deficit irrigation led to sweeter, harder, and crispier almonds with higher nutty and almond-ID flavors. These results corroborate those of volatiles in which compounds with nutty (pentanal and 2,6-dimethyl-3-ethylpyrazine) and almond (benzaldehyde, furfural, etc.) flavors were higher in hydroSOStainable almonds. However, the roasted flavor was higher in fully irrigated samples, according to the trained panel and volatile analysis (2-ethyl-3-methylpyrazine).

3.5 Principal component analysis

Principal component analysis (PCA) showed that irrigation treatments roasted at the same temperature were grouped together, but the samples roasted at different temperature were separated. Almonds roasted at 150 ºC were characterized by ketones, alcohols, alkanes (fresh, fruity, herbal, sweet and green notes) and sensory attributes such as adhesiveness, sweetness, and almond ID; characteristics of raw almonds. Almonds roasted at 170 ºC were described as hard, with nutty flavor and aldehydes aromatics, and with light inside color; aldehydes was the chemical family closer to the 170 ºC almonds, with descriptors such as nutty, chocolate, bready and almond notes (The Good Scent Company, 2018; Xiao et al., 2014). Finally, almonds roasted at 190 ºC were surrounded by burnt, woody, benzaldehyde, and roasted notes, long aftertaste maybe due to their astringency and bitterness, and volatiles such as pyrazines and furans. It was reported that some pyrazines (2,5-dimethyl-3-ethylpyrazine, 2,3-dimethyl-5-ethylpyrazine) and furans (furaneol) might provide flavor notes such as burnt, roasted, coffee and burnt brown (The Good Scent Company, 2018). Thus, roasting conditions must be adjusted to control the amount of these compounds and avoid non desirable flavors such as burnt (Hojjati et al., 2016). For instance, 190 ºC for 10 min can be considered an excessive treatment.

3.6 Consumers acceptability, drivers of liking and attributes improvement

One hundred consumers were used to assess the samples to determine the preference, drivers of liking, and attributes improvement. Regarding sample preference, most consumers chose T1 (27%) and T2 (24%) samples roasted at 170 ºC as the best almonds due to their roasted almond flavor (62 %), aftertaste (43%), texture (38%) and sweetness (29%).

Considering the drivers of liking assessment (PLS), consumers overall liking was positively linked to specific volatiles (alkanes, alcohols, aldehydes and furans characterized by fruity, creamy, fresh, fatty, bready, nutty, caramel-like and cocoa chocolate notes) and sensory attributes (sweetness, roasted, almond ID, overall nut flavors together with a hard and crispy texture) corresponding to a mild roasting. On the contrary, a negative correlation was observed between consumers overall liking and pyrazines, bitterness, astringency and woody and burnt flavor notes. These volatiles are considered key compounds of roasted almonds formed during heating which contribute to desirable nutty and toasty odors in roasted nuts, if they are in a proper concentration (Alasalvar et al., 2003; Vázquez-Araújo et al., 2009). However, high concentrations of pyrazines is not associated to high quality roasted almonds, if they smell and taste burnt (Vázquez-Araújo et al., 2009).

Finally, to check which of the sensory attributes might need improvements a Penalty analysis was conducted using JAR scores and consumer liking. The attributes susceptible of improvement were those with negative impact on the sample liking for at least 20% of consumers and caused a drop of at least 1 unit for liking. The results revealed the need to improve samples roasted at 150 and 190 ºC. The samples roasted at 150 ºC were penalized due to low roasted color, almond flavor, aftertaste, sweetness, and hardness while those roasted at 190 ºC due to excess of bitterness, aftertaste, color, and roasted notes. Finally, no improvements were needed for the almonds roasted at 170 ºC.

Conclusions of the 4th objective

The present study revealed information on volatile composition, sensory profile and consumer acceptance of almonds grown under deficit irrigation conditions roasted at 150, 170 and 190 ºC. Generally, 170 ºC was the optimum roasting temperature for almonds cv. Vairo regarding aromatic, descriptive and affective parameters. These samples were characterized by a proper total content of volatile compounds with 2.5-dimethyl pyrazine and furfural being the main predominant compounds contributing to the almond, baked bread, roasted nuts notes; and also by possessing intense almond and nutty flavors with a hard and crispy texture. Penalty analysis showed that almonds roasted at 150 ºC were penalized because of their low roasted aroma and hardness, while almonds roasted at 190 ºC were perceived as over-roasted, with too intense color and burn notes. Almonds roasted at 170 ºC did not have any attribute which penalized liking and, therefore, did not need to be improved. Regarding the irrigation treatments, hydroSOStainable almonds (T2) roasted at 170 ºC were characterized by a higher volatile compounds content compared to the control sample, being also sweeter, harder, with intense almond and roasted notes.

9. CONCLUSIONS / CONCLUSIONES

CONCLUSIONS

According to the studied irrigation strategies, it can be concluded that moderate stress during the kernel filling phase, substantially improves the morphological, physicochemical, and functional properties, maintaining an adequate yield similar to that obtained under optimal water endowments. Thus, this strategy allows to increase the antioxidant activity and polyphenols, as well as the volatile compounds, polyunsaturated fatty acids, phytoprostanes and phytofurans.

