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

HydroSOStainable Table Olives and Olive Oil: Quality, Functionality and Acceptance in the European Market

Doctoral Thesis

Lucía Sánchez Rodríguez

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HydroSOStainable Table Olives and Olive Oil: Quality, Functionality and Acceptance in the European Market

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Thesis for the Degree of Doctor from the Miguel Hernández University of Elche

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HydroSOStainable Table Olives and Olive Oil: Quality, Functionality and Acceptance in the European Market

Thesis presented by Lucía Sánchez Rodríguez to qualify for Doctor degree from Miguel Hernández University of Elche

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Andaluces de Jaén,
aceituneros altivos,
decidme en el alma, ¿quién,
quién levantó los olivos?

No los levantó la nada,
ni el dinero, ni el señor,
sino la tierra callada,
el trabajo y el sudor.

Unidos al agua pura
y a los planetas unidos,
los tres dieron la hermosura
de los troncos retorcidos.

Levántate, olivo cano,
dijeron al pie del viento.
Y el olivo alzó una mano
poderosa de cimiento.

Andaluces de Jaén,
aceituneros altivos,
decidme en el alma ¿quién
quién amamantó los olivos?

Vuestra sangre, vuestra vida,
no la del explotador
que se enriqueció en la herida
generosa del sudor.

No la del terrateniente
que os sepultó en la pobreza,
que os pisoteó la frente,
que os redujo la cabeza.

Árboles que vuestro afán
consagró al centro del día
eran principio de un pan
que sólo el otro comía.

¡Cuántos siglos de aceituna,
los pies y las manos presos,
sol a sol y luna a luna,
pesan sobre vuestros huesos!

Andaluces de Jaén,
aceituneros altivos,
pregunta mi alma: ¿de quién,
de quién son estos olivos?

Jaén, levántate brava
sobre tus piedras lunares,
no vayas a ser esclava
con todos tus olivares.

Dentro de la claridad
del aceite y sus aromas,
indican tu libertad
la libertad de tus lomas.

Miguel Hernández

PUBLICATION QUALITY INDEX

This doctoral thesis, as a **compendium of publications**, is presented to be qualified for the Doctoral degree from Miguel Hernández University of Elche.

For that purpose, the selected research articles and their quality indexes, in accordance with the 2018 and 2019 edition of Journal Citation Reports® (JCR®), are presented:

PUBLICATION 1

Sánchez-Rodríguez, L., Corell, M., Hernández, F., Sendra, E., Moriana, A., Carbonell-Barrachina, Á.A. 2019. Effect of Spanish-style processing on the quality attributes of *HydroSOStainable* green olives. *Journal of Science of Food and Agriculture*. 99(4):1804-1811. DOI: 10.1002/jsfa.9373.

Published: 31 October 2018

Publisher: Wiley, 111 River St, Hoboken 07030-5774, NJ USA

ISSN: 0022-5142

Research Domain: Agriculture, Multidisciplinary; Chemistry. Applied; Food Science & Technology

JCR® category	Quartile in Category	Rank	Impact Factor	5-year impact factor
Agriculture, Multidisciplinary	Q1	9/57	2.422	2.733

PUBLICATION 2

Sánchez-Rodríguez, L., Lipan, L., Andreu, L., Martín-Palomo, M.J., Carbonell-Barrachina, Á.A., Hernández, F., Sendra, E. 2019. Effect of regulated deficit irrigation on the quality of raw and table olives. *Agricultural Water Management*. 221:415-421. DOI: 10.1016/j.agwat.2019.05.014

Published: 20 July 2019

Publisher: Elsevier Science BV, PO Box 211, 1000 AE Amsterdam, Netherlands

ISSN: 0378-3774

Research Domain: Agriculture; Water Resources

JCR® category	Quartile in Category	Rank	Impact Factor	5-year impact factor
Water Resources	Q1	10/94	4.021	4.469

PUBLICATION 3

Sánchez-Rodríguez, L., Cano-Lamadrid, M., Carbonell-Barrachina, Á.A., Wojdyło, A., Sendra, E., Hernández, F. 2019. Polyphenol Profile in "Manzanilla" Table Olives as Affected by Water Deficit during Specific Phenological Stages and Spanish-Style Processing. *Journal of Agricultural and Food Chemistry*. 67: 661-670. DOI: 10.1021/acs.jafc.8b06392.

Published: 20 December 2018

Publisher: ACS Publications 1155 Sixteenth St NW Washington DC 20036

ISSN: 0021-8561

Research Domain: Chemistry, applied; Foods Science & Technology; Agriculture, Multidisciplinary.

JCR® category	Quartile in Category	Rank	Impact Factor	5-year impact factor
Food Science & Technology	Q1	28/135	3.571	3.991

PUBLICATION 4

Sánchez-Rodríguez, L., Cano-Lamadrid, M., Carbonell-Barrachina, Á.A., Sendra, E., Hernández, F. 2019. Volatile Composition, Sensory Profile and Consumer Acceptability of HydroSOStainable Table Olives. *Foods*. 8: 470. DOI: 10.3390/foods8100470

Published: 10 October 2019

Publisher: MDPI St. Alban-Angale, 66 Basel, Switzerland 4052

ISSN: 2304-8158

Research Domain: Food Science & Technology.

JCR® category	Quartile in Category	Rank	Impact Factor	5-year impact factor
Food Science & Technology	Q1	27/139	4.092	n/a

PUBLICATION 5

Sánchez-Rodríguez, L., Cano-Lamadrid, M., Carbonell-Barrachina, Á.A., Sendra, E., Hernández, F. 2020. Impact of gastrointestinal in vitro digestion and deficit irrigation on antioxidant 1 activity and phenolic content bioaccessibility of "Manzanilla" table olives. *Journal of Food Quality*. Volume 2020, Article ID 6348194, 6 pages DOI: 10.1155/2020/6348194

Published: 10 October 2019

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ISSN: 2304-8158

Research Domain: Food Science & Technology.

JCR® category	Quartile in Category	Rank	Impact Factor	5-year impact factor
Food Science and Technology	Q3	83/139	1.763	1.781

PUBLICATION 6

Sánchez-Rodríguez, L., Kranjac, M., Marijanović, Z., Jerković, I., Carbonell-Barrachina, Á.A., Sendra, E., Hernández, F. 2019. Quality Attributes and Fatty Acid, Volatile and Sensory Profiles of "Arbequina" *hydroSOStainable* Olive Oil. *Molecules*. 24 (11):2148. DOI: 10.339/molecules24112148

Published: 6 June 2019

Publisher: MDPI St. Alban-Angale, 66 Basel, Switzerland 4052

ISSN: 1420-3049

Research Domain: Biochemistry & molecular biology; Chemistry, multidisciplinary

JCR® category	Quartile in Category	Rank	Impact Factor	5-year impact factor
Chemistry, multidisciplinary	Q2	70/177	3.267	3.589

PUBLICATION 7

Sánchez-Rodríguez, L., Kranjac, M., Marijanović, Z., Jerković, I., Carbonell-Barrachina, Á.A., Sendra, E., Hernández, F. 2019. "Arbequina" olive oil composition is affected by the application of regulated deficit irrigation during pit hardening stage. *Journal of the American Oil Chemists' Society.* 97(5): 449-462. DOI: 10.1002/aocs.12332

Published: 6 January 2020

Publisher: Wiley, 111 River St, Hoboken 07030-5774, NJ USA

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Research Domain: Chemistry, applied; Food Science & Technology

JCR® category	Quartile in Category	Rank	Impact Factor	5-year impact factor
Food Science and Technology	Q3	86/139	1.659	1.869

La Tesis Doctoral titulada "***HydroSOStainable table olives and olive oil: quality, functionality and acceptance in the European market***", de la que es autora la Máster en Nutrición y Seguridad Alimentaria Dña. **Lucía Sánchez Rodríguez**, ha sido dirigida por la Dra. Dña. Esther Sendra Nadal, Catedrática de Universidad del Departamento de Tecnología de los Alimentos de la Universidad Miguel Hernández de Elche y codirigida por la Dra. Dña. Francisca Hernández García, Catedrática de Universidad del Departamento de Producción Vegetal y Microbiología de la Universidad Miguel Hernández de Elche

En Orihuela, a 13 de mayo de 2021

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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 "**Aceitunas de mesa y aceite de oliva hidroSOStenibles: calidad, funcionalidad y aceptación en el mercado Europeo"** de la que es autora la graduada en Ciencia y Tecnología de los Alimentos **Dña. Lucía Sánchez Rodríguez**, ha sido realizada bajo la dirección de la **Dra. Esther Sendra Nadal** y la codirección de la **Dra. Francisca Hernández García**, actuando como tutora de la misma la Dra. María Estrella Sayas Barbera. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA, siendo por tanto apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a 13/05/2021.

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INDEX

INDEX

1. DOCTORAL THESIS STRUCTURE

This Doctoral Thesis follows University Miguel Hernández de Elche internal regulation for the presentation of Doctoral Thesis as a Compendium of Publications, the structure is as follow:

1. **Abstract and *Resumen*.** The hypothesis, main objectives and most relevant results obtained are detailed.
2. **Introduction.** The state of the art of olive tree production have been reviewed, as well as the relevance of olive products, the introduction on deficit irrigation strategies and which is the unifying thread of this Doctoral Thesis.
3. **Objectives.** The main objective and the specific ones are presented in this section.
4. **Material and Methods.** The vegetable material, the agronomic practices and the analytical methods and data processing used to carry out this Doctoral Thesis are summarized and referenced.
5. **Publications**. The seven publications included in this Thesis are presented:
   - 5.1. In the First Publication, (Journal of the Science of Food and Agriculture. 99: 1804-1811. DOI: 10.1002/jsfa.9373), the effect of Regulated Deficit Irrigation (RDI) during pit hardening stage and Spanish-Style of "Manzanilla" green olives during 2015 and 2016 seasons have been studied.
   - 5.2. In the Second Publication, (Agricultural Water Management. 221: 415-421. DOI: 10.1016/j.agwat.2019.05.014), "Manzanilla" raw olives and table olives after Spanish-style process growing under two RDI strategies during rehydration stage (2015 and 2016 seasons) have been studied.
   - 5.3. In the third publication (Journal of Agricultural and Food Chemistry. 67: 661-670. DOI: 10.1021/acs.jafc.8b06392), it has been studied the polyphenolic profile of "Manzanilla" raw olives and table olives after Spanish-style process grown under three RDI during pit hardening stage and two RDI during rehydration stage.
   - 5.4. In the fourth publication (Foods. 8: 470. DOI: 10.3390/foods8100470), volatile composition, descriptive sensory analysis, affective sensory analysis and consumers' willingness to pay for "Manzanilla" table olives after Spanish-style process grown under three RDI during pit hardening stage have been studied.

5.5. The fifth publication (Journal of Food Quality. Volume 2020, Article ID 6348194, 6 pages DOI: 10.1155/2020/6348194) addressed the total polyphenols bioaccessibility and antioxidant potential after gastrointestinal *in vitro* digestion simulation of “Manzanilla” table olives grown under three RDI during pit hardening stage and two RDI during rehydration stage.

5.6. In the sixth publication (Molecules. 24 (11): 2148. DOI: 10.339/molecules24112148), “Arbequina” olive oil grown from trees submitted to two RDI during pit hardening and one Sustained Deficit Irrigation (SDI) strategies in 2017 (Sevilla) have been studied.

5.7. The seventh publication (Journal of American Oil Chemist´s Society. 97(5): *449-462.* DOI: 10.1002/aocs.12332), studied “Arbequina” olive oil grown from trees submitted to three RDI during pit hardening in 2017 (Ciudad Real).

6. **Results and Discussion**. In this section, main results obtained in this Doctoral The thesis is summarized, explained, and discussed.
   6.1. “Manzanilla” green olives and table olives
      6.1.1. Experiment A, where publications 1, 3, 4 and 5 are commented.
      6.1.2. Experiment B, where publications 2,3 and 5 are explained.
   6.2. “Arbequina” olive oil
      6.2.1. Experiment C, which is about publication 6.
      6.2.2. Experiment D, where the seventh publication is explained.
7. **Conclusions and *Conclusiones*.** The main conclusions reached after the work carried out that were raised in the objectives, have been listed, as well as the future possible research.
8. **References.** Bibliographic references used for writing and justifying this Doctoral Thesis have been references following APA 6th edition.

2. ABBREVIATIONS

AA	Antioxidant activity
AGS	Ácidos grasos saturados
AI	Atherogenic index
ANOVA	One-way analysis of variance
AOVE	Aceite de oliva virgen extra
DAD	Diode-array detector
EC	Enzyme commission
ESI	Electrospray ionization
EVOO	Extra virgin olive oil
FAME	Fatty acid methyl esters
GAE	Gallic acid equivalents
GC-MS	Gas chromatograph-mass spectrometer
GS	Gastric step
GSS	Gastric solution simulation
HPLC	High-performance liquid chromatography
IOC	International olive oil council
IS	Intestinal step
ISS	Intestinal solution simulation
JAR	Just about right
LPO	Lupoxygenase
MS	Mouth step

MTT	Magness-Taylor test
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
PLS	Partial least squares regression
PT	Puncture test
RDI	Regulated deficit irrigation
RF	Residual fraction
RID	Refractive index detector
RO	Raw olives
SDI	Sustained deficit irrigation
SF	Soluble fraction
SFA	Saturated fatty acids
SSS	Salivary soluble fraction
SI	Stress integral
TI	Thrombogenic index
TO	Table olives
TPC	Total phenolic content
UV	Ultraviolet
VOO	Virgin olive oil
Ψ_{stem}	Midday stem water potential

3. ABSTRACT AND RESUMEN

3.1. ABSTRACT

Olive oil and table olives demand has been continuously increasing during the last decades due to their acclaimed health benefits, so, in order to increase productivity, the area under olive groves has increased and irrigation was implemented to enhance productivity. Nowadays, one of the main world challenges is the scarcity of water resources, and, given that agriculture is one of the most water demanding sectors it is important to develop water saving techniques to face this problem. When plants suffer water restrictions, the stress induced on its system can lead to an increased production of some functional and nutritional components. Based on that fact, the main hypothesis of this Doctoral thesis is that "The implementation of deficit irrigation strategies during different phenological stages of olive trees leads to the increase on functional and nutritional properties of table olives and olive oil". These fruits were labelled as HydroSOStainable. For that purpose, "Manzanilla" raw and table olives after Spanish-style processing and "Arbequina" olive oil from water saving techniques had been studied (morphological, functional, nutritional and sensory characteristics).

Concerning "Manzanilla" olives, two experiments were carried out:

- Experiment A was run in olive trees located in Sevilla, in Dos Hermanas (Spain), irrigation deficit strategies with different stress levels were applied during the pit hardening stage (stage II). Main results of these experiments indicated that saving water techniques applied during stage II made rounder, harder, lighter and greener olives than full irrigation. Minerals, antioxidants, total phenols, organic acids and sugars did not show statistical differences with the full irrigated olives. Polyphenolic profile was affected by water saving techniques and a moderate stress was the one producing the biggest changes as it increased the concentration of oleoside, elenoic acid glucoside, oleoside diglucoside, oleuropein and comselogoside, while the concentration of some polyphenols was decreased at higher stress levels. Regarding volatile composition and sensory analysis, some compounds were affected; esters were reduced with the stress while terpenes increased. Alcohols and phenolic compounds also increased on some samples. These changes affected the descriptive sensory analysis, hydroSOStainable table olives presented modified intensity of some attributes such as green-olive flavor, sourness, aftertaste, bitterness or crunchiness. Consumers did not perceive

relevant differences among samples, except when olives were labelled with the HydroSOStainable logo. Those were the preferred table olives, and consumers were willing to pay higher prices for them. Finally, phenols bioaccessibility was not affected by the application of water saving techniques.

- Experiment B was run in olive trees located in Sevilla as well, in Coria del Río (Spain). Water deficit with different stress levels was applied during rehydration stage, just before harvest (stage III). In this experiment, hydroSOStainable olives presented a slight smaller size than control but the pulp:pit proportion was maintained. Antioxidant activity, total phenolic content and monounsaturated fatty acid (MUFA) content were highest with the highest water stress. Regarding the polyphenolic profile, hydroSOStainable table olives presented higher concentration of some polyphenols than control, such as luteolin-3-*O*-rutinoside, oleoside diglucoside, comselogoside, elenoic acid glucoside, dihydro-oleuropein and oleuropein. Finally, phenol bioaccessibility was not affected by the water stress.

With respect to "Arbequina" olive oil, also two experiments took place:

- Experiment C: olive trees were located in Sevilla, in Carmona (Spain). Water restrictions were applied during pit hardening stage and one of the treatments was applied during the whole season. HydroSOStainable olive oils from Experiment C were classified as extra virgin olive oil (EVOO) and at moderate stress level applied during pit hardening the total phenolic content was increased, as well as oleic acid increase ~ 3.5 % of concentration and decreased the saturated fatty acids (SFAs). Some volatile compounds and sensory attributes also increased their concentration and intensity, respectively.
- Experiment D: olive trees were located in Ciudad Real (Spain), and the water saving techniques were applied during pit hardening stage. HydroSOStainable olive oils also were classified as EVOO and showed increased antioxidant activity and total phenolic content. These olive oils also improved the fatty acid profile as increased the MUFAs and decreased the SFAs. Volatile compounds and sensory descriptors were more balanced than in control oil.

3.2. RESUMEN

La demanda de aceite de olive y aceitunas de mesa ha sufrido un incremento durante las últimas décadas debido a los aclamados beneficios que aporta su consumo para la salud, por lo que su producción se ha visto incrementada y el cultivo del olivo se ha introducido en el regadío para poder incrementar su productividad. Como uno de los principales retos a los que nos enfrentamos hoy en día está la falta de recursos hídricos, siendo la agricultura uno de sus principales consumidores, por lo que es muy importante desarrollar técnicas de ahorro de agua en el campo para poder afrontar este problema. Al aplicar restricciones hídricas a las plantas, se produce un estrés en sus sistemas que puede derivar en un incremento en algunos de sus compuestos nutricionales y funcionales, por lo que la principal hipótesis de esta tesis doctoral es el incremento de las propiedades funcionales y nutricionales de las aceitunas de mesa y aceite de oliva procedentes de cultivos con estrategias de riego deficitario que se aplican durante diferentes estados fenológicos de crecimiento de las aceitunas. Estas aceitunas se han llamado HydroSOStenibles. Para llevarla a cabo, se han analizado aceitunas crudas y aderezadas siguiendo el estilo español de la variedad "Manzanilla" y aceites de oliva de la variedad "Arbequina" que proceden de estrategias de riego deficitario (características morfológicas, funcionales, nutricionales y sensoriales).

Con respecto a las aceitunas "Manzanilla", se han llevado a cabo dos experimentos:

- <u>Experimento A</u>: los olivos se sitúan en Sevilla, en Dos Hermanas (España). Las técnicas de riego deficitario, con diferentes niveles de estrés, se aplicaron durante la fase de endurecimiento del hueso (estadío II). Los principales resultados de este experimento indicaron que las estrategias de riego deficitario aplicadas durante el estadío II dieron aceitunas más redondas, duras, luminosas y verdes, lo que las hace más atractivas para los consumidores. Los minerales, antioxidantes, fenoles totales, ácidos orgánicos y azúcares no sufrieron cambios, mientras que el perfil polifenólico sí se vio afectado por el riego deficitario: con un estrés moderado se incrementó la concentración de oleósido, glucósido de ácido elenócio, diglucósido oleosido, oleuropeína y comselogósido mientras que un mayor estrés produjo el descenso de concentración de algunos polifenoles. Con respecto a los compuestos volátiles y el análisis sensorial, algunos compuestos se vieron afectados; los ésteres se redujeron con el estrés, mientras que los terpenos aumentaron. Los

alcoholes y compuestos fenólicos aumentaron en algunas muestras. Estos cambios produjeron cambios en las intensidades de algunos atributos sensoriales como el aroma verde-aceituna, la acidez, el postgusto, el amargor o la crujibilidad. Los consumidores no percibieron estas diferencias entre muestras, pero al ser sometidos al efecto del logo, prefirieron las aceitunas de mesa hidroSOStenibles e indicaron que estaban dispuestos a pagar más por ellas. Por último, la bioaccesibilidad de fenoles no se vio afectada por las estrategias de riego deficitario.

- Experimento B: los olivos se sitúan en Sevilla también, concretamente en Coria del Río (España). Las estrategias de riego deficitario se llevaron a cabo durante la fase de rehidratación, justo antes de la cosecha (estadío III). Se aplicaron tratamientos con diferentes niveles de estrés. En este experimento, las aceitunas hidroSOStenibles fueron más pequeñas que el control, pero se mantuvo la proporción de pulpa. La actividad antioxidante, el contenido total de polifenoles y los ácidos grasos monoinsaturados aumentaron según aumentó el estrés hídrico. Con respecto al perfil polifenólico, las aceitunas hidroSOStenibles presentaron una mayor concentración de algunos polifenoles como la luteolina-3-*O*-rutinosido, diglucósido oleósido, comselogósido, glucósido de ácido elenoico, dihidro-oleuropeína y oleuropeína. Finalmente, la bioaccesibilidad de fenoles totales no se vio afectada por el riego deficitario.

Los aceites de oliva de la variedad "Arbequina" también se dividen en dos experimentos:

- Experimento C: los olivos se sitúan en Sevilla, en la localidad de Carmona (España). Las restricciones se aplicaron durante la fase II y en uno de los tratamientos durante toda la temporada. Los aceites hidroSOStenibles se clasificaron como aceite de oliva virgen extra (AOVE) y, con un estrés moderado durante el endurecimiento del hueso, aumentó el contenido total de fenoles y la concentración de ácido oleico (~ 3.5 %). También disminuyó la concentración de ácidos grasos saturados (AGSs). Algunos compuestos volátiles y atributos sensoriales también aumentaron sus concentraciones e intensidades respectivamente.
- Experimento D: los olivos se sitúan en Ciudad Real (España). El riego deficitario se aplicó durante el endurecimiento del hueso. Los aceites de este experimento se

clasificaron como AOVE. Se produjo un aumento de la actividad antioxidante y contenido total de polifenoles con el estrés hídrico. Se mejoró el perfil de ácidos grasos, ya que aumentó el contenido de ácidos grasos monoinsaturados y disminuyó el de saturados. Los compuestos volátiles y atributos sensoriales fueron más equilibrados que en el control.

4. INTRODUCTION

The olive tree is one of the oldest agricultural evergreen tree crops. It belongs to the plant family *Oleaceae* (*Olea europaea* L.). Olive tree can grow up to 15 m tall and can live for hundreds of years (Lavee, 2011; Guo et al., 2017). During olive fruit development three differentiated phenological stages are defined (Goldhamer, 1999):

- Stage I: from beginning of fruit growth until pit hardening starts (~ 10 weeks).
- Stage II: pit hardening (fruit growth is stopped) (~ 7 weeks).
- Stage III: rehydration. Period of oil accumulation and maturation (9-17 weeks).

Olive tree, original from the Mediterranean basin and parts of Asia (Guo et al., 2017) was spread around the world, and, nowadays is cultivated in all continents (Figure 1) having Europe the 65 % of the total production, followed by Africa (20 %), Asia (11.6 %), America (3.1 %) and finally Oceania (0.3 %) (FAOSTAT, 2020).

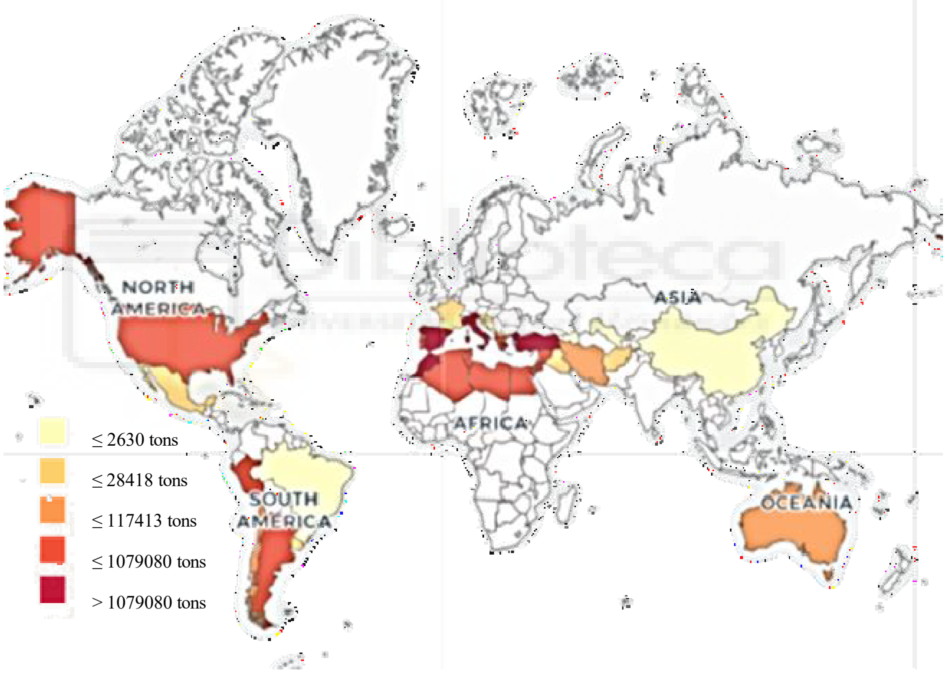

Figure 1. World olives production on 2017. (FAOSTAT, 2020)

During the last years, both the area under olive groves and its yield have been progressively increased (Figure 2) and, in 2018, Spain was the main producer with 9,819,569 t, followed by Italy with 1,877,222 t, Morocco with 1,561,465 t and Turkey (1,500,467 t). On 2018, the total production worldwide was 10,513,320 t with an area of 21,066,062 hectares (FAOSTAT, 2020).

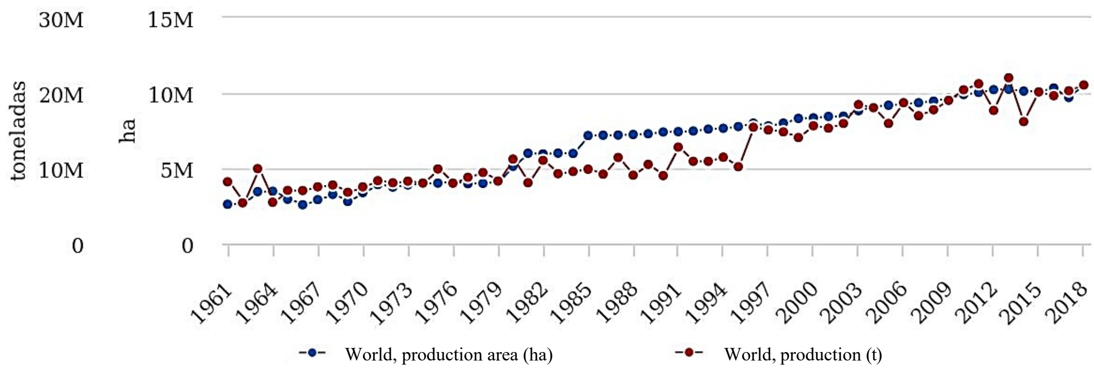

Figure 2. World production (t) and world production area (ha) since 1961 to 2018 of olives (FAOSTAT, 2020)

The most common olive product is olive oil, although table olives are also a popular consumed appetizer. There are 139 main varieties recognized around the world, but some of them are employed for olive oil production ant others for table olives, although there are some varieties suitable for both purposes. Spain, is the country with the highest number of olive tree varieties, for instance, at least 37 olive varieties are grown in Spain (Gómez-Escalonillas et al., 2006). “Manzanilla” variety is one of the most used for table olive production, as olives’ size is highly appreciated by consumers due to symmetry of fruits and its high productivity. Regarding olive oil, “Arbequina”, “Arbosana” and “Koroneiki” are the three most used olive tree varieties for super high-density because of their fast entry in production, good productivity every year, their high production since early age and their good olive oil quality (Aparicio et al., 2013).

Olives need processing to be edible because of the firmness and bitterness (due to oleuropein) of raw fruits. There are several processing styles for olives, the most common being Spanish-Style green olives, California-Style black olives and Greek-Style (Figure 3). The last one is a simple, natural process that does not use any chemical while the first two involve more steps. Both, Spanish and Californian Styles, involve lye treatment through which oleuropein is converted into hydroxytyrosol, elenoic acid glucoside and oleuropein aglycone to reduce bitterness (Guo et al., 2017). Then, Californian-Style produces an air oxidation followed by a fixing color step. Then, olives are submitted to bringing, and, in the case of Spanish-Style olives, to a fermentation by *Lactobacillus plantarum* and *Lactobacillus pentosus*. After that, table olives are ready to eat, in each style with different sensory properties (Guo et al., 2017).

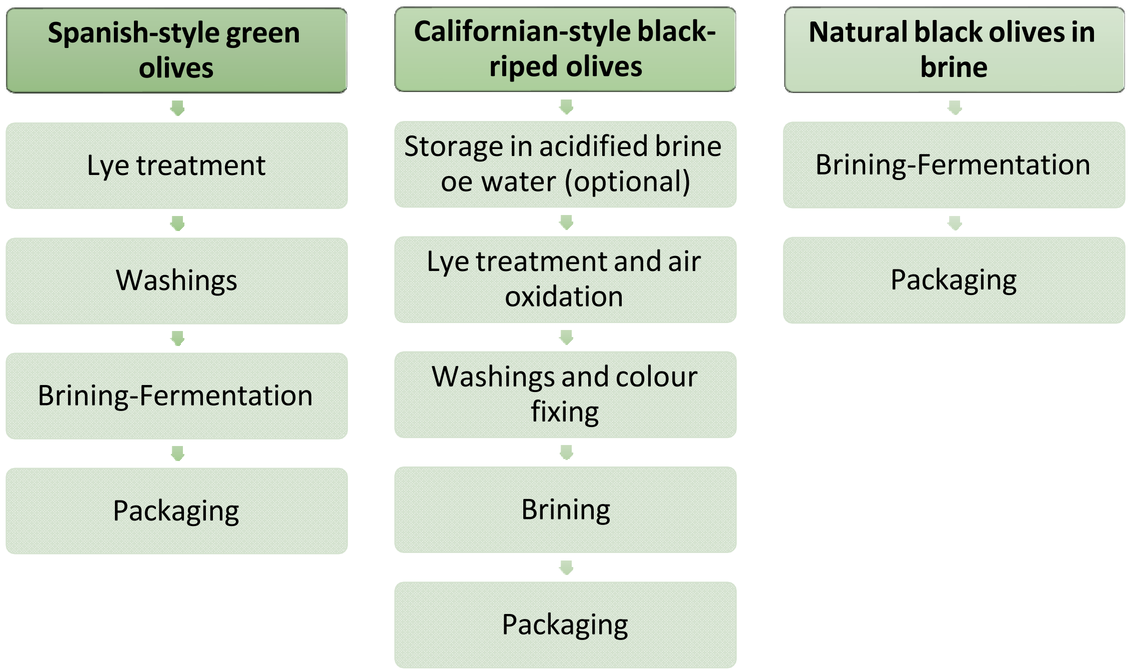

Figure 3. Production process flow charts for Spanish-style green olives, Californian-style black-ripe olives, and naturally black olives in brine (Guo et al., 2017).

Table olives composition and properties could vary depending on several factors, such as: style of processing, maturity index, variety employed, soil quality, climate, irrigation strategies, etc. All types of table olives share some characteristics such as their low sugar content (2-5 %), balanced fat content (made up mainly by monounsaturated oleic acid), fiber, vitamins, minerals, antioxidants (polyphenols) and attractive sensory quality for consumers (Guo et al., 2017), so olives are a healthy appetizer (Boskou et al., 2015; Guo et al., 2017).

Olive oil composition and quality also depend on some factors like the variety, maturity index, extraction procedure, climate, soil, irrigation, etc. Regarding quality, there are different commercial categories according to International Olive Oil Council (IOC) and European Regulation (EEC, 2568/91). From highest to lowest quality, on the market it could be found: *i)* extra virgin olive oil (EVOO), *ii)* virgin olive oil (VOO), *iii)* refined olive oil, and *iv)* pomace oil. They are categorized as a function of some chemical and sensory parameters established by regulation EEC (2568/91). The composition of olive oil ensures the consumption of essential molecules such as polyunsaturated and monounsaturated fatty acids (ω-3 and ω-9) as well as some vitamins like E and K and some minerals such calcium, potassium or sodium and also polyphenols. In fact, olive oil is the only food with a health claim approved by the European Regulation related to polyphenols: olive oil polyphenols contribute to the protection of blood lipids from oxidative stress (5 mg of hydroxytyrosol and its derivatives *per* 20 g of olive oil) (EU, 2012).

One of the main worldwide-accepted goals is the need to reduce pollution and generate environmentally friendly systems in order to preserve natural resources and still be able to benefit from them. One of the activities with a high demand of proportion of natural resources is agronomy, for instance, it covers ~ 43 % of the world's ice- and desert-free land whose ~ 87 % is for food production (Poore et al., 2018). Furthermore, water is highly consumed by agricultural practices: approximately two thirds of freshwater withdrawals are used for irrigation and this water is rarely returned to rivers or groundwater as industry done (Poore et al., 2018). Regarding Mediterranean diet sustainability, it has become during last decades a focus of attention, it is based on high quantities of olive oil and olives, fruits, vegetables, cereals, legumes, nuts and moderate amounts of fish and dairy products and low amount of meat products. These foods are traduced in a plant-based diet with low greenhouse-gas emissions and low water footprints as compared to western dietary patterns (Dernini et al., 2015).

During the last decades, olive products demand has experienced a high increase due to their nutritional and functional properties. For that reason, traditional non-irrigated olive orchards were adapted to intensive production and new highly productive orchards were planted. Changes include the incorporation of some agricultural practices such as irrigation for increasing production (Lavee, 2011). In fact, nowadays, Spain olive orchards under irrigation practices count with 818,505 ha (only exceeded by cereals) (MAPAMA, 2018).

Deficit irrigation strategies are being studied to save water without affecting productivity. Tested strategies are Regulated Deficit Irrigation (RDI) which reduces the water irrigation in a specific plant growth stage, and Sustained Deficit Irrigation (SDI) which reduces the water irrigation in a uniform way during the whole season (Fereres, et al., 2012). Several studies focused on the study of the agronomic aspects of the application of this strategies on olive tree orchards have been reported (Dell'Amico et al., 2012; Moriana et al., 2013; Girón et al., 2015, Girón et al., 2016, Corell et al., 2016, Corell et al., 2017), however, such studies did not find a clear trend in the effects of water restrictions on nutritional, functional and sensory quality of olive oil and table olives due to the application of deficit irrigation strategies.

HydroSOStainable products have been defined as vegetables coming from deficit irrigation strategies that have unique characteristics (Noguera-Artiaga et al., 2016). When using less water than the optimum, the trees suffer from a hydric stress, so if the stress is moderate, the trees generate a higher content of some compounds. For that reasons, hydroSOStainable vegetables are expected to contain high concentrations of bioactive compounds and improved sensory characteristics. Therefore, farmers and consumers would be highly interested in cultivate under such conditions and in consuming hydroSOStainable foods (Cano-Lamadrid et al., 2015; Collado-González et al., 2015; Noguera-Artiaga et al., 2016; Cano-Lamadrid et al., 2017)

This Doctoral thesis in included in the research project AGL2016-75794-C4-1-R (Agencia Estatal de Investigación/Ministerio de Ciencia, Innovación y Universidades) from "Food Quality and Safety" Research Group (Universidad Miguel Hernández de Elche). From this project, this Doctoral thesis is focused on studying the effect of different deficit irrigation strategies on "Manzanilla" raw and table olives after Spanish-style processing and on "Arbequina" olive oil with the hypothesis of the enhancement of bioactive compounds on olive products due to the stress produced on olive tree because of the water restrictions. For that purpose, morphological, nutritional, functional and sensory aspects have been studied to be able to inform farmers and consumers about benefits of applying these agronomic strategies.

The outline objectives and the results obtained during the course of this Doctoral thesis, have produced seven research publications (Figure 4):

1. In the First Publication, "Manzanilla" raw olives and table olives after Spanish-style process growing under three RDI strategies during pit hardening stage (2015 and 2016 seasons) have been studied [morphological parameters (weight, size, color and texture), nutritional parameters (minerals, organic acids and sugars) and functional (antioxidant activity and total phenolic content)].

Sánchez-Rodríguez, L., Corell, M., Hernández, F., Sendra, E., Moriana, A., Carbonell-Barrachina, Á.A. 2019. Effect of Spanish-style processing on the quality attributes of HydroSOStainable green olives. *Journal of Science of Food and Agriculture. 99(4):1804-1811. DOI: 10.1002/jsfa.9373.*

2. In the Second Publication, "Manzanilla" raw olives and table olives after Spanish-style process growing under two RDI strategies during rehydration stage (2015

and 2016 seasons) have been studied [morphological parameters (weight, size, color and texture), nutritional parameters (organic acids and sugars) and functional (fatty acids, antioxidant activity and total phenolic content)].

Sánchez-Rodríguez, L., Lipan, L., Andreu, L., Martín-Palomo, M.J., Carbonell-Barrachina, Á.A., Hernández, F., Sendra, E. 2019. Effect of regulated deficit irrigation on the quality of raw and table olives. *Agricultural Water Management. 221:415-421. DOI: 10.1016/j.agwat.2019.05.014.*

3. In the third publication, polyphenolic profile of "Manzanilla" raw olives and table olives after Spanish-style process grown under three RDI during pit hardening stage and two RDI during rehydration stage have been studied.

Sánchez-Rodríguez, L., Cano-Lamadrid, M., Carbonell-Barrachina, Á.A., Wojdyło, A., Sendra, E., Hernández, F. 2019. Polyphenol Profile in "Manzanilla" Table Olives As Affected by Water Deficit during Specific Phenological Stages and Spanish-Style Processing. *Journal of Agricultural and Food Chemistry. 67: 661-670. DOI: 10.1021/acs.jafc.8b06392.*

4. In the fourth publication, volatile composition, descriptive sensory analysis, affective sensory analysis and consumers' willingness to pay for "Manzanilla" table olives after Spanish-style process grown under three RDI during pit hardening stage have been studied.

Sánchez-Rodríguez, L., Cano-Lamadrid, M., Carbonell-Barrachina, Á.A., Sendra, E., Hernández, F. 2019. Volatile Composition, Sensory Profile and Consumer Acceptability of HydroSOStainable Table Olives. *Foods. 8: 470. DOI: 10.3390/foods8100470.*

5. The fifth publication is about total polyphenols bioaccessibility and antioxidant potential after gastrointestinal *in vitro* digestion simulation of "Manzanilla" table olives grown under three RDI during pit hardening stage and two RDI during rehydration stage.