The moderate deficit irrigation treatment leads to almonds with intense red brown color, almond flavor, sweetness, and crispy texture; all these sensory attributes will help in having higher acceptance by international consumers.

Parameters such as, color coordinates (*L*, a*, b**), minerals (K, Fe and Zn), organic acids (citric acid), sugars (sucrose, fructose), antioxidant activity ($ABTS^{\bullet+}$, FRAP), fatty acids (oleic, linoleic) and sensory attributes (size, bitterness, benzaldehyde and woody) can be considered important markers for hydroSOStainable almonds detection.

Considering the consumer willingness to pay a higher price for a product obtained using environmentally friendly strategies, the government and industry actions might emphasize on providing the right information to consumers regarding hydroSOStainable products, while agricultural sector might produce these foods helping to reduce the water scarcity worldwide.

A temperature of 170 ºC for 10 min were the optimum conditions for the roasting of the hydroSOStainable almonds. In addition, the effects of deficit irrigation on the almond quality were not limited only to the raw kernel, but also after the roasting process; these almonds were sweeter, harder, crispier, had a greater intensity of roasted almond notes, and a higher content of volatile compounds when compared to the full irrigated almonds.

Thus, using moderate regulated deficit irrigation strategy the farming sector can save up to 45% of irrigation water, obtaining a product with differentiated quality and environmentally friendly.

Overall, these results reinforce the statement that, water savings strategies in almond crop help in obtaining a high-quality product with a higher consumer

acceptance and willingness to pay. These circumstances will increase the final added value of hydroSOStainable almonds allowing farmers to recover their investment in implementing deficit irrigation strategies, the low economic losses caused by yield reductions, but offering a product with a higher competitiveness and marketability.

CONCLUSIONES

De acuerdo con las estrategias de riego estudiadas, se puede concluir que un estrés moderado durante el periodo de llenado de grano mejora sustancialmente las propiedades morfológicas, fisicoquímicas y funcionales, manteniendo además un nivel de producción adecuado y similar a los obtenidos bajo dotaciones hídricas óptimas. Así, esta estrategia permite incrementar la actividad antioxidante y los polifenoles, además de los compuestos volátiles, ácidos grasos polinsaturados, fitoprostanos y fitofuranos.

El tratamiento de riego deficitario moderado da lugar a almendras con una intensidad de color, sabor a almendra y dulzor más alta, así como a una textura crujiente; por tanto, todos estos atributos sensoriales ayudarán a tener una mayor aceptación por parte de los consumidores internacionales.

Parámetros tales como coordenadas de color *(L*a*b*)*, minerales (K, Fe, y Zn), ácidos orgánicos (ácido cítrico), azúcares (sacarosa y fructosa), actividad antioxidante ($ABTS^{\bullet+}$, FRAP), ácidos grasos (oleico y linoleico) y atributos sensoriales (tamaño, amargor, benzaldehído y amaderado) pueden considerarse marcadores importantes para la detección de almendras hidroSOStenibles.

Considerando una mayor predisposición por parte de los consumidores internacionales a pagar un precio más alto por un producto que ha sido obtenido siguiendo estrategias de sostenibilidad y conservación de los recursos hídricos, las acciones del gobierno y de la industria podrían brindar información adecuada con respecto a los productos hidroSOStenibles, promocionando la producción de alimentos que contribuyan a combatir la escasez de agua en el mundo.

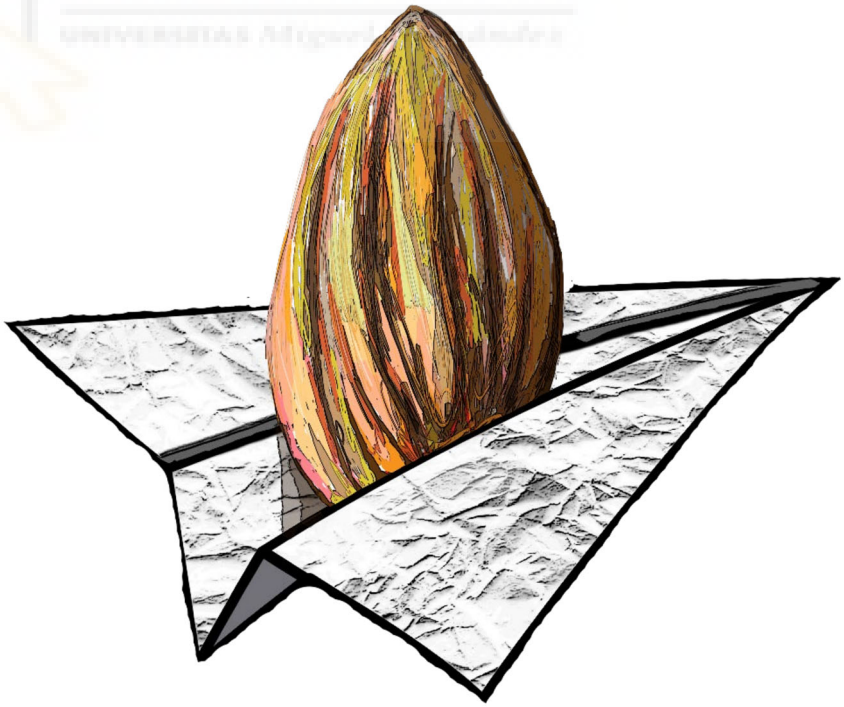
Las condiciones óptimas recomendadas para el proceso de tostado de las almendras hidroSOStenibles son una temperatura de 170 ºC durante 10 min. Además, los efectos del riego deficitario en la calidad de la almendra no se ciñeron tan sólo a la almendra cruda, sino también se mantuvieron tras el proceso de tostado. Las