Sánchez-Rodríguez, L., Cano-Lamadrid, M., Carbonell-Barrachina, Á.A., Hernández, F., Sendra, E. 2020. Impact of gastrointestinal in vitro digestion and deficit irrigation on antioxidant activity and phenolic content bioaccessibility of

"Manzanilla" table olives. *Journal of Food Quality*. Volume 2020, Article ID 6348194, 6 pages DOI: 10.1155/2020/6348194

6. In the sixth publication, "Arbequina" olive oil grown from trees submitted to two RDI during pit hardening and one SDI strategies in 2017 (Sevilla) have been studied [analytical parameters for olive oil grading, nutritional parameter (volatile compounds), functional parameters (fatty acids, antioxidant activity and total phenolic content) and sensory analysis (descriptive)].

Sánchez-Rodríguez, L., Kranjac, M., Marijanović, Z., Jerković, I., Carbonell-Barrachina, Á.A., Sendra, E., Hernández, F. 2019. Quality Attributes and Fatty Acid, Volatile and Sensory Profiles of "Arbequina" hydroSOStainable Olive Oil. *Molecules. 24 (11):2148. DOI: 10.339/molecules24112148.*

7. In the seventh publication, "Arbequina" olive oil grown from trees submitted to three RDI during pit hardening in 2017 (Ciudad Real) have been studied [analytical parameters for olive oil grading, nutritional parameter (volatile compounds), functional parameters (fatty acids, antioxidant activity and total phenolic content) and sensory analysis (descriptive)].

Sánchez-Rodríguez, L., Kranjac, M., Marijanović, Z., Jerković, I., Pérez-López, D., Carbonell-Barrachina, Á.A., Hernández, F., Sendra, E. 2020. "Arbequina" olive oil composition is affected by the application of regulated deficit irrigation during pit hardening stage. *Journal of American Oil Chemist's Society.* 97(5): 449-462. DOI: 10.1002/aocs.12332.

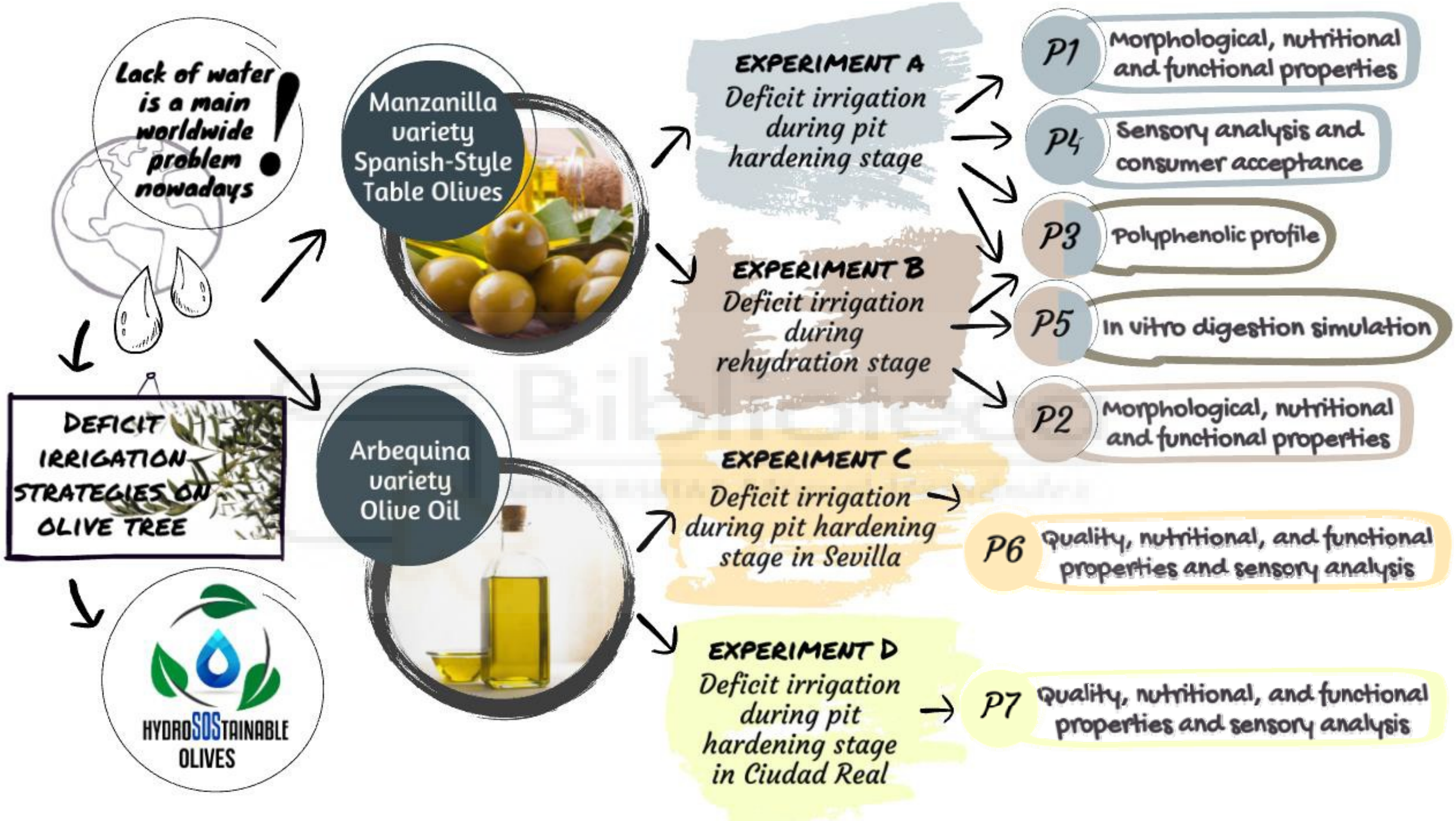

Figure 4. Graphical summary of the experiments and publications included in the Doctoral Thesis.

5. OBJECTIVES

The **overall objective** of this doctoral thesis was to determine the differential functionality and sensory quality of *hydroSOStainable* table olives and olive oil, as well as consumer attitude, to create the knowledge to properly inform consumers and farmers about the benefits involving the use of deficit irrigation strategies.

For that purpose, the **specific objectives** were:

- To determine deficit irrigation strategies effect on morphological, nutritional, functional and sensory properties of *hydroSOStainable* table olives and olive oil. For this end, these parameters were evaluated: (i) morphological (weight, size, color and texture), (ii) nutritional (minerals, organic acids, sugars and volatile compounds), (iii) functional [fatty acids, antioxidant activity by three assays ($ABTS^{+}$, $DPPH^{\cdot}$ and FRAP) and total phenolic compounds] and (iv) sensory (descriptive sensory analysis).

- To compare functional quality (based on polyphenol profile) of *hydroSOStainable* olives submitted to different deficit irrigation treatments of: (i) raw olives (RO) and (ii) table olives (TO) after Spanish-style processing.

- To study *hydroSOStainable* table olives affective sensory analysis and consumers' willingness to pay in different locations.

- To study phenols bioaccessibility and antioxidant activity after gastrointestinal *in vitro* digestion simulation of *hydroSOStainable* table olives.

6. MATERIAL AND METHODS

This doctoral thesis comprises four experiments with olives (*Olea europaea* L.). Two of them with "Manzanilla" olive trees for table olives, held in Sevilla, Spain, during 2015 and 2016 seasons. (Experiment A: RDI during pit hardening stage; Experiment B; RDI during rehydration stage); and, other two experiments with "Arbequina" olive trees for olive oil elaboration during 2017 season (Experiment C: 2 RDI during pit hardening stage, and 1 SDI, both held in Sevilla, Spain; Experiment D: RDI during pit hardening stage held in Ciudad Real, Spain) (Figure 5). The present section summarizes the methodology used in the thesis.

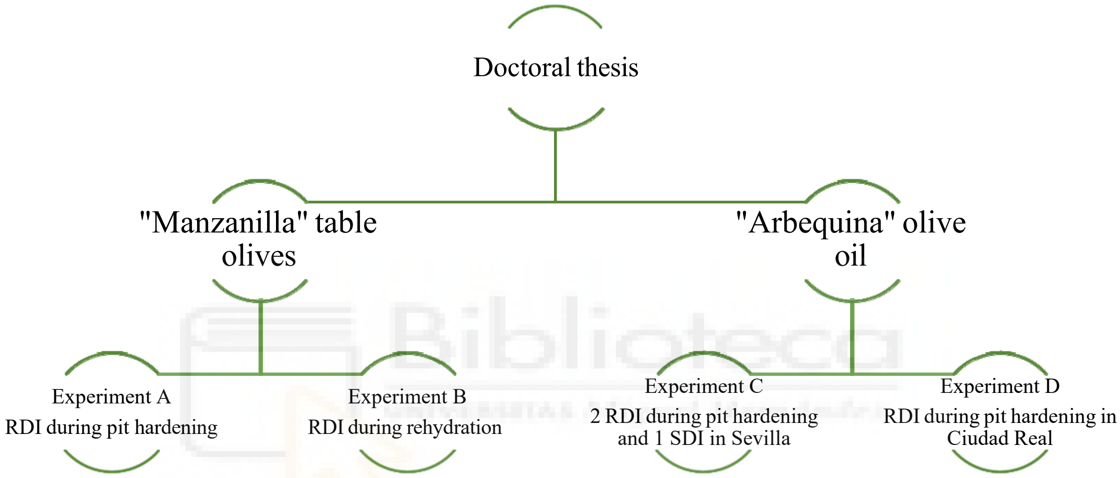

Figure 5. Structure of the experiments run in the Doctoral Thesis

6.1. "Manzanilla" raw and table olives

6.1.1. Experimental design and plant material

Experimental design was randomized completely in blocks with three repetitions and two control trees per plot. Irrigation scheduling was performed using the threshold values of midday stem water potential (ψ_{stem}) using a pressure chamber (PMS Instrument Company, Albany, OR, U.S.A.) during all season. Stress Integral (SI), as defined by Myers (1988) and using ψ_{stem} data during the period of beginning pit hardening until harvest (Eq. (1)), was used to describe the accumulative effect of the water deficit irrigation treatments (Corell et al., 2017). The expression was:

$$\text{(1)} \qquad SI = |\sum(\Psi - (-0.2)) \times n|$$

Where: SI is the stress integral, Ψ is the average midday stem water potential for any interval, n is the number of the days in the interval.

Pest control, pruning, and fertilization practices were those commonly used by growers, and weeds were chemically removed in the orchard. Climatic conditions of both experiments are almost equal because the distance between the orchards is only around 10 km, and both of them are at the same level in the Guadalquivir Valley. Winter minimum temperatures were slightly above 0 °C, and spring temperatures determine that flowering happens around mid-April. Weather conditions make this area perfect for olive tree growth. Olives were hand-harvested in September 2015 and 2016 at their mature-green stage. Two olive farms took part in the study of “Manzanilla” raw and table olives study: plots were located in Dos Hermanas (Experiment A) and Coria del Río (Experiment B) (both in Sevilla with similar bioclimatic conditions).

6.1.1.1. Experiment A

Olives were collected from a farm, Doña Ana, which is located in Dos Hermanas (Sevilla, Spain, 37° 25′ N, 5° 95′ W). Olive trees (“Manzanilla” variety) were 30 years old. The tree spacing followed a 7 × 4 m square pattern (Image 1). Experimental design was randomized completely in blocks with 4 replicates and 2 control trees per plot. The loam soil was characterized by a volumetric water content of 0.31 m^3 m^{-3} at field capacity and 0.14 m^3 m^{-3} at the permanent wilting point and a bulk density of 1.40 g cm^{-3} (0−30 cm) and 1.35 g cm^{-3} (30−90 cm). Irrigation was performed during the night by drip, using one lateral pipe per row of trees and four emitters per plant, split between the two rows (each delivering 2 L h^{-1}). Three different irrigation treatments and a control were carried out:

- *optimum water status* (A0) full irrigated,
- *moderate deficit irrigation* (A1), where the threshold value (ψ_{stem}) was −2 MPa during the pit-hardening stage,
- *severe deficit irrigation (short time)* (A2), where the threshold value (ψ_{stem}) was −3 MPa during half of the period of the pit-hardening stage; and,

- *severe deficit irrigation (long time)* (A3), where the threshold value (ψ_{stem}) was −3 MPa until the end of the period of the pit-hardening stage.

Image 1. Doña Ana Orchard (Dos Hermanas, Sevilla, Spain)

6.1.1.2. Experiment B

Olives were collected from La Hampa, the experimental farm of the *Instituto de Recursos Naturales y Agrobiología* (IRNAS−CSIC) located in Coria del Río (Sevilla, Spain, 37° 17′ N, 6° 3′ W, 30 m altitude) (Image 2). Olive trees ("Manzanilla" variety) were 43 years old. The tree spacing followed a 7 × 5 m square pattern. Experimental design was randomized completely in blocks with 3 replicates and 2 control trees per plot. The sandy loam soil was characterized by a volumetric water content of 0.33 $m^3\ m^{-3}$ at field capacity and 0.10 $m^3\ m^{-3}$ at the permanent wilting point and a bulk density of 1.30 g cm^{-3} (0−10 cm) and 1.50 g cm^{-3} (10−120 cm). Irrigation was performed during the night by drip, using one lateral pipe per row of trees and five emitters per plant, split between the two rows (each delivering 8 L h−1). Two different irrigation treatments and a control were carried out:

- *optimum water status* (B0) full irrigated,
- *moderate deficit irrigation before harvest (short time)* (B1), where the threshold value (ψ_{stem}) was reduced to −2 MPa at the beginning of September without a rehydration period, and

- *moderate deficit irrigation (long time)* (B2), where the threshold value (ψ_{stem}) was −2 MPa from mid-August without a rehydration period.

Image 2. La Hampa Orchard (Coria del Río, Sevilla, Spain)

6.1.2. Spanish-style processing of raw to table olives

"Manzanilla" olives from all of the trees of each block of each RDI treatment from the two experiments were systematically mixed, and a sample of approximately 5 kg in 2015 and 50 kg in 2016 per block was used to prepare TO. Fruits were transported the day after their picking at the farm to the *Cooperativa Nuestra Señora de las Virtudes* (La Puebla de Cazalla, Sevilla, Spain) to be processed as TO according to the Spanish-style method. In this method, RO were submitted to lye treatment in a dilute NaOH solution (1.3-2.6 % weight:volume) during 6-8 h followed by washings during 12 h and then olives were put in brine (12 % NaCl) (Image 3), where lactic acid fermentation occurred until table olives reach an equilibrium with brine (pH<4.2, 8 % NaCl, 0.8 % lactic acid and residual alkalinity <0.120 N). A part of each batch was kept as RO to be analyzed. Once

RO and TO arrived to our laboratories, a representative sample of olives from each treatment was lyophilized for further analysis.

Image 3. Olives submitted to brine for fermentation

6.1.3. Morphological analyses of raw and table olives

Twenty-five olives from each batch for each irrigation treatment were randomly selected to conduct measurements. Thus, 100 olives per irrigation treatment in Experiment A and 75 olives per irrigation treatment in Experiment B.

6.1.3.1. Weight and size

Whole olives were weighed (Image 4. A) (model AG204 scale; Mettler Toledo, Barcelona, Spain) and, then, each pit was removed (Image 4. B) and weighted for calculation of fruit/pit ratio. Size of each fruit (longitudinal and equatorial diameters) was measured using a digital caliper (Image 4. C) (model 500-197-20 150 mm; Mitutoyo Corp., Aurora, IL, USA).

6.1.3.2. Color determination

Color determinations were made using a colorimeter (model CR-300, Minolta, Osaka, Japan) (Image 4. D) using an illuminant D65 and 10∘ observer. Color was measured three times per olive at 25 ± 1 ∘C. Color results were given as CIE L*a*b* coordinates. This system defines color in a three-dimensional space: (i) L* indicates lightness (0 – 100 values); (ii) a* is green-red coordinate, taking green and red color negative and positive values, respectively; and, b* is blue-yellow coordinate, taking blue and yellow color negative and positive values, respectively.

6.1.3.3. Texture

Analysis of texture were conducted at 25 ± 2 °C using a Texture Analyser TA-XT2i (Stable Micro Systems, Godalming, UK) (Image 4. E) according to (Szychowski et al., 2015). Units were given in N.

Two textural analysis were carried out:

- *Puncture test* (PT), (to study peel firmness) using a stainless-steel needle probe P/2 N (2 mm thickness) that punched the whole fruit (0.5 mm s^{-1}; 7 mm of penetration) trying to avoid the needle touching the pit.
- *Magness–Taylor test* (MTT) (to measure pulp firmness) with a stainless-steel cylindrical probe SMSP/2 of 2 mm diameter (Image 4. F). To conduct this test, the peel of the olives was removed in approximately 1 cm^2 (0.3 mm s^{-1}; 5 s of penetration) on olive flesh.

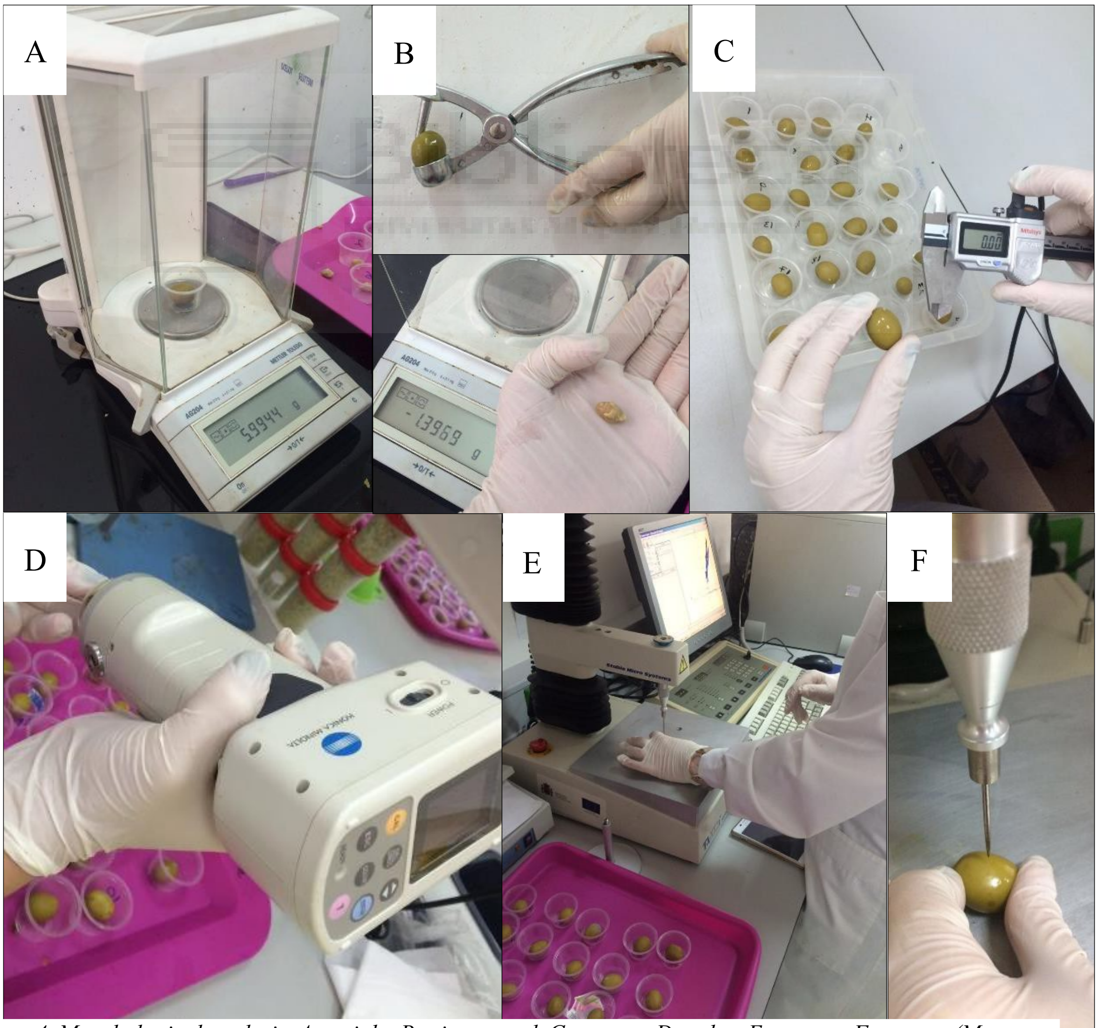

Image 4. Morphological analysis. A: weight. B: pit removal. C: texture. D: color. E: texture. F: texture (Magness-Taylor)

6.1.4. Mineral analysis of raw and table olives

Mineral analysis was carried out using a multi-place digestion block (Digest 20, Selecta) to digest 0.5 g of freeze-dried olives. Samples were digested for 2 h at 130 ∘C using 5 mL of 65 % nitric acid (HNO_3) (w/v). Later, samples were diluted with ultra-high-purity deionized water (1:10 and 1:50) and afterwards cooled at room temperature (Carbonell-Barrachina et al., 2002).

Determination of calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), and zinc (Zn) in the mineralized samples was performed using a Unicam Solaar 969 atomic absorption-emission spectrometer (Unicam Ltd., Cambridge, UK). Potassium was analyzed using atomic emission, while the rest of the elements were analyzed by atomic absorption. Calibration curves and blank reagent were used in each analytical batch. The analyses were run in triplicate.

6.1.5. Antioxidant activity (AA) and total phenol content (TPC) in raw and table olives

Antioxidants and phenols extraction was done with MeOH/H_2O (80:20 v/v) + 1 % HCl as described by Cano-Lamadrid et al. (2017). Three methods were used to evaluate the antioxidant activity (AA) of the studied olive samples. Radical scavenging activity was evaluated using $DPPH^{•}$ radical (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams et al., 1995). The absorbance decrease was measured at 515 nm. The free radical scavenging capacities were determined using the $ABTS^{+}$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) method (Re et al., 1999) at 734 nm and FRAP (ferric reducing antioxidant power) method, as described by (Benzie and Strain, 1996) at 593 nm. Analysis was carried out in a UV-visible spectrophotometer (Helios Gamma model, UVG 1002E). Calibration curves were done with Trolox. Analyses were run in triplicate and results were expressed as mmol Trolox kg^{-1} fresh weight (fw).

Total phenolic content (TPC) was quantified using Folin – Ciocalteu reagent (Gao et al., 2000) Absorbance was measured using an UV-visible spectrophotometer (Helios Gamma model, UVG 1002E) at 765 nm. Gallic acid was used to prepare calibration curves. Analyses were run in triplicate and results were expressed as gallic acid equivalents (GAE) kg^{-1} fw.

6.1.6. Organic acids and sugars in raw and table olives

For organic acids and sugars determination 2 g of freeze-dried olive sample were mixed with phosphate buffer 50 mM (pH 7.8) and centrifuged (Sigma 3 – 18 K; Sigma Laborzentrifugen, Osterode and Harz, Germany). Then, 10 μL of the filtered supernatant (0.45 μm filter) were injected into a Hewlett Packard (Wilmington, DE, USA) series 1100 high-performance liquid chromatography (HPLC) system using 0.1 % ortophosphoric acid elution buffer. Organic acid separation was made 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, USA). Absorbance was measured with a diode-array detector (DAD) at 210 nm for organic acids. Sugars were separated and detected in the same run but they were monitored using a refractive index detector (RID). Calibration curves were made 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} fw (Cano-Lamadrid et al., 2017).

6.1.7. Fatty acids of raw and table olives

Fatty acid methyl esters (FAMEs) were *trans*-methylated by adding dichloromethane, methanolic NaOH solution and BF_3-methanol and boiling during 10 min. Then, FAMEs extraction was done with hexane (Cano-Lamadrid et al., 2017). Organic layer was injected on a gas-chromatograph coupled with a mass spectrometer detector (GC-MS) (Shimadzu GC-17A and GC-MS QP-5050A) (Shimadzu Corporation, Kyoto, Japan) coupled with a Suprawax-280 column 30 m × 0.25 mm × 0.25 µm (Teknokroma). GC-MS programme was previously described by Marina Cano-Lamadrid et al. (2017). FAME standards from Sigma-Aldrich were used for identification of peaks by their retention time. Results are expressed as percentage of the total area of methylated fatty acids.

6.1.8. Polyphenol extraction, identification and quantification by LC-PDA-MS-QTof on raw and table olives

For polyphenol extraction 1 g of lyophilized olives were mixed with 10 mL of acetone:water:MeOH (1:2:2 v/v) (HPLC-grade) mixture. Doubled extraction was performed by incubation during 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland). The upper phase was centrifuged at 19,000 g during 10 min and filtered with Hydrophilic PTFE 0.20 µm membrane (Millex Samplicity Filter, Merck, Darmstadt, Germany). This extract was used for analysis. The compound identification was done using an Acquity ultraperformance LC system equipped with a photodiode detector (PDA; UPLC)) with binary solvent manager (Waters Corp., Milford, MA, USA) series with a mass detector G2 QTof Micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative and positive modes. The chromatographic conditions for the identification and quantification were previously reported by Wojdyło et al. (2016)

6.1.9. Gastrointestinal *in vitro* digestion simulation of table olives

In vitro digestion simulation was carried out following the method described by Minekus et al. (2014). Mouth, gastric and intestinal phase were done preparing salivary solution simulation (SSS), gastric solution simulation (GSS) and intestinal solution simulation (ISS) with KCl (0.5 M), KH_2PO_4 (0.5 M), $NaHCO_3$ (1 M), NaCl (2 M), $MgCl_2(H_2O)_6$ (0.15 M) and $(NH_4)_2CO_3$ (0.5 M). As well, SSS was mixed with 1 mL of α-amylase (1500 U/mL) (Enzyme Commission (EC) Number 3.2.1.1), GSS with pepsin (2500 U/mL) (EC Number 3.4.23.1) and ISS with pancreatin (800 U/mL) (EC Number 232.468.9) and bile salts (160 mM). In mouth step (MS), 10 g of table olives were mixed with 10 mL of SSS and homogenized in a bag mixer (Bagmixer 400, Intersience, France) during 1 minute to simulate mastication and then, it was transferred to a glass bottle in a 37 ºC bath with agitation (170 rpm) with addition of 15 mL of GSS (pH 3) for gastric step (GS). Then, intestinal step (IS) began with the addition of 20 mL of ISS (pH 7). After IS, liquid soluble fraction (SF) and solid residual fraction (RF) were collected. The SF was centrifuged at 10,000 g for 10 min at 4 ºC (Sigma 3–18 K; Sigma Laborzentrifugen, Germany) for later analysis. RF antioxidants and phenolic compounds were extracted as previous described (Cano-Lamadrid et al., 2017). Antioxidant activity ($DPPH^{\cdot}$, $ABTS^{+}$ and FRAP) and total phenolic content of SF and RF were measured as previous described.

TPC bioaccessibility expressed as the percentage of total polyphenols liberated from the test matrix after the gastrointestinal digestion, was calculated as previously reported by D'Antuono et al. (2018).

6.1.10. Volatile compounds profile of table olives

Volatile compounds extraction was done using headspace solid phase micro-extraction (HS-SPME). Five g of table olives were mixed with 15 mL of ultrapure water and 1.5 g of NaCl and placed into a vial. The vial was put in a bath at 40 ºC and, after equilibration, a 50/30 µm DVB/CAR/PDMS fiber was exposed to the headspace during 50 min. Volatiles were desorbed from the fiber into the injection port of the GC-MS during 3 min.

Volatile compounds separation and identification was performed in a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A equipped with a Restek Rxi-1301 2016 column (Restek Coropration, Bellefonte, USA) (30 m × 0.25 mm internal diameter × 1 µm thickness). Helium was used as carrier gas with same program previously reported by Cano-Lamadrid et al. (2015). Identification was done using: retention indices, GC-MS retention times, and mass spectra [Wiley 09 MS library (Wiley, New York, NY, USA) and NIST14 (Gaithersburg, MD, USA)]. Results were expressed as percentage of the total area represented by each one of the volatile compounds.

6.1.11. Sensory analysis of table olives

6.1.11.1. Descriptive sensory evaluation

Ten trained panelists (aged from 25-55 years) from the Food Quality and Safety research group (Miguel Hernández University of Elche, Alicante, Spain) carried out the descriptive sensory analysis of table olives under study. Each panelist had more than 600 h of experience with a variety of products, mostly, vegetal products. Three training sessions (1 h each) were carried out to train the panel on the use of Olive Oil Council table olive lexicon (IOOC, 2011). After these sessions, the panel agreed on the useful lexicon for the table olives under study: color (from yellow to green), green-olive flavor, saltiness, bitterness, sourness, sweetness, aftertaste, hardness, crunchiness and fibrousness, as well as off-flavors. Three sessions were run for the descriptive sensory evaluation of olives (each sample was evaluated in triplicate). Panelists used a 0-10 scale (0: no intensity; and 10: extremely strong).

6.1.11.2. Affective sensory evaluation

For affective sensory evaluation (Image 5) a randomized block design was used. Table olives, with three-digits codification, were served using odor-free disposable 100 mL plastic cups at room temperature (~20 ºC) and covered. Distilled water and crackers were used to clean palates between samples. Questionnaires used a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely) for global acceptability, degree of color, flavor, bitterness, saltiness, sourness, hardness, crunchiness, fibrousness, aftertaste and overall attributes. Just About Right (JAR) scale was used for scoring intensity attributes.

One-hundred regular consumers of table olives from 3 locations were selected. Locations were:

i) *L1: El Esparragal* (Murcia, Spain).
ii) *L2*: *Elche* (Alicante, Spain).
iii) *L3*: *Los Desamparados* (Alicante, Spain)

L1 and L3 were chosen as representatives of people living in countryside and L2 as people living in a city. Demographic questions were added to the questionnaire. The consumers' age range was 18-24 (13 %), 25-35 (14 %), 36-45 (19 %), 45-55 (26 %) and more than 55 (28 %) with a 62:38 gender ratio (women:men). Forty-six percent of consumers taking part in the study were full-time workers, 17 % part--time, 17 % were students and 20 % were unemployed. Consumers were asked for labelling interest, and 79 % of them admitted paying attention to the label of foods, specially valuing Spanish-product (64 %), healthy product (57 %) and sustainable product (25 %).

Image 5. Affective sensory evaluation

6.1.11.3. Consumer willingness to pay

Commercial Spanish-style "Manzanilla" table olives were purchased from Mercadona supermarket (Mercadona is one of the most popular food supermarkets in the Mediterranean area of Spain). Table olives were splitted in two batches and labeled as "conventional" and "*HydroSOStainable"*, with its logo (Image 6), so the same product was presented to the consumers but labeled as different. Consumers were informed about the *hydroSOStainable* concept.

A B

Image 6. Hydrosostainable logo. A: Spanish version. B: English version

The study was run in the same three locations than affective sensory evaluation but using 100 consumers in each location. Consumers were asked to taste both table olive samples and answer about overall liking, flavor, saltiness and hardness, as well as willingness to pay. They were given a price for conventional table olives of 1.35 €/200 g (Mercadona price) and four options to pay for *hydroSOStainable* table olives: ≤ 1.35 €, 1.35-1.75 €, 1.75-2.50 €, and >2.50 €.

6.2. "Arbequina" olive oil

6.2.1. Experimental design and plant material

Stem water potential at midday (ψ) was determined using a pressure chamber (PMS Instrument Company, Albany, OR, USA) in 4 trees per irrigation treatment, weekly during the experiments. Water stress integral (SI) was calculated (Equation 1) (Myers, 1988) to describe the accumulative effect of deficit irrigation strategies, from the beginning of pit hardening to harvest. Farms located in Sevilla (Experiment C) and in Ciudad Real (Experiment D) took part the study.

6.2.1.1. Experiment C

Olive tree orchard, located at Carmona (37.49◦ N, −5.67◦ W, Sevilla, Spain), is super-high density (4.0 m × 1.5 m) with 360 m^2 and has 60 trees organized in 3 liens (30 m) (Image 8. A). Olive trees, variety "Arbequina" were 11-year-old. The design was done with randomized blocks with 4 repetitions per treatment. Harvesting was done with a mechanical harvester, like at super-intensive farming (Image 8. B). The trees from the inside row (20) of each orchard were harvested for olive oil production. Harvest was carried out when olives had 1.9 maturity index. Each block was collected in one day, and the average yield was 7117 kg ha^{-1}.

Following the pressure chamber technique and the threshold values of midday stem water potential before and after the pit hardening period, 4 irrigation treatments were carried out:

- *Control* (C0): trees were watered to supply the 100 % crop evapotranspiration (ETc).
- *Optimal RDI* (C1): trees were under non-limited water conditions during stage I and III while regulated deficit irrigation was applied during stage II (58 % of reduction of total water irrigation amount).
- *Confederation RDI* (C2): the same way was followed as in T1 but with the limitation of water dotation of Guadalquivir hydrographic confederation (66 % of reduction of total water irrigation amount).
- *Confederation SDI* (C3): sustained deficit irrigation with the water amount allowed by the Guadalquivir hydrographic confederation (66 % of reduction of total water irrigation amount).

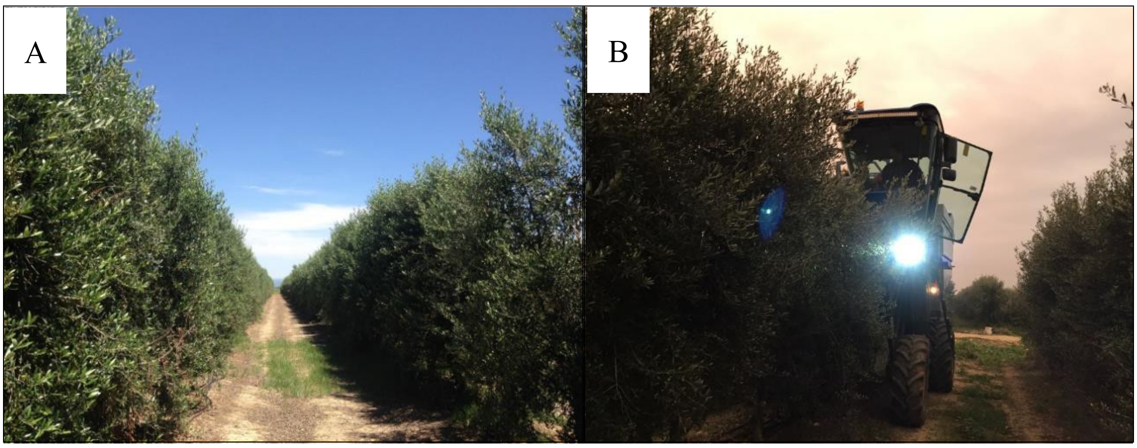

Image 1. A: Carmona Orchard. B: mechanical harvester

6.2.1.2. Experiment D

"Arbequina" olive trees (planted in 1999 with 7 m x 4.76 m tree spacing) were located near Ciudad Real (39º N, 3º,56' W; altitude 640 m). Irrigation was performed daily using a drip irrigation system with four self-compensating emitters (each delivering 8 L h^{-1}) per tree and irrigation water with an electrical conductivity of 2.6–2.9 dS cm^{-1}. The distance between drippers was 1 m, and the distance from trunk to drippers was 0.5 m.

The experimental plot consisted of eleven rows *per* five columns of olives trees. The two rows and columns in the outer part of the field were maintained as line borders and fruits from these trees were not harvested. Thus, measurements were made in representative olive trees and their fruits from the inner rows and columns (9 × 3). The experimental design consisted of randomized blocks with four repetitions. Around the experimental orchard, two rows and columns made of border of the orchard.

Four irrigation treatments were applied:

- *Control treatment* (D0) served to determine potential yield. Control plants (T0 treatment) were irrigated at 100 % of crop irrigation requirements (ETc) of the previous week.
- *Treatment 1* (D1) reduced irrigation water to produce water stress during pit hardening, maintaining ψ at -2 MPa during this phase.
- *Treatment 2* (D2) reduced irrigation water in a more severe way to maintain ψ at -3 MPa during the same phase.
- *Treatment 3* (D3) was the one producing the strongest water stress conditions, because no irrigation was done during the pit hardening stage.

6.2.2. Olive oil extraction

Olive oil was elaborated in a mini-mill model Frantoino Bio (Toscana Enologica Mori, Florence, Italy) at 40–50 kg h^{-1}, with oil extraction 2 phases technique. Each sample milled was 100 kg of olives per plot (4 per irrigation treatment). After olives were cleaned and washed they were transferred to the milling, which was held in a mill mixer at <28 ◦C during 20 min with 1 % (w:w) talc and 2 % (w:w) water, for the extraction of water flow meter 5 L h^{-1}. Olive oil samples were stored at 4 ºC in the absence of light until analysis were done.

6.2.3. Analytical parameters for olive oil grading

Acidity, peroxide value and UV absorption characteristics (K_{232}, K_{270} and ΔK) were analyzed following the procedure described by European Union Commission Regulation (EEC, 2568/91).

Rancimat (Metrohm, model 743, Switzerland) was used to evaluate oxidative stability of olives oil. It was carried out with 3 g of oil, at 120 ºC with air flow rate of 20 L h^{-1}. Results were expressed as induction time (h).

6.2.4. Antioxidant activity (AA) and total phenolic content (TPC) in olive oil

Extractions for olive oil AA and TPC analysis were done as previous described by Tuberoso et al. (2007). Briefly, 3 g of olive oil was mixed with 5 mL of methanol/water (80:20, v/v). The mixture was shaken for 2 min, and the hydrophilic phase was filtered with a GD/X 0.45 µm cellulose acetate septa (25 mm, Sartorius, Madrid, Spain). This procedure was repeated twice with the lipophilic phases, and all the hydrophilic extracts were evaporated in a rotary evaporator at 35 ºC. Finally, the residue was dissolved in 1.5 mL of methanol.

$DPPH^{\cdot}$, $ABTS^{+}$ and TPC were determined as previous described for "Manzanilla" olives.

6.2.5. Fatty acids of olive oil

Fatty acid methyl esters (FAMEs) were determined (ISO-12966-2, 2017). C13:0 (0.04 mg mL^{-1}) was added as internal standard for fatty acid concentration calculation. Gas chromatography (C-17A; Shimadzu Corporation, Kyoto, Japan) connected to a flame ionization detector (FID) was used to inject oils as described by ISO-12966-4 (2015) with some modifications. The capillary column used was CPSil-88 (100 m × 0.25 mm ID. 0.2 µm film thickness; J&W 112-88A7; Agilent Technologies, Santa Clara, CA, USA), which is appropriate for olive oil fatty acids separation. Detector temperature was 260 ºC, and oils were injected with a 1:20 split ratio. The oven temperature was 175 ºC for 10 min, then raised to 220 ºC (3 ºC min^{-1}) and kept at 220 ºC for 5 min. The carrier gas was helium, and detector gases were hydrogen (30 mL min^{-1}) and air (350 mL min^{-1}), and helium (30 mL min^{-1}) was used as make-up gas. Standard solutions (FAME 37 MIX, Supelco; Bellefonte, PA, USA), were injected under the same conditions as oils for the identification of compounds.