almendras tostadas resultaron más dulces, con una mayor intensidad aromática a almendra tostada, más crujientes, y con un mayor contenido de compuestos volátiles en comparación con las almendras procedentes de riego óptimo.

Así, utilizando esta estrategia de riego deficitario controlado en un nivel moderado el sector agrícola puede ahorrar un 45% del agua de riego, obteniendo un producto de calidad diferenciada y amistoso con el medio ambiente.

En conjunto, estos resultados refuerzan la afirmación de que las estrategias de ahorro de agua en el cultivo de la almendra contribuyen positivamente en la obtención de un producto final de mayor calidad y con una mayor aceptación por parte de los consumidores. Es decir, ayudan a generar un valor añadido final que permitiría recuperar las pérdidas económicas provocadas por la implementación de estrategias de riego deficitario controlado y por las reducciones de rendimiento, ya que dan lugar a un producto con mayor competitividad y comerciabilidad.

10. FURTHER RESEARCH



Once this research has finalized and based on the obtained results, further work must be done to make the hydroSOStainable concept reality.

1. First, individual phenolic compounds, proanthocyanidins, phytoprostanes and phytofurans must be analyzed for more than one seasons to confirm that they are surely good markers for hydroSOStainable identification.
2. More cultivars might be evaluated and mainly those most cultivated such as Marcona and Desmayo Largueta in Spain or Nonpareil in USA.
3. The accumulation of the bioactive compounds during the kernel filling phase must be also an interesting further research; because analyzing how fruit composition varies based on stress levels could allow establishing thresholds to improve/optimize the content of these compounds depending on the final use of the almond.
4. *In vitro* and *in vivo* studies must be carried out to check the almonds phytochemical bioavailability.
5. Another research should focus on the protein profile assay and the study *in vivo* of the tryptophan levels in human blood after diets with hydroSOStainable *versus* conventional almonds.
6. Finally, a methodology of certification of the hydroSOStainable labelled almonds and almond based products must be developed to protect them and to offer farmers an added value which will encourage them to bet on deficit irrigation strategies and consequently to reach an efficient use of water in agriculture.

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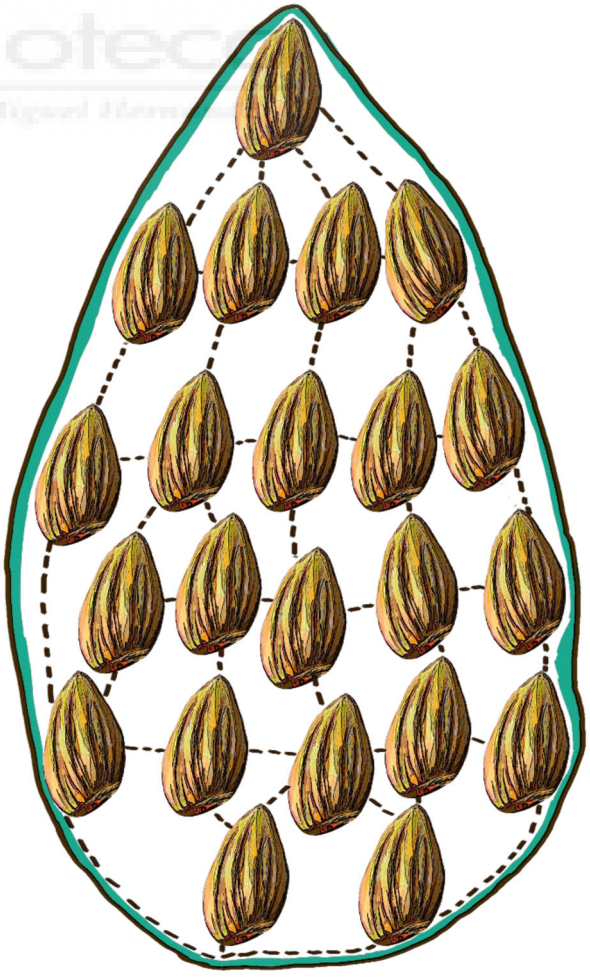

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