Atherogenic index (AI) and thrombogenic index (TI) were calculated as indicated in Equations (2) and (3) (Ulbricht and Southgate, 1991):

(2) $AI = (4 \times C14{:}0 + C16{:}0) / [\Sigma PUFA\ (n - 3) + \Sigma PUFA\ (n - 6) + \Sigma MUFA]$

where C14:0 is myristic acid, C16:0 is palmitic acid, PUFA means polyunsaturated fatty acids, and MUFA is monounsaturated fatty acids.

(3) $TI = (C14{:}0 + C16{:}0 + C18{:}0) / [0.5 \times \Sigma MUFA + 0.5 \times \Sigma PUFA\ (n - 6) + 3 \times \Sigma PUFA\ (n - 3) + (n - 3) / (n - 6)]$

where C18:0 is stearic acid.

6.2.6. Volatile compounds of olive oil

For Headspace Solid-Phase Microextraction, 5 mL of olive oil was added into a 15 mL glass vial with the addition of 2 µL of carvacrol (325.6 mg carvacrol in 1 L of olive oil) as an internal standard and 1 g NaCl. The vial was hermetically sealed with polytetrafluorethilenesilicone septa and maintained in a water bath at 40 ºC during equilibration (15 min) and extraction (40 min) and was partially submerged such that the liquid phase of the oils was below the water level. All the experiments were performed under constant stirring (500 rpm) with a magnetic stirrer. After sampling, the SPME fiber was inserted into the injector (250 ºC) of the GC-MS for 7 min, where the extracted volatiles were thermally desorbed directly into the GC column. Two fibers were used: divinylbenzene/carboxen/polydimethylsiloxane (50/30 µm DVB/CAR/PDMS, Supelco, Bellefonte, USA) and polydimethylsiloxane/divinylbenzene (65 µm PDMS/DVB, Supelco, Bellefonte, USA).

An Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7890A equipped with the mass selective detector, model 5977E, and capillary column HP-5MS (5 %-phenyl)-methylpolysiloxane (Agilent J & W; Santa Clara, CA, USA) GC column, 30 m, 0.25 mm i.d., coating thickness 0.25 µm was used. The flow rate of the helium carrier gas was 1.5 mL min^{-1}. The injector was operated in split mode (2:1 split ratio) at 260 ºC. The column was maintained at 40 ◦C for 3 min, heated to 100 ºC at a rate of 5 ºC min^{-1}, heated to 260 ºC at a rate of 3 ºC min^{-1}, and held to 260 ºC for 3 min. MS conditions were as follows: source temperature 230 ºC; quadrupole temperature 150 ◦C; transfer line temperature 270 ºC; acquisition mode electron impact (EI 70 eV) by 3 scans s^{-1}, and mass range m/z 29–350. The analyses were carried out in triplicate. The individual peaks were identified by comparison of their retention indices (relative to C9–C25 n-alkanes for HP-

5MS) to those of authentic samples and literature as well as by comparing their mass spectra with the Wiley v9-MS library (Wiley, New York, NY, USA) and NIST14 (National Institute of Standards and Technology; Gaithersburg, MD, USA) mass spectral database.

6.2.7. Descriptive sensory analysis of olive oil

Four olive oils of each irrigation treatment were analyzed by an accredited sensory panel with the purpose to determine olive oils commercial quality as described by the European regulation (EEC, 2568/91). With that objective, oils were sent to the Laboratorio Agroalimentario de Granada (Granada, Spain) (ENAC number: 276/LE 507).

Additionally, 8 panelists from the Research Group “Food Quality and Safety” (Universidad Miguel Hernández; Alicante, Spain) analyzed the same oils to fully understand how deficit irrigation techniques affected the olive oil sensory characteristics (Image 9). This panel had more than 600 h of training in sensory analysis, especially of fruit and vegetables, and it consisted of 4 males and 4 females aged from 25 to 55 years old.

The panelists did three orientation days in order to determine the scales of each attribute and the reference product. A previous lexicon developed by this panel was used (Vazquez-Araujo et al., 2015) following International Olive Council (IOC) (IOC, 2007) and European Regulation (EEC, 2568/91). The scale ranged from 0 to 10, and the reference products were adapted to the Spanish market These descriptors were divided into 3 categories: (i) Flavor (positive attributes): fruity-olive, fruity-green, fruity-ripe, floral, green-artichoke, green-avocado, green-banana, green-herbs, green-grass, green-peppery, apple, buttery, almond, walnut, woody, piney, sweet, sour, and bitter; (ii) Flavor (negative attributes): oxidized, painty, rancid, musty, and muddy; (iii) Mouthfeel: astringent, pungent, and viscosity.

Image 2. Descriptive sensory analysis by "Food Quality and Safety" Research Group.

6.3. Statistical analysis

The present section is a summary of the data analysis run in the thesis: one-way analysis of variance (ANOVA) and Tukey's multiple range test were performed to compare experimental data and determine significant differences among irrigation treatments. Differences were considered statistically significant at three levels: (i) $p< 0.05$ (*), (ii) $p<0.01$ (**) and (iii) $p<0.001$. A three-way ANOVA was used (factor 1: irrigation treatment; factor 2: session; factor 3: panelist) to study the effect of these three factors on the composition, quality, and functionality of the olive oils under study and to check panel consistency. Pearson correlation and correlation coefficients were done to correlate all data with water stress integral. XLSTAT (Version 2016.02.27444, Addinsoft, Paris, France) was used to perform all statistical analysis.

7. PUBLICATIONS

7.1. First Publication

Publication 1 (literal transcription)

Effect of Spanish-style processing on the quality attributes of *HydroSOStainable* green olives

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Effect of Spanish-style processing on the quality attributes of *HydroSOStainable* green olives

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Abstract

BACKGROUND: Three treatments of regulated deficit irrigation (RDI) were assayed on olive trees for table olive production. RDI provides *hydroSOStainable* crops. The effect of RDI treatments on the quality of raw and table olives was determined based on their: weight, pit weight, fruit/pit ratio, size, texture, colour, mineral content, antioxidant activity, total phenol content and organic acid and sugar profile.

RESULTS: *HydroSOStainable* olives showed the most attractive shape and colour: highest fruit weight, roundest fruit, hardest texture and a lightest and greenest colour than control olives. Minerals, antioxidants, phenols and organic acids and sugars of *hydroSOStainable* olives were similar to control olives. After processing to table olives, calcium, potassium, antioxidants and phenols contents decreased, whereas sugars and organic acids profiles changed in both types of olives.

CONCLUSIONS: *HydroSOStainable* table olives offer environmental and quality advantages over control olives given the reduced use of fresh water and favourable morphological traits, which are more attractive for consumers.

Keywords: '*Manzanilla*'; *Olea europea* L.; regulated deficit irrigation; antioxidant activity; total phenol content; minerals

INTRODUCTION

Olive trees are a millenarian crop that was extended by Romans, Phoenicians and Arabs through the Mediterranean countries. There are different olive varieties; some of them are used to extract olive oil and others for table olives because of their physical properties (volume, shape, firmness, etc.). Table olives are one of the most consumed appetizers in the world; in fact, in the last five years, an amount of 2.5 million tons per year have been consumed. Twenty-one per cent of these table olives were produced in Spain, which is the main producer and exporter of table olives in the world; moreover, Andalusia (in particular, Seville) is the main region in Spain producing table olives.[1]

'*Manzanilla*' is the most valued table olive variety because of its high productivity and its good fruit quality. In Spain, it is typical to harvest olives when they are green to process them following the Spain-style while in the United States, it is traditional to process them using the Californian-style by oxidation of mature olives. '*Manzanilla*' olives have medium aptitude for oil extraction whereas its oil has good quality and stability; therefore, this variety is perfect to be processed to table olives.[2] '*Manzanilla*' olives have a thin peel and the flesh is delicate, hard, pulpy, tasty, and non-fibrous.[3] Furthermore, the removal of the flesh from the pit is very easy[2]; this characteristic is important for the industry because it makes the pitting process easier.

Raw olives are firm and bitter, so some processes are necessary to make them edible. Table olives can be processed using different techniques, and the Spanish-style is the most common in Spain. It consists of: (i) treatment of debittering, to hydrolyse ouleoperin, (ii) washing process, to remove alkali, and (iii) lactic acid fermentation.[4] During alkali treatment, some components, such as tocopherol and free fatty acids, diffuse from the olive peel to the surrounding liquid.; this process increases the cellular membrane permeability and contributes to a decreased peel firmness.[5]

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Table olives composition is different from other fermented vegetables due to their high content of phenolic compounds and fatty acids, mainly oleic acid (monounsaturated), thus, it can be considered a functional food. Table olives bioactivity can be influenced by many factors, such as cultivation technique and type of process to turn raw olives into table olives.[6]

Although olive trees were traditionally rain-fed, some years ago periodic irrigation was implemented in this crop because of the intensification of agriculture. This practice generates some benefits on olive production and was established following FAO (Food and Agriculture Organization of the United Nations) recommendations[7]; and, it is essential in regions where rain is concentrated only in autumn – winter (specific microclimatic conditions),[8,9]. Nowadays, regulated deficit irrigation (RDI) is a watering technique that is widely studied due to an increasing water scarcity. Many studies have concluded that different RDI treatments can affect some table olives characteristics, such as phenolic composition, antioxidant activity, fatty acids composition, volatile compounds, phytoprostanes, etc.[10]

RDI table olives belong to a group of vegetable products named *hydroSOStainable*. These products are characterized by having unique characteristics: (i) high intensity of some key sensorial attributes, (ii) high content of some nutritional and functional components, and (iii) reduced use of water, which is a benefit for both farmers (economic benefit) and for the environment (water sustainability).[11,12]

Different studies have been carried out to improve the quality of different varieties of RDI olives at different locations,[13,14] and it is very important to determine the effect of each new watering technique on fruit quality. Therefore, the main aim of this research was to investigate the effect of three new RDI treatments applied to olive trees on the quality attributes of olives, particularly their morphology, physico-chemical properties, antioxidant activity, mineral composition, organic acids, and sugar composition. These parameters were studied in raw and table olives (after processing using the Spanish-style); thus, the second aim of this research was to investigate the effects of processing on the quality attributes of '*Manzanilla*' olives.

MATERIALS AND METHODS

Plant material, growing conditions and experimental design

Olives were collected from a farm, Doña Ana, which is located in Dos Hermanas (Seville, Spain). Table olive trees (*Olea europea* L. cv Manzanilla) were 30-years-old. Further detail of collection and experimental design can be found in Corell *et al.*[14]

Irrigation scheduling was performed following pressure chamber technique and the threshold values of midday stem water potential before and after pit hardening period. Three different irrigation treatments and a control were carried out:

1 *Optimum water status* (T0): trees were watered to avoid any water stress (−1.2 MPa before pit hardening and −1.4 MPa after pit hardening).
2 *Moderate deficit irrigation* (T1): trees were watered following same way as T0 but during pit hardening period threshold value was −2 MPa.
3 *Severe deficit irrigation (short time)* (T2): same scheduled as for control trees was followed, but threshold value was −3 MPa during half time of pit hardening.
4 *Severe deficit irrigation (long time)* (T3): trees were watered following same way as T2 but threshold value of −3 MPa was maintained until end of pit hardening period.

Table 1. Minimum midday stem water potential (min ψ_{stem}) and water stress integral (SI) in each regulated deficit irrigation treatment in each year

	Treatment	Min ψ_{stem} (MPa)	SI (MPa × day)
ANOVA[a]			
	2015	***	***
	2016	***	*
Multiple range Tukey test			
2015	T0	−1.76 a[b]	1.4 b
	T1	−1.96 ab	2.7 ab
	T2	−1.84 ab	1.2 b
	T3	−2.4 b	4.7 a
Multiple range Tukey test			
2016	T0	−2.34 a	29.6 b
	T1	−2.76 ab	62.6 ab
	T2	−2.74 ab	50.4 ab
	T3	−3.52 b	87.4 a

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

Stem water potential at midday (Ψ) was measured using a pressure chamber (PMS Instrument Company, Albany, OR, USA), Table 1 shows the average of the minimum values observed in each treatment. In order to describe the accumulative effect of the water deficit, the water stress integral was calculated from the Ψ data[15] during the period of beginning pit hardening until harvest (Eqn 1). Equation (1) used a reference of −1.4 MPa. The expression used was:

$$SI = \left| \sum (\Psi - (-0.2)) \times n \right| \quad (1)$$

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

Sample processing

Two harvesting seasons were evaluated: 2015 and 2016. For each RDI treatment and season, four batches were completed for later analysis as raw fruit (raw olives). Each batch consisted of 5 kg of olives in 2015 and 50 kg in 2016 season. Remaining harvested fruits were mixed and transported to *Cooperativa Nuestra Señora de las Virtudes* (La Puebla de Cazalla, Seville, Spain) to be processed as table olives using the Spanish-style method as described by Cano-Lamadrid *et al.*[3] Once processed sampling of table olives followed the same procedure described for raw olives.

Morphological analyses

Each treatment was performed on four batches. Twenty-five olives from each batch were randomly selected to conduct measurements. Thus, 100 olives for each treatment were analysed and used for all physico-chemical analyses in each of the two seasons.

Weight and size

Longitudinal and equatorial diameters were measured on each olive using a digital calliper (model 500-197-20 150 mm; Mitutoyo Corp., Aurora, IL, USA). Whole olives were weighed (model AG204 scale; Mettler Toledo, Barcelona, Spain) and, then, each pit was removed and weighted.

Colour determination
Colour determinations were made using a colorimeter (model CR-300, Minolta, Osaka, Japan) which uses an illuminant D65 and 10° observer. Colour was measured three times per olive at 25 ± 1 °C. Colour results were given as CIE $L^*a^*b^*$ coordinates. This system defines colour in a three-dimensional space: (i) L^* indicates lightness (0 – 100 values); (ii) a^* is green-red coordinate, taking green and red colour negative and positive values, respectively; and, b^* is blue-yellow coordinate, taking blue and yellow colour negative and positive values, respectively.

Texture
Texture analysis was carried out according to Szychowski *et al.*[16] Briefly, two methods were used:

1 Puncture test (PT), using a stainless-steel needle probe P/2 N (2 mm thickness) into the whole fruit (0.5 mm s^{-1}; 7 mm of penetration) trying to avoid the needle touching the pit.
2 Magness– Taylor test (MTT) with a stainless-steel cylindrical probe SMSP/2 of 2 mm diameter. To conduct this test, the peel of the olives was removed in approximately 1 cm^2 (0.3 mm s^{-1}; 5 s of penetration) on olive flesh.

Analysis of texture were conducted at 25 ± 2 °C using a Texture Analyser TA-XT2i (Stable Micro Systems, Godalming, UK). Analyses were conducted in 25 olives per batch and results were expressed in *N*.

Mineral analysis
A representative amount of olives from each batch was freeze dried. Mineral analysis was carried out as described by Carbonell-Barrachina *et al.*[17] A multi-place digestion block (Digest 20, Selecta) was used to digest 0.5 g of freeze-dried olives. Samples were digested for 2 h at 130 °C using 5 mL of 65% nitric acid (HNO_3) (*w*/*v*). Later, samples were diluted with ultra-high-purity deionized water (1:10 and 1:50) and afterwards cooled at room temperature.

Determination of calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), and zinc (Zn) in the mineralized samples was performed using a Unicam Solaar 969 atomic absorption-emission spectrometer (Unicam Ltd., Cambridge, UK). Potassium was analysed using atomic emission, while the rest of the elements were analysed by atomic absorption. Calibration curves and blank reagent were used in each analytical batch. The analyses were run in triplicate.

Antioxidant activity (AA) and total phenol content (TPC)
Extraction of antioxidants was done with methanol as described previously by Cano-Lamadrid *et al.*[10] Three methods were used to evaluate the antioxidant activity (AA) of the studied olive samples. Radical scavenging activity was evaluated using DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams *et al.*[18] The absorbance decrease was measured at 515 nm. The free radical scavenging capacities were determined using the $ABTS^+$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) method described by Re *et al.*,[19] and FRAP (ferric reducing antioxidant power) method, as described by Benzie and Strain.[20] Absorbance was measured at 734 nm and 593 nm for $ABTS^+$ and FRAP, respectively. Analysis was carried out in a UV-visible spectrophotometer (Helios Gamma model, UVG 1002E). Calibration curves (3.5 – 5.0 mmol Trolox L^{-1}) with good linearity ($R^2 \geq 0.999$) were used for the quantification of the AA by these methods. Analyses were run in triplicate and results were expressed as mmol Trolox per kilogram of fresh weight (fw).

The extracts used to measure total phenolic content (TPC) were the same as those previously describe for the AA analysis.[10] TPC was quantified using Folin – Ciocalteu reagent, as described by Gao *et al.*[21] Absorbance was measured using an UV-visible spectrophotometer (Helios Gamma model, UVG 1002E) at 765 nm. Gallic acid was used to prepare calibration curves. This analysis was run in triplicate and results were expressed as gallic acid equivalents (GAE) per kilogram of fw.

Organic acids and sugars
Organic acid and sugar profiles were determined according to Cano-Lamadrid *et al.*[10] Briefly, 2 g of freeze-dried olive sample were mixed with phosphate buffer 50 mM (pH 7.8) and centrifuged (Sigma 3 – 18 K; Sigma Laborzentrifugen, Osterode and Harz, Germany). Then, 10 μL of the filtered supernatant (0.45 μm filter) were injected into a Hewlett Packard (Wilmington, DE, USA) series 1100 high-performance liquid chromatography (HPLC) system using 0.1% ortophosphoric acid elution buffer. Organic acid separation was made 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, USA). Absorbance was measured with a diode-array detector (DAD) at 210 nm for organic acids. Sugars were separated and detected in the same run but they were monitored using a refractive index detector (RID). Calibration curves were made using standards of different organic acids and sugars provided by Sigma (Poole, UK). Analyses were run in triplicate and results were expressed as grams per kilogram of fw.

Statistical analysis
Results were the average results from two seasons, and include, three replications for each one of the four batches included in each irrigation treatment. One-way analysis of variance (ANOVA) was carried out to study the effect of RDI treatments on table olives and raw olives, and then Tukey's multiple range test was used to compare the means. The effect of the Spanish-style processing was also studied. Statistical differences were considered significant when $P < 0.05$. All statistical analyses were performed using IBM SPSS Statistics v21.0 Core System software package (SPSS Inc. an IBM Company, Chicago, IL, USA).

RESULTS AND DISCUSSION

Irrigation
Apply RDI was possible using crop water status, through midday stem water potential. Four irrigation treatments were applied with different levels and durations of stress. As Table 1 shows, irrigation treatments showed different levels of stress, with T0 showing the lowest values of minimum stem water potential and stress integral, and T3 showed significantly higher levels of stress. Levels of stress varied mainly depending on tree load, in 2015 the load of the trees was 15% of the 2016 load, this could explain the difference between the values of the two seasons studied.

Morphological analysis
Weight, size, dry matter content (DMC), texture (peel and flesh) and colour of raw olives and table olives are shown in Table 2. Raw

Table 2. Morphological analyses [fruit weight (fw), pit weight, fruit/pit ratio, equatorial diameter, longitudinal diameter, dry matter content (DMC), puncture test (PT), Magness-Taylor test (MTT), CIE *L*a*b**] of '*Manzanilla*' raw olives (ROs) and table olives (TOs) as affected by regulated deficit irrigation (RDI) treatments

	Fruit weight (g)[c]	Pit weight (g)[c]	Fruit/ pit ratio[c]	Equatorial diameter (mm)[c]	Longitudinal diameter (mm)[c]	DMC (g dw kg^{-1} fw)[d]	Texture[c]		Colour[c]		
							PT(*N*)	MTT (*N*)	*L**	*a**	b*
ANOVA[a]											
Irrigation RO	***	***	***	**	*	**	***	***	*	***	NS
Irrigation TO	***	***	***	*	*	*	***	**	**	*	**
Spanish-style processing	***	NS	***	*	*	*	***	**	**	***	**
Multiple range Tukey test ROs											
T0	4.43 b[b]	0.76 a	5.83 b	19.3 b	21.3 a	328 b	1.28 b	13.1 b	57.3 ab	−12.9 a	38.3
T1	4.45 b	0.73 a	6.09 ab	19.2 b	21.3 a	341 a	1.35 b	12.9 b	56.9 b	−12.5 a	38.0
T2	4.66 a	0.74 a	6.29 ab	19.5 a	21.5 a	321 b	1.44 b	10.2 c	57.2 b	−12.4 a	37.9
T3	4.13 c	0.64 b	6.45 a	19.7 a	20.4 b	341 a	2.54 a	19.1 a	59.9 a	−19.1 b	38.2
Multiple range Tukey test TOs											
T0	4.20 a	0.75 a	5.60 a	19.0 ab	19.5 a	330 b	1.07 b	6.52 b	55.6 b	0.64 ab	36.4 ab
T1	4.02 a	0.73 a	5.51 a	18.7 b	19.2 a	338 a	1.28 b	5.95 b	55.3 b	0.54 b	36.9 a
T2	3.97 a	0.73 a	5.44 a	18.8 ab	18.9 a	332 b	1.40 b	4.92 c	55.4 b	0.70 a	36.2 b
T3	2.81 b	0.61 b	4.60 b	19.2 a	14.9 b	341 a	1.85 a	7.25 a	56.4 a	0.62 ab	37.0 a
Multiple range Tukey test Spanish-style processing											
ROs	4.42 a	0.72	6.14 a	19.4 a	21.1 a	317 a	1.65 a	13.8 a	57.8 a	−14.2 b	38.1 a
TOs	3.75 b	0.71	5.28 b	18.9 b	18.1 b	309 b	1.40 b	6.03 b	55.7 b	0.62 a	36.7 b

[a] NS = not significant at $P < 0.05$; *, **, and ***, significant at $P < 0.05$, 0.01, and 0.001, respectively.
[b] Values followed by the same letter within the same column and analysis of variance (ANOVA) treatments (RO, TO, Spanish-style processing), were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.
[c] $n = 200$.
[d] $n = 16$.

Table 3. Minerals (macro-elements and micro-elements) content of '*Manzanilla*' raw olives (ROs) and table olives (TOs) as affected by regulated deficit irrigation (RDI) treatments

	Macro-elements[c]		Micro-elements[c]		
	Ca (g kg^{-1} fw)	K (g kg^{-1} fw)	Mg (mg kg^{-1} fw)	Zn (mg kg^{-1} fw)	Cu (mg kg^{-1} fw)
ANOVA[a]					
Irrigation RO	NS	NS	NS	NS	NS
Irrigation TO	NS	NS	NS	NS	NS
Spanish-style processing	***	***	NS	NS	NS
Multiple range Tukey test ROs					
T0	0.47[b]	4.96	0.13	2.07	1.72
T1	0.51	4.84	0.14	2.17	1.87
T2	0.54	4.70	0.12	2.29	2.06
T3	0.54	4.75	0.13	2.07	1.62
Multiple range Tukey test TOs					
T0	0.40	0.95	0.15	2.01	1.98
T1	0.27	1.07	0.14	2.12	1.72
T2	0.40	1.10	0.13	1.83	1.45
T3	0.37	1.12	0.14	2.19	1.80
Multiple range Tukey test Spanish-style processing					
ROs	0.52 a	4.81 a	0.13	2.15	1.79
TOs	0.36 b	1.06 b	0.14	2.03	1.74

[a] NS = not significant at $P < 0.05$; *, **, and ***, significant at $P < 0.05$, 0.01, and 0.001, respectively.
[b] Values followed by the same letter within the same column and analysis of variance (ANOVA) treatments (RO, TO, Spanish-style processing), were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.
[c] $n = 16$.

olives from T2 had the highest fruit weight (4.66 g), while T3 had the lowest weight (4.13 g). Previous studies reported similar results showing that strongest RDI treatments decreased the weight of olive fruits, but moderate treatments yielded larger fruits.[3,7,10] T3 olives had the highest fruit/pit ratio (6.45); this ratio increased as irrigation decreased.

The shape of raw olives changed with RDI; the differences between the longitudinal and the equatorial diameters were bigger for T0 and T1 fruits as compared to fruits from the other treatments. However, T3 showed the roundest olives (highest equatorial and smallest longitudinal diameter). Although not many differences were found in diameters, there was a trend showing that RDI treatments lead to rounder olives than control; this can be an advantage of RDI on fruit shape because consumers usually prefer rounded '*Manzanilla*' olives.[10]

Regarding DMC of raw olives, T0 and T2 had the lowest contents while T1 and T3 had the highest. These results were logical because a higher moisture content is expected in fruits from trees with abundant irrigation volume, leading to higher moisture content, and consequently, lower content of dry matter. These results agreed with the results previously reported by Cano-Lamadrid *et al.*[3,10]

The texture tests showed that T3 raw olives had the hardest peel (PT) and flesh (MTT), while no differences were found among the hardness of the other types of olives. Flesh was the softest while the hardest one was that from T3 trees; which again is logical due to the highest DMC of T3 fruits. Texture of olives is a main attribute for consumer's acceptance and for the industry; thus, from a texture point of view, T3 raw olives are very interesting because they had strong peel and hard flesh.

Colour results showed differences in lightness (L^*) and the green-red coordinate (a^*). T3 olives were the lightest and greenest ones. These results agreed with those of a previous study with the same olive variety (but different irrigation treatments) in which the most severe treatment yielded the lightest and greenest olives.[10]

Table olives morphological characteristics were similar to those of raw olives. T3 yielded the roundest, hardest, lightest, and greenest table olives, although T3 fruits had the lowest fruit and pit weight. The fruit/pit ratio in table olives did not show differences among control, T1, and T2; whereas T3 fruits had the smallest ratio.

Clear differences have been found before and after processing for: fruit weight, fruit/pit ratio, equatorial and longitudinal diameters, DMC, texture attributes (PT and MTT), lightness, green and yellow colours intensity; in all of them the values of these parameters decreased after processing. These differences could be due to osmotic dehydration by the effect of addition of sodium chloride (NaCl)[22] and the solubilization of the components from the olive flesh to the surrounding fermentation liquid.

Mineral analysis

Mineral composition of raw olives and table olives is shown in Table 3. Magnesium, Zn and Cu showed mean contents of approximately 0.10 g kg^{-1} fw, 2.10 mg kg^{-1} fw and 1.70 mg kg^{-1}, respectively; no statistically significant differences were found for RDI treatments or processing. Calcium and K were also not affected by irrigation, but processing decreased their concentration (from 0.52 to 0.36 g kg^{-1} fw and from 4.81 to 1.06 g kg^{-1} fw, respectively).

The decrease on Ca content could be explained because of the ion exchange and, consequently, the formation of some salts like calcium chloride ($CaCl_2$) or calcium lactate.[23] Calcium and K are highly soluble in the acidic surrounding media, and it can be replaced by sodium (Na) in the olive flesh and can be lost during the washing step. These results agreed quite well with

Table 4. Antioxidant activity (AA) and total phenol content (TPC) of '*Manzanilla*' raw olives (ROs) and table olives (TOs) as affected by regulated deficit irrigation (RDI) treatments.

	ABTS+ (mmol Trolox kg^{-1} fw)[c]	DPPH• (mmol Trolox kg^{-1} fw)[c]	FRAP (mmol Trolox kg^{-1} fw)[c]	TPC (g GAE kg^{-1} fw)[c]
ANOVA[a]				
Irrigation RO	NS	NS	NS	NS
Irrigation TO	NS	NS	NS	NS
Spanish-style processing	***	***	***	***
Multiple range Tukey test ROs				
T0	27.1[b]	48.7	24.8	19.4
T1	26.3	48.9	25.1	19.6
T2	26.3	48.1	24.5	20.4
T3	26.3	49.2	24.7	19.6
Multiple range Tukey test TOs				
T0	6.67	9.55	15.5	5.77
T1	6.88	9.38	15.2	5.81
T2	6.70	9.71	15.2	5.74
T3	6.87	9.75	15.3	5.82
Multiple range Tukey test Spanish-style processing				
ROs	26.5 a	48.7 a	24.8 a	19.8 a
TOs	6.78 b	9.60 b	15.3 b	5.79 b

[a] NS = not significant at $P < 0.05$; *, **, and ***, significant at $P < 0.05$, 0.01, and 0.001, respectively.
[b] Values followed by the same letter within the same column and analysis of variance (ANOVA) treatments (RO, TO, Spanish-style processing), were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.
[c] $n = 16$.

those of a study with different olive varieties, in which K and Ca concentrations during fermentation were reported to also decrease.[24] In this study the decrease was explained by elution due to the washing steps of processing.

Antioxidant activity (AA) and total phenol content (TPC)

Antioxidants can be defined as compounds that have the ability to reduce pro-oxidant agents. This property is important to consumers, so it is interesting to analyse their concentration in food matrices. Antioxidant compounds can act by different mechanisms; thus, the combination of different analytical protocols in the same food matrix is the best way to describe AA in detail.[25] Therefore, three different electron-transfer-based methods (ABTS+, DPPH•, FRAP) were used in this study.

Fresh olives are recognized as a highly antioxidant fruit. In fact, high values of AA (27.1, 48.7, and 24.8 mmol Trolox kg^{-1} fw for ABTS+, DPPH•, FRAP, respectively) and total phenol content (TPC) (19.4 g GAE kg^{-1} fw) were found in 'Manzanilla' raw olives (Table 4). In addition, TPC showed a significant positive relationship (Pearson correlation) with all antioxidant methods (0.432 for ABTS+, 0.582 for DPPH• and 0.386 for FRAP).

During processing to table olives, the TPC and the values of AA decreased, and the type of processing significantly influenced such losses. The Spanish-style green olives experienced loss of AA of 72, 80, and 38% for ABTS+, DPPH•, and FRAP, respectively, and 70% for TPC. According to the literature,[26] the Spanish-style processing of green olives affects the loss of TPC depending on the ripening stage of fresh olives, the debittering method employed, and the fermentation type used. During fermentation, the contents of some phenols (hydroxytyrosol, tyrosol, and oleoside-11-methyl ester) decrease because of diffusion to the preservation liquid due to acidic pH. Another important change during fermentation is the conversion of oleoside-11-methyl ester to elenoic acid, which is rapidly broken down due to the acidic conditions.[26]

Regarding irrigation treatments, no significant statistical differences were found for raw or table olives. Other authors reported similar results also in table olives, but after application of different RDI treatments.[10]

Organic acids and sugars

Table 5 shows the contents of organic acids and sugars in raw olives. Citric, tartaric, malic, and succinic acids were found at concentrations of 0.27, 0.11, 0.46 and 0.16 g kg^{-1} fw, respectively. However, sucrose (1.72 g kg^{-1} fw), glucose (2.55 g kg^{-1} fw), and fructose (1.39 g kg^{-1} fw) were the sugars identified in this raw material. Concentrations of organic acids and sugars in table olives are shown in Table 6, and it can be observed that no significant effects were found as a consequence of the irrigation treatment; thus, mean values of all four treatments will be discussed. In this matrix, the organic acids found were phytic acid (mean value of all treatments 6.88 g kg^{-1} fw), lactic acid (1.60 g kg^{-1} fw) and acetic acid (0.62 g kg^{-1} fw), while maltoheptaose, mannitol, and glycerol were the main sugars found (2.16, 2.70 and 0.98 g kg^{-1} fw, respectively).

Concentration of organic acids and sugars was not significantly affected by any RDI treatment for either the raw olives (Table 5) or the table olives (Table 6). The main observed changes were found in the profiles of organic acids and sugars due to processing. Lactic acid bacteria transform sugars in raw olives into CO2, lactic acid and other organic acids.[27] It is important to study the profile of organic acids in raw olives because some of them are related to degradation or synthesis of other compounds, such as malic and citric acids. These two organic acids play an important role in oil accumulation during the Krebs cycle. Similar results were found in a study that identified citric, malic and succinic acids in

Table 5. Sugars and organic acids of '*Manzanilla*' raw olives (ROs) as affected by regulated deficit irrigation (RDI) treatments

Irrigation treatment	Citric acid[b] (g kg^{-1} fw)	Tartaric acid[b]	Malic acid[b]	Succinic acid[b]	Sucrose[b]	Glucose[b]	Fructose[b]
ANOVA[a]	NS	NS	NS	NS	NS	NS	NS
Multiple range Tukey test							
T0	0.25	0.12	0.43	0.14	1.59	2.55	1.34
T1	0.30	0.11	0.48	0.15	1.83	3.07	1.54
T2	0.27	0.11	0.45	0.16	1.75	1.84	1.30
T3	0.27	0.11	0.47	0.20	1.71	2.75	1.39

[a] NS = not significant at $P < 0.05$.
[b] $n = 16$.

Table 6. Sugars and organic acids of '*Manzanilla*' table olives (TOs) as affected by regulated deficit irrigation (RDI) treatments

Irrigation treatment	Phytic acid[b] (g kg^{-1} fw)	Lactic acid[b]	Acetic acid[b]	Maltoheptaose[b]	Mannitol[b]	Glycerol[b]
ANOVA[a]	NS	NS	NS	NS	NS	NS
Multiple range Tukey test						
T0	7.53[b]	1.64	0.53	2.30	3.15	1.23
T1	6.44	1.57	0.63	2.10	2.52	0.91
T2	6.82	1.61	0.66	2.14	2.58	0.89
T3	6.73	1.59	0.64	2.10	2.55	0.90

[a] NS = not significant at $P < 0.05$.
[b] $n = 16$.

different varieties of raw olives.[28] In raw olives, sucrose, glucose, and fructose were identified and quantified. These sugars are naturally present in the olive flesh because of transport by phloem from mature leaves and by formation by photosynthesis and they are very important for the fruit growth and lipid biosynthesis. These three sugars were also identified in other studies as the main sugars in raw olives.[29]

After processing, the profile of sugars and organic acids changed. Phytic acid, lactic acid, acetic acid, maltoheptaose, mannitol and glycerol appeared in table olives. Previous studies also identified these compounds in different table olive varieties.[4,10,30]

CONCLUSIONS

The present study is the first to evaluate the effects of three watering techniques on olive trees to produce *HydroSOStainable* table olives. RDI treated olives had higher fruit weight, rounder fruits with hardest texture, with a lighter but greener colour than control olives; therefore, the *HydroSOStainable* table olives were attractive to consumers. Regarding mineral composition, AA, TPC, and organic acids and sugars profiles, RDI fruits showed no statistical differences from control olives; thus, it can be said that *HydroSOStainable* table olives had equivalent composition to that of the control or conventional fruits. Furthermore, the 'Spanish-style' processing induced decreases in the contents of some minerals (Ca and K), AA and TPC concentrations due to fermentation in all types of olives regardless of the watering system. Other effects of processing were significant changes in the organic acid and sugar profiles.

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7.2. Second Publication

Publication 2 (literal transcription)

Effect of regulated deficit irrigation on the quality of raw and table olives

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Effect of regulated deficit irrigation on the quality of raw and table olives

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A B S T R A C T

Fresh water scarcity is a major worldwide issue. There is a need to reduce water use whereas preserving the quality of food products. Regulated deficit irrigation (RDI) is a strategy to reduce fresh water consumption. The aim of this work was to study the effect of RDI on olives when applied before harvesting, without a rehydration period, on the quality of table olives. The experiment was performed in "La Hampa", the experimental farm of IRNAS-CSIC at Coria del Río (Seville, Spain) during 2015 and 2016. Two deficit irrigation treatments were compared with a full irrigated control. Treatment 1 (T1) reduced irrigation from early September, about 2 weeks before harvest, until values of midday stem water potential were around −2 MPa. Treatment 2 (T2) reduced irrigation from mid-August, about 4 weeks before harvest, with a similar water stress level. The duration and level of water stress was described with the stress integral (SI). Fruit features were studied, before and after the industrial process to obtain Spanish-style table olives, in order to evaluate differences due to irrigation on raw olives and due to processing on table olives. Water stress conditions slightly changed olive characteristics, affecting size and composition. The industrial processing to table olives masked differences between irrigation treatments, though some features such as total polyphenols content (TPC) were still different. SI was significantly related with fruit weight, pit weight, equatorial diameter, linolenic acid and MUFAs content and (MUFA/PUFA)/SFA ratio.

1. Introduction

Olive tree has been, traditionally, one of the most cultivated trees under rainfed conditions, but in the recent years, the intensification of agriculture forced farmers to implement irrigation following Food and Agriculture Organization of the United Nations (FAO) recommendations. Nowadays, fresh water resources are scarce even for non-agricultural applications. Regulated deficit irrigation (RDI) is a technique that reduces the use of water and it has been already tested on olive crops. Several benefits on oil composition such as the improvement of phenolic compounds content, phytoprostanes, fatty acids, etc. have been related with RDI of olive crops (Cano-Lamadrid et al., 2015; Collado-González et al., 2015; Sánchez-Rodríguez et al., 2019a).

"HydroSOStainable" vegetables are products with unique characteristics, including the reduced use of water (economic benefit for farmers and water sustainability for the environment), and the enhanced concentration of some functional components of the vegetables (Carbonell-Barrachina et al., 2015; Noguera-Artiaga et al., 2016).

Spanish-style process is one of the most typical methods for preparing table olives (TO) in Spain. It is based on the lye treatment and fermentation of green olives to allow raw olives (RO) to become edible. This type of TO is one of the most consumed appetizer in the Mediterranean countries due to their taste and functional properties. Table olives are a nutritional rich food because they provide proteins, carbohydrates, lipids, dietary fiber, minerals (phosphorous, iron, calcium, magnesium, potassium, copper, zinc, manganese) and vitamins (vitamin E, B-complex, β-carotene) (Boskou et al., 2015). Some of the most valued functional properties are due to the fatty acid profile (high content of oleic acid) and the non-dietary constituents. These healthy compounds lend to table olives some health benefits associated with

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cardiovascular, immune, nervous, respiratory and digestive systems (Boskou et al., 2015).

It is well known that the olive fruit development in the tree could be divided in three stages: *stage I* fruit growth, *stage II* pit hardening, and *stage III* oil accumulation and maturation (Goldhamer, 1999). In previous studies, when water stress techniques were applied during stage II, the effect in yield was not significant and some beneficial properties were reported on "hydroSOStainable" table olives (Cano-Lamadrid et al., 2015). However, if the stress is applied during stage III, it has to be taken into account that it is a critical period for compounds synthesis, and so, special attention needs to be paid to the changes in the concentration of some compounds. Thus, it is necessary to study the effect of applying RDI during stage III of fruit growth on the both RO and TO composition after Spanish-style process. Therefore, the aim of this work was to study the effect of two RDI treatments in the morphological parameters (fruit and pit weight, fruit/pit ratio, equatorial and longitudinal diameters, texture and color), antioxidant activity ($ABTS^{•+}$, $DPPH^{·}$ and FRAP), total phenol content (TPC), fatty acid profile, organic acids and sugars of RO and TO. Experiments and analyses were carried out in two seasons (2015 and 2016).

2. Materials and methods

2.1. Experimental conditions, treatments and sample processing

Olives were collected from La Hampa, the experimental farm of the *Instituto de Recursos Naturales y Agrobiología* (IRNAS-CSIC). This orchard is located in Coria del Río, near Seville (Spain) (37°17″N, 6°3′W, 30 m altitude). Table olive trees (*Olea europaea* L. cultivar Manzanilla) were 43-year-old. Experimental design was randomized completely in blocks with 3 replicates and 2 control trees per plot. Irrigation scheduling was performed following pressure chamber technique and the threshold values of midday stem water potential before and after pit hardening period. Two different irrigation treatments and a control were carried out:

i) *Control* (T0), no water stress conditions. Irrigation was scheduled using pressure bomb technique according to the recommendations of (Moriana et al., 2012)
ii) *Moderate deficit irrigation before harvest* (T1) irrigation was reduced at the beginning of September to reach a water stress level around −2 MPa.
iii) *Moderate deficit irrigation for long time* (T2) a period of restriction from mid-August with the same water stress level than T1 (Table 1).

Olives were hand-harvested in September 2015 and 2016 at their mature-green stage. Raw olives were processed to obtain table olives using Spanish-style method in *Cooperativa Nuestra Señora de las Virtudes* (La Puebla de Cazalla, Seville, Spain). Firstly, olives were cleaned and selected by size; then, raw olives were treated during 6–8 h with 1.3–2.6 % (weight:volume) of NaOH to remove ouleoperin. After lye penetrated ¾ through the flesh, olives were washed with water during 12–14 h. After cleaning, olives were put on 10–12 % NaCl concentration for fermentation process. At the end of fermentation, table olives reached an equilibrium with fermentation brine (pH < 4.2, 8–9 % (weight:volume) of NaCl, 0.7–1.0 % lactic acid and residual alkalinity < 0.120 N).

2.2. Morphological analyses

Seventy-five raw olives and table olives from each irrigation treatment [25 fruits of each irrigation replicate (3)] were randomly selected to characterize physicochemical properties. Same olives were used for all determinations.

2.2.1. Weight and size

Whole fruits and pits were weighed (Mettler balance model AG204 scale; Mettler Toledo, Barcelona, Spain). The size of each fruit: longitudinal and equatorial diameters were measured (digital caliper, model 500-197−20 150 mm; Mitutoyo Corp., Aurora, IL, USA) (Sánchez-Rodríguez et al., 2019b).

2.2.2. Color determination

Three measurements were made around the equatorial diameter of each olive using a digital colorimeter (D65 illuminant and 10° observer references) (model CR-300, Minolta, Osaka, Japan). Results were given following a system which define color in a three-dimensional space (CIE $L^*a^*b^*$), where L^* defined lightness, a^* reddish (positive values) and greenish (negative values) and b^* yellowish (positive values) and bluish (negatives values) (Sánchez-Rodríguez et al., 2019b).

2.2.3. Texture

Two different texture measurements were carried out in each olive using a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, U.K.) at 25 ± 2 °C. Puncture test (PT) was used to study peel firmness and Magness-Taylor test (MTT) to measure pulp firmness of raw olives and table olives. PT was performed with a stainless-steel needle probe P/2 N (2 mm thickness) and MTT with a stainless-steel cylindrical probe SMSP/2 of 2 mm diameter. PT was done on whole olives and MTT on olives removing 1 mm of peel. Results were expressed in N (Szychowski et al., 2015).

After measuring the previous parameters, olive pulp was removed and pits were weighed while the pulp was freeze-dried and stored frozen at -80 °C under vacuum packaging. The following determinations were run on freeze dried olive powder.

2.3. Antioxidants and total phenol content

Antioxidant activity (AA) was measured in RO and TO. Extracts were done with MeOH/water (80:20 v/v) + 1% HCl as described by Sánchez-Rodríguez et al. (2019b). AA was measured by three methods: $DPPH^{•}$, $ABTS^{•+}$ and d FRAP. Radical scavenging activity was evaluated using $DPPH^{•}$ radical (2,2-diphenyl-1-picrylhydrazyl) as described by Brand-Williams et al. (1995), $ABTS^{•+}$ radical [2,2-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] as described by Re et al. (1999) and the ferric reducing antioxidant power (FRAP) as described by Benzie and Strain (1996); Trolox was used to performed calibration curves. Furthermore, total phenol content (TPC) was quantified using Folin-Ciocalteu reagent as described by Gao et al. (2000). Gallic acid was used to carry out the calibration curve. Antioxidant activity and TPC

Table 1
Minimum ψ_{stem} (min ψ_{stem}) and water stress integral (SI) as affected by regulated deficit irrigation treatments.

Stress parameter	2015				2016				ANOVA†	2015	2016
	ANOVA†	T0	T1	T2	ANOVA†	T0	T1	T2			
Min ψ_{stem} (MPa)	NS	−2.07‡	−1.99	−2.20	NS	−2.44	−2.44	−2.66	***	−2.08 b	−2.51 a
SI (MPa x day)	NS	34.3	26.4	33.8	*	75.7 ab	62.9 b	85.5 a	**	31.5 b	74.8 a

† NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (n = 3) followed by the same letter within the same row and season were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

Table 2
Morphological parameters [fruit weight, pit weight, fruit/pit ratio, equatorial diameter, longitudinal diameter, puncture test (PT), Magness-Taylor test (MTT), CIE $L^*a^*b^*$] of "*Manzanilla*" raw and table olives as affected by regulated deficit irrigation treatments.

		2015				2016				ANOVA	2015	2016
		ANOVA†	T0	T1	T2	ANOVA	T0	T1	T2			
Raw olives												
Fruit weight (g)		NS	5.03‡	5.27	5.07	NS	3.40†	3.06	3.05	***	5.13 a	3.17 b
Pit weight (g)		NS	0.89	0.82	0.85	NS	0.65	0.62	0.62	***	0.85 a	0.63 b
Fruit/pit ratio		NS	5.65	6.43	6.00	NS	5.24	4.90	4.89	***	6.04 a	5.03 b
Equatorial diameter (mm)		NS	19.4	19.7	19.5	NS	16.4	16.1	15.9	NS	19.5	19.5
Longitudinal diameter (mm)		NS	23.5	23.6	23.5	NS	19.7	19.6	19.2	***	23.5 a	16.1 b
Texture	Puncture Test (N)	NS	2.67	2.36	2.85	NS	2.57	2.86	2.85	NS	2.71	2.75
	Magness Taylor (N)	**	19.2 a	9.02 b	10.7 b	***	17.7 a	10.3 b	10.4 b	NS	12.5	12.8 b
Color	L^*	NS	55.9	57.7	57.3	NS	59.9	58.6	59.9	NS	56.9	59.5
	a^*	NS	−18.8	−18.4	−18.3	NS	−19.2	−17.8	−18.9	NS	−18.5	−18.7
	b^*	NS	37.9	39.6	39.3	NS	41.1	39.5	41.3	NS	38.9	40.7
Table olives												
Fruit weight (g)		*	5.51 a	4.75 b	5.07 ab	NS	2.87	2.98	2.82	***	5.11 a	2.89 b
Pit weight (g)		NS	0.87	0.79	0.83	NS	0.60	0.61	0.59	***	0.83 a	0.60 b
Fruit/pit ratio		NS	6.33	6.01	6.11	NS	4.76	4.87	4.76	***	6.16 a	4.79 b
Equatorial diameter (mm)		NS	18.4	18.9	18.2	NS	15.9	15.7	15.5	***	18.5 a	15.7 b
Longitudinal diameter (mm)		***	22.4 b	23.2 a	22.1 b	NS	20.0	19.4	19.4	***	22.6 a	19.6 b
Texture	Puncture Test (N)	NS	1.21	1.06	1.23	NS	1.24	1.10	1.22	NS	1.17	1.18
	Magness Taylor (N)	NS	8.29	8.92	10.1	*	8.59 b	9.16 b	10.5 a	NS	9.10	9.44
Color	L^*	NS	53.3	51.6	55.4	NS	54.6	55.7	57.9	NS	53.4	56.1
	a^*	**	1.17 ab	1.65 a	0.78 b	NS	1.43	1.19	0.87	NS	1.20	1.16
	b^*	*	34.2 b	33.6 b	37.6 a	NS	33.3	33.8	36.2	NS	35.1	34.4

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

were measured by a UV–vis spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK).

2.4. Fatty acids

Fatty acid profile was determined according to Cano-Lamadrid et al. (2015) on freeze-dried olive pulp. Concisely, fatty acid methyl esters (FAMEs) were *trans*-methylated *in situ* by adding dichloromethane and methanolic NaOH solution followed by BF_3-methanol and boiling for 10 min and, followed by the extraction of the FAMEs using hexane. The organic layer of samples was injected on a gas chromatograph coupled with a mass spectrometer detector (GC–MS) (Shimadzu GC-17A and GC–MS QP-5050A) (Shimadzu Corporation, Kyoto, Japan) coupled with a Suprawax-280 column 30 m ×0.25 mm ×0.25 μm (Teknokroma). The GC–MS program was the same as described by Cano-Lamadrid et al. (2015). FAME standards (Sigma-Aldrich) were used for identification of peaks by their retention time. Results are expressed as percentage of the total area of methylated fatty acids. Analyses were done in triplicate.

2.5. Organic acids and sugars

Organic acids and sugars profiles were quantified using high-performance liquid chromatography (HPLC-DAD-RID) (Hewlett Packard 1100 series; Willmington, DE, USA) according to Sánchez-Rodríguez et al. (2019b). A supelcogel TM C-610H column 30 cm × 7.8 mm and Supelguard 5 cm × 4.6 mm; pre-column (Supelco, Bellefonte, PA) were used for separation: the absorbance was measured using a diode-array detector (DAD) at 210 nm for organic acids detection. A refractive index detector (RID) was used for the detection of sugars. Organic acids and sugars were analyzed in triplicate. Calibration curves were obtained from the analysis of pure standards of organic acids and sugars (Sigma. Poole, UK).

2.6. Statistical analyses

One-way analysis of variance (ANOVA) and Tukey's multiple range test were carried out for the results of each season. Results were expressed as means of triplicate analysis from each batch in each treatment. Statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD) and differences were considered statistically significant at three levels: (i) $p < 0.05$ (*), (ii) $p < 0.01$ (**), and (iii) $p < 0.001$ (***). Additionally, correlation coefficients were calculated to study the relationship between SI and all analyses done in raw and processed olives of both seasons.

3. Results and discussion

3.1. Irrigation

Minimum stem water potential (min Ψ_{stem}) and stress integral (SI) results of each year for each treatment under study are shown in Table 1. During 2015 season, both parameters were smaller than during 2016. For the period of 2015, although non-statistical differences were found due to the high variability of data, moderate deficit irrigation during long time (T2) showed the highest min Ψ_{stem} and the SI was the same than control. Regarding 2016 season, also T2 showed the highest min Ψ_{stem} and, in this case, SI showed statistical significant differences. It could be seen that T2 was the most stressed treatment while T1 was the least one. It has to be considered that even though researchers could control the applied irrigation, other natural factors due to real on-field conditions (rain, overall weather, soil differences among areas of the same field, among others) significantly affect SI and modified the targeted values; thus, statistical differences were found between both seasons, being 2016 significantly more stressed than 2015.

3.2. Morphological analyses

Morphological parameters of raw and table olives are shown in Table 2. Regarding RO, fruit weight, pit weight, equatorial and longitudinal diameters and color were not affected by RDI treatments at any season; only the results of one of the texture tests (MTT) was affected by irrigation. In both seasons, RDI decreased the hardness of the pulp of RO.

In relation to TO, in 2015 season, fruit weight was significantly affected by RDI treatments, with T1 and T0 having the smallest and highest values, respectively. Regarding longitudinal diameter, T1 table olives were longer than the other ones. With respect to color, *a** and *b** parameters showed statistical differences; T2 provided the greenest and less yellow table olives. During 2016 season, the only morphological parameter affected by RDI treatments was MTT, showing that T2 table olives had the hardest pulp. If both seasons are compared, it could be found that the highest stress (2016) produced smallest olives, affecting the weight and the diameters.

Previous studies were done with the same cultivar but applying the deficit irrigation during stage II of fruit growth and it was found that the higher the stress the higher the differences of olives in comparison with the control (Sánchez-Rodríguez et al., 2019b). Therefore, the timing of application of RDI is a highly relevant variable given that when the stress was applied during stage III, a different behavior was reported: the higher the stress, the lower the morphological differences between olives.

Fruit/pit ratio is one of the most important quality factors for table olive production and also for olive oil extraction (Gucci et al., 2009). In the current study, no statistically significant differences were found for fruit/pit ratio. This result agreed with that by Gucci et al. (2019), who studied the effect of RDI applied before pit hardening period and during rehydration phase.

3.3. *Antioxidants and total phenol content*

Antioxidants (ABTS^{+}, DPPH$^{\bullet}$ and FRAP assays) and TPC results of RO and TO during the two studied seasons are shown in Table 3. Regarding RO, in ABTS^{+} assay, T2 performed as the control, and in FRAP assay, T2 had the highest values in both studied seasons, although for DPPH$^{\bullet}$ radical, the highest values were found for T1. Treatment 2 showed the same TPC values than control, while T1 had the lowest values. In general, T1 olives had the lowest values of AA and TPC, as compared to T0 and T2 fruits. If both seasons are compared, 2015 showed highest values of AA regarding FRAP assay, while, DPPH^{+} and TPC showed highest values on 2016 season.

In relation to TO, AA was affected by irrigation in both seasons; in the DPPH$^{\bullet}$ assay, T1 and T2 presented higher antioxidant power than control; while in the FRAP assay, T2 had the same concentration than control. In table olives, also both RDI treatments yielded higher concentrations of TPC than T0. No statistical differences were found in TO between seasons.

No significant differences were found in previous studies with the same cultivar but different irrigation strategies (Cano-Lamadrid et al., 2017; Sánchez-Rodríguez et al., 2019b). In such studies, RDI was applied during stage II, consequently, the timing of RDI application is very important regarding antioxidants and TPC. Stage III of fruit growth corresponds to the maturation and oil accumulation period, and when the stress was applied in that period, phenol content and antioxidant power increased their concentration. A similar result was also obtained when the polyphenol profile was studied (Sánchez-Rodríguez et al., 2019a). In such study it was reported that the application of RDI at stage III improved the polyphenolic profile of table olives because several polyphenols increased their concentration: oleuropein, oleoside di-glucoside or comselogoside, among others.

Many studies have demonstrated that the increase of polyphenol concentration on olive oil is due to RDI. Gucci et al. (2019) in a study on the effect of irrigation time on the polyphenolic compounds of olive oil in the Frantoio cultivar, reported the highest increase in the concentration of polyphenols when RDI was applied before the hardening period of the pit. Differences with the current work could be due to agronomic conditions, cultivar, water stress conditions, etc.

3.4. *Fatty acids*

The fatty acids profile of the olives under study are shown in Table 4. Regarding RO, during 2015 season no statistical differences were found among irrigation treatments; whereas in 2016, the percentages of stearic and oleic acids slightly changed. It was found that stearic acid increased in T2 (3.40%) and oleic increased in T1 (70.7%) as compared to 2.90% and 68.8% in the control treatment, T0. This observation may be related to the fact that during 2016, the midday stem water potential (Ψ_{stem}) values were smaller than in 2015 and the stress integral values were larger; that means that the stress in the trees was higher than in 2015; thus, only under a high water stress of the trees the fatty acid profile was altered.

Regarding TO, when the stress in trees was smaller (2015), no differences were found in the fatty acid profile (following the same trend reported for RO) although total saturated fatty acids (SFA) slightly decreased in T2. However, in 2016 season, palmitic acid percentage decreased in both RDI treatments, and oleic acid concentration increased in T2 (71.4%) as compared to 70.1% in the control olives. Therefore, these results showed a trend to a positive functional quality of table olives under water stress, as saturated fatty acids (SFA) decreased while simultaneously monounsaturated fatty acids (MUFA) increased on the total fatty acid profile under water stress.

Other studies were done on olive trees with different RDI treatments (Cano-Lamadrid et al., 2015, 2017) in which the RDI was conducted during pit hardening stage (non-critical stage), and no differences were found on antioxidant activity or total phenol content, although MUFA percentage increased with high stress and PUFA with moderate stress. Thus, similar results were found when *moderate deficit irrigation during long time* was applied during stage III because MUFA content increased, although, in this situation, also the SFA content decreased, leading to an improvement of the fatty acid profile. However, enhanced functional quality needs to be evaluated considering changes in other components

Table 3
Antioxidant activity and total phenol content (TPC) of "*Manzanilla*" raw and table olives as affected by regulated deficit irrigation treatments.

Antioxidant parameter	2015				2016				ANOVA	2015	2016
	ANOVA†	T0	T1	T2	ANOVA	T0	T1	T2			
Raw olives											
ABTS^{+} (mmol Trolox kg^{-1})	***	28.6 a ‡	24.9 b	28.1 a	*	27.6 ab	25.2 b	28.8 a	NS	27.2	27.2
DPPH$^{\cdot}$ (mmol Trolox kg^{-1})	***	48.9 b	52.0 a	46.7 c	*	47.70 b	53.94 a	48.58 b	**	49.5 b	50.1 a
FRAP (mmol Trolox kg^{-1})	***	24.3 b	23.7 c	28.4 a	**	23.6 b	23.2 b	27.9 a	***	25.5 a	24.9 b
TPC (g GAE kg^{-1})	***	32.4 a	21.4 b	32.2 a	***	32.6 a	21.6 b	33.4 a	***	28.6 b	29.2 a
Table olives											
ABTS^{+} (mmol Trolox kg^{-1})	NS	9.04	9.10	9.19	NS	8.74	9.41	9.34	NS	9.11	9.16
DPPH$^{\cdot}$ (mmol Trolox kg^{-1})	***	7.41 b	8.61 a	8.42 a	*	7.31 c	9.64 a	8.62 b	NS	8.15	8.52
FRAP (mmol Trolox kg^{-1})	*	19.2 a	18.1 b	20.1 a	**	19.1 a	17.8 b	19.3 a	NS	19.1	18.73
TPC (g GAE kg^{-1})	***	5.46 b	5.87 a	5.82 a	**	5.46 c	5.90 a	5.83 b	NS	5.72	5.73

† NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (n = 9) followed by the same letter within the same row and season were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

Table 4
Fatty acid profile of "*Manzanilla*" raw and table olives expressed as percentage of the total profile as affected by regulated deficit irrigation treatments.

Fatty acid (%)	2015				2016				ANOVA	2015	2016
	ANOVA†	T0	T1	T2	ANOVA†	T0	T1	T2			
Raw olives											
Palmitic acid (C16:0)	NS	16.2‡	16.9	16.8	NS	18.6‡	17.8	18.2	*	16.6 b	18.2 a
Stearic acid (C18:0)	NS	2.72	2.59	2.85	**	2.90 b	3.01 b	3.40 a	**	2.72 b	3.11 a
Oleic acid (C18:1)	NS	73.4	70.7	70.5	*	68.8 b	70.7 a	69.5 ab	NS	71.5	69.7
Linoleic acid (C18:2)	NS	5.08	6.82	6.69	NS	5.62	4.37	4.88	NS	6.19	4.95
Linolenic acid (C18:3)	NS	0.94	0.94	1.11	NS	1.30	1.26	1.16	*	0.99 b	1.24 a
Araquidic acid (C20:0)	NS	0.42	0.38	0.48	NS	0.44	0.56	0.60	NS	0.43	0.53
Σ SFA	NS	19.3	19.9	20.1	NS	21.9	21.4	22.2	**	19.8 b	21.8 a
Σ MUFA	NS	73.4	70.7	70.5	*	68.8 b	70.7 a	69.5 ab	NS	71.5	69.7
Σ PUFA	NS	6.01	7.75	7.79	NS	6.92	5.63	6.04	NS	7.18	6.20
(MUFA + PUFA)/SFA	NS	4.11	3.94	3.89	NS	3.45	3.57	3.40	NS	3.98	3.47
Table olives											
Palmitic acid (C16:0)	NS	16.9	16.8	16.5	*	18.7 a	17.7 b	17.0 b	***	16.7 b	17.8 a
Stearic acid (C18:0)	NS	2.74	2.93	2.84	NS	2.73	2.62	3.06	NS	2.84	2.80
Oleic acid (C18:1)	NS	70.82	69.28	70.47	*	70.1 b	70.3 b	71.4 a	*	70.2 b	70.6 a
Linoleic acid (C18:2)	NS	6.41	7.87	7.23	NS	6.72	7.53	6.87	NS	7.17	7.04
Linolenic acid (C18:3)	NS	1.02	0.94	0.99	NS	1.18	1.26	1.29	**	0.98 b	1.24 a
Araquidic acid (C20:0)	NS	0.47	0.46	0.45	NS	0.48	0.49	0.46	NS	0.46	0.48
Σ SFA	**	20.1 a	20.2 a	19.7 b	*	22.0 a	20.8 b	20.5 b	***	20.0 b	21.1 a
Σ MUFA	NS	70.82	69.28	70.47	*	70.1 b	70.3 b	71.4 a	*	70.2 b	70.6 a
Σ PUFA	NS	7.42	8.81	8.22	NS	7.90	8.79	8.16	**	8.15 b	8.28 a
(MUFA + PUFA)/SFA	**	3.89 b	3.86 b	3.99 a	*	3.57 b	3.81 a	3.87 a	***	3.91 a	3.75 b

† NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (n = 9) followed by the same letter within the same row and season were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

on the olives.

There are some studies that demonstrated that timing of olive tree irrigation influenced the fruit tissues evolution (Gucci et al., 2009; Rapoport et al., 2004), although there is small information about timing. Gucci et al. (2019) did not found a clear trend on Frantoio olive oil fatty acid composition after applying RDI before stage II and during III.

3.5. Organic acids and sugars

Organic acids and sugars profiles of RO are shown in Table 5. The main organic acids found in RO were citric, tartaric, malic, and succinic acids while the main sugars were sucrose, glucose, and fructose. Both seasons under study yielded similar contents of organic acids and sugars, so it could be said that differences between the stress levels in both seasons did not affect the organic acids and sugars profiles. Regarding differences between irrigation treatments, the two seasons showed the same trend; only tartaric and succinic acids decreased their concentration when both RDI treatments were applied. Tartaric acid decreased from 0.14 g kg^{-1} fw (T0) to 0.07 g kg^{-1} fw (T1) and 0.08 g kg^{-1} fw (T2) and succinic acid decreased from 0.50 g kg^{-1} fw (T0) to 0.14 g kg^{-1} fw (T1 and T2).

Regarding TO (data not shown in Table 5), phytic (6.42 g kg^{-1} fw), lactic (1.50 g kg^{-1} fw), and acetic acids (0.79 g kg^{-1} fw) were found as major organic acids while maltoheptaose (2.03 g kg^{-1} fw), mannitol (2.46 g kg^{-1} fw) and glycerol (0.77 g kg^{-1} fw) as sugar. No statistical differences were found on TO due to irrigation treatments and seasons. Same profiles of organic acids and sugars were previously found in RO and TO (Sánchez-Rodríguez et al., 2019b) in "Manzanilla" olives. The differences among the organic acid and sugar profiles in RO and TO were the consequence of the transformation of RO into TO via fermentation during the Spanish-style process.

3.6. Correlation

For further information about the effect of the water stress in the parameters studied, correlations were done among all parameters (for both RO and TO) and SI including both seasons (Fig. 1). Negative correlations among SI and (i) fruit weight (Fig. 1A), (ii) pit weight (Fig. 1B), and (iii) equatorial diameter (Fig. 1C). Although non-statistically significant, regarding morphological characteristics (Table 2), it was observed that the higher the stress applied during stage III, the higher the decrease in fruit and pit weight. Thus, the relation fruit/pit was maintained, and similar fruit quality was obtained. As it was

Table 5
Sugars and organic acids of "*Manzanilla*" raw olives as affected by regulated deficit irrigation treatments.

Organic acid or sugar (g kg^{-1} fw)		2015				2016				ANOVA	2015	2016
		ANOVA†	T0	T1	T2	ANOVA†	T0	T1	T2			
Raw olives												
Organic acids	Citric acid	NS	0.255 ‡	0.255	0.230	NS	0.253	0.263	0.237	NS	0.247	0.251
	Tartaric acid	**	0.140 a	0.067 b	0.084 b	*	0.143 a	0.066 b	0.083 b	NS	0.097	0.098
	Malic acid	NS	0.490	0.487	0.432	NS	0.516	0.487	0.450	NS	0.469	0.484
	Succinic acid	*	0.500 a	0.144 b	0.137 b	*	0.505 a	0.145 b	0.137 b	NS	0.260	0.262
Sugars	Sucrose	NS	1.758	1.696	1.677	NS	1.764	1.690	1.677	NS	1.710	1.710
	Glucose	NS	3.905	3.283	3.482	NS	3.915	3.427	3.528	NS	3.556	3.623
	Fructose	NS	1.455	1.626	1.934	NS	1.478	1.756	1.966	NS	1.672	1.733

†NS = not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (n = 9) followed by the same letter within the same row and season were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

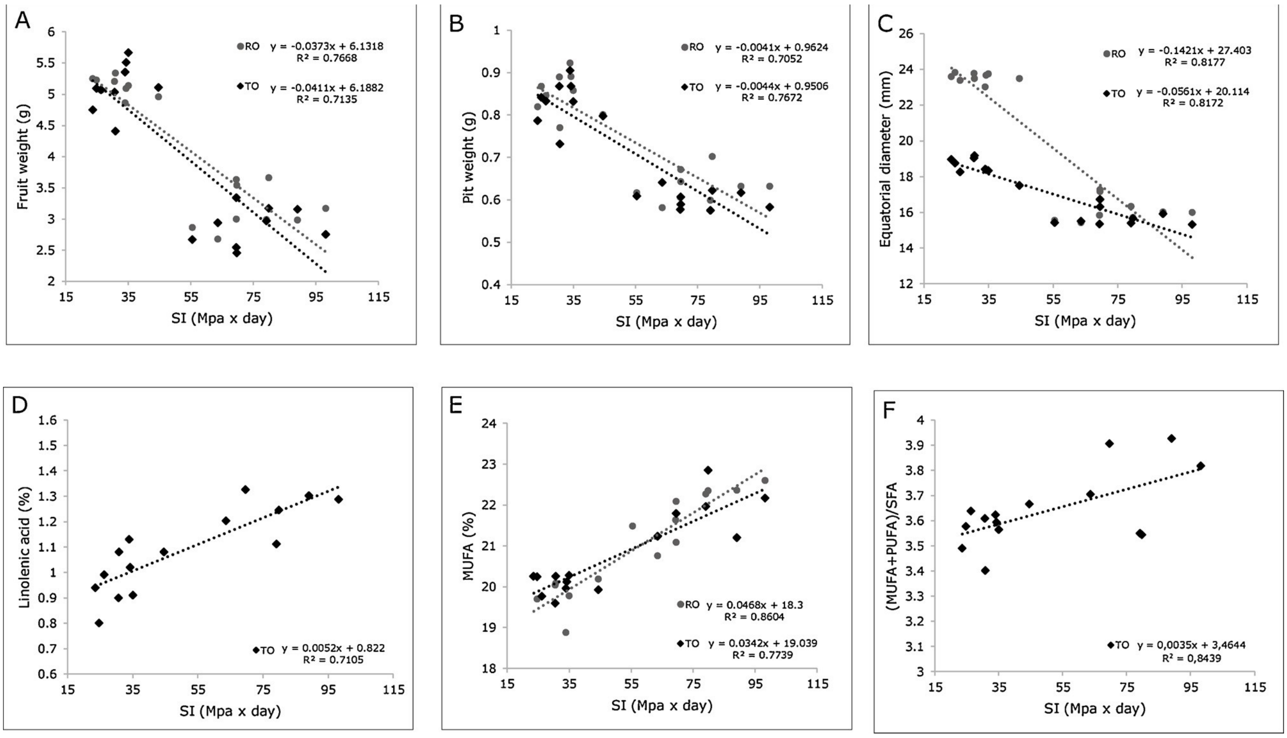

Fig. 1. Correlations between stress integral (SI) and different variables [RO (grey circles) and TO (black diamonds)] of 2015 and 2016 seasons. A: fruit weight; B: pit weight; C: equatorial diameter; D: linolenic acid; E: monounsaturated fatty acids (MUFA); F: (monounsaturated fatty acids + polyunsaturated fatty acids)/saturated fatty acids [(MUFA + PUFA)/SFA].

expected, equatorial diameter was also negatively correlated with SI, and it was definitely linked to the weight loss. Regarding fatty acids, positive correlations among SI and (i) MUFAs (Figure E) for RO and TO, (ii) linolenic acid (Fig. 1D), and (iii) (MUFA + PUFA)/SFA (Fig. 1F) percentages only for TO; only TO data are represented in such figures. The higher the stress applied during stage III, the higher the concentration of linolenic acid in TO, also the sum PUFA + MUFAs slightly increased due to water stress. The higher the stress applied, the higher the content on MUFA in RO and TO olives.

4. Conclusions

This is the first study investigating functional parameters of raw olives and table olives under the effect of a RDI applied just before harvest and without a rehydration period. After processing raw into table olives by the Spanish-style process, the differences due to RDI treatments were reduced. In general, the quality of fruit morphological parameters was maintained (reduced size but maintained pulp proportion), while the antioxidant activity and total phenolic content were increased due to RDI, and the fatty acid profile was improved (enhanced MUFA content) when the stress integral was higher (2016 season). Consequently, and after application of RDI strategies just before olives harvesting, the higher the SI, the better the nutritional quality of the *hydroSOStainable* table olives obtained. Hence, farmers interested on saving water techniques, now have more information about effect on timing: *i)* moderate stress applied during stage II led to maintained fruit size and yield with no significant differences on composition, and *ii)* high stress applied during stage III led to reduced olive size but improved the nutritional quality of fruits.

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7.3. Third Publication

Publication 3 (literal transcription)

Polyphenol Profile in "Manzanilla" Table Olives As Affected by Water Deficit during Specific Phenological Stages and Spanish-style Processing

Sánchez-Rodríguez, L., Cano-Lamadrid, M., Carbonell-Barrachina, Á.A, Wojdyło, A., Sendra, E., Hernández, F

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Polyphenol Profile in Manzanilla Table Olives As Affected by Water Deficit during Specific Phenological Stages and Spanish-Style Processing

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ABSTRACT: Reducing water consumption on agriculture is a worldwide goal toward sustainability. In this scenario, two experiments of regulated deficit irrigation (RDI) were carried out on olive trees, cultivar Manzanilla. With regard to experiment A, three RDI techniques were applied during the olive pit hardening period (stage II), while in experiment B, two RDI treatments were applied during the rehydration phase (stage III). Table olives under RDI are so-called hydroSOStainable. The effect of water deficit and Spanish-style processing was studied on the polyphenol profile, antioxidant capacity, and total polyphenol content (TPC) of both raw olives (RO) and table olives (TO). The TPC decreased after processing of TO. It could be due to osmotic mechanisms. However, many individual polyphenols, such as oleuropein (main polyphenol) or oleoside diglucoside, increased their concentrations in hydroSOStainable TO. Additionally, the TPC content was correlated to the phenological stage of the fruit when the stress is applied. A moderate stress during pit hardening and an intense stress during the rehydration phase were the treatments that best improved the polyphenol profile.

KEYWORDS: *regulated deficit irrigation, pit hardening, rehydration phase, oleuropein, hydroSOStainable olives*

1. INTRODUCTION

Fruit cultivated under regulated deficit irrigation (RDI) have been called "hydroSOStainable" products; their main characteristic is that they are environmentally friendly because of the optimization of the irrigation water use. In addition, their derived products present unique properties (high content of bioactive compounds, high intensity of key sensory attributes, etc.).[1−3] Although olive tree is one of the most drought-resistant species, its physiology is also affected by water deficit. The effect of RDI on olives depends upon the phenological stage of the plant when the water deficit is applied. A modification of morphological, chemical, functional, and sensory properties has been observed in previous studies. For instance, when water stress was applied during pit hardening (stage II), an enhanced content of polyunsaturated fatty acids and consumer satisfaction have been reported.[2] However, the application of water stress is mainly limited to the most stress-tolerant phenological stage of the olive tree, pit hardening.[4]

The cultivar Manzanilla is mainly used to produce table olives (TO). Among the different processes used in the preparation of TO, the Spanish style is by far the most popular process. Previous studies indicated that the polyphenol profile in raw olives (RO) and TO was characterized by the presence of high contents of sercoiridoids, including especially oleuropein, whereas flavonoids (rutin, luteolin, etc.) represent only a minor fraction.[5,6] It is worth mentioning that the content of those compounds in raw fruit has been observed far higher than in processed fruit. Besides being responsible for olive bitterness, the phenolic compounds are also involved in the color changes happening during fruit processing.[7]

Previous research indicated that the deficit irrigation applied during pit hardening followed by Spanish-style processing of Manzanilla de Sevilla TO enhanced potential health benefits by increasing their phytoprostane content.[8] On the other hand, a reduction of total polyphenol content (TPC) and antioxidant capacity has been reported as a result of the Spanish-style processing, whereas no statistical differences were found among the studied irrigation treatments when RDI was applied during the pit-hardening stage.[9,10] It is worth mentioning that no previous data exist on the simultaneous effect of deficit irrigation treatments and Spanish style on the polyphenol profile.

Consumer awareness of the impact of food on health and well-being is increasing in the recent years. Although polyphenols have a healthy image, there is only one food with authorized health claims regarding its polyphenol content within the European Union (European regulation 432/2012), and this is the presence of polyphenols in olive oil for protection of low-density lipoprotein (LDL) particles from

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oxidative damage (5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil). It should be emphasized that consumers are interested in Mediterranean and environmentally friendly foods as a healthy and better choice. At present, there is no authorized health claim related to TO within the European Union regulations.

To our knowledge, no studies have been carried out about the changes induced by RDI during pit hardening (experiment A) or just before the harvest without rehydration (experiment B) and Spanish-style processing. The aim of this study was to evaluate the effect of different irrigation conditions and the effect of Spanish-style processing on raw and processed 'Manzanilla' TO polyphenol profile [determined by ultra-performance liquid chromatography with photodiode array (UPLC−PDA) after identification by liquid chromatography−mass spectrometry−quadrupole time of flight (LC−MS−QTof)] obtained from two different water stress strategies. These analyses were completed by evaluating antioxidant capacity (DPPH$^•$, FRAP, and ABTS$^+$) and TPC. In addition, the relationship between all analyzed parameters and the stress integral (SI) was also studied.

2. MATERIALS AND METHODS

2.1. Plant Material and Experimental Design. Two experiments were run in this research. Experimental design was randomized completely in blocks with three repetitions and two control trees per plot. Irrigation scheduling was performed using the threshold values of midday stem water potential using a pressure chamber (PMS Instrument Company, Albany, OR, U.S.A.) during all season. SI, as defined by Myers,[11] was used to describe the effect of the irrigation treatments.[12] Pest control, pruning, and fertilization practices were those commonly used by growers, and weeds were chemically removed in the orchard. Climatic conditions of both experiments are almost equal because the distance between the orchards is only around 10 km, and both of them are at the same level in the Guadalquivir Valley. Winter minimum temperatures were slightly above 0 °C, and spring temperatures determine that flowering happens around mid-April. Weather conditions make this area perfect for olive tree growth. Olives were hand-harvested in 2016 at their mature-green stage.

2.1.1. Experiment A. Olives were collected from a farm, DoñaAna, which is located in Dos Hermanas (Seville, Spain, 37° 25′ N, 5° 95′ W). Olives trees (cultivar Manzanilla) were 30 years old. The tree spacing followed a 7 × 4 m square pattern. The loam soil was characterized by a volumetric water content of 0.31 m^3 m^{-3} at field capacity and 0.14 m^3 m^{-3} at the permanent wilting point and a bulk density of 1.40 g cm^{-3} (0−30 cm) and 1.35 g cm^{-3} (30−90 cm). Irrigation was performed during the night by drip, using one lateral pipe per row of trees and four emitters per plant, split between the two rows (each delivering 2 L h^{-1}). Three different irrigation treatments and a control were carried out: (i) optimum water status (A0) full irrigated, (ii) moderate deficit irrigation (A1), where the threshold value was −2 MPa during the pit-hardening stage, (iii) severe deficit irrigation (short time) (A2), where the threshold value was −3 MPa during half of the period of the pit-hardening stage; and, (iv) severe deficit irrigation (long time) (A3), where the threshold value was −3 MPa until the end of the period of the pit-hardening stage.

2.1.2. Experiment B. Olives were collected from La Hampa, the experimental farm of the Instituto de Recursos Naturales y Agrobiologia(IRNAS−CSIC) located in Coria del Rio(Seville, Spain, 37° 17′ N, 6° 3′ W, 30 m altitude). Olive trees (cultivar Manzanilla) were 44 years old. The tree spacing followed a 7 × 5 m square pattern. The sandy loam soil was characterized by a volumetric water content of 0.33 m^3 m^{-3} at field capacity and 0.10 m^3 m^{-3} at the permanent wilting point and a bulk density of 1.30 g cm^{-3} (0−10 cm) and 1.50 g cm^{-3} (10−120 cm). Irrigation was performed during the night by drip, using one lateral pipe per row of trees and five emitters per plant, split between the two rows (each delivering 8 L h^{-1}). Two different irrigation treatments and a control were carried out: (i) optimum water status (B0) full irrigated, (ii) moderate deficit irrigation before harvest (short time) (B1), where the threshold value was reduced to −2 MPa at the beginning of September without a rehydration period, and (iii) moderate deficit irrigation (long time) (B2), where the threshold value was −2 MPa from mid-August without a rehydration period.

2.2. Spanish-Style Processing. 'Manzanilla' olives from all of the trees of each block of each RDI treatment from the two experiments were systematically mixed, and a sample of approximately 50 kg per block was used to prepare TO. Fruit were transported the day after their picking at the farm to the Cooperativa Nuestra Señora de las Virtudes (La Puebla de Cazalla, Seville, Spain) to be processed as TO according to the Spanish-style method. In this method, RO were dipped in a dilute NaOH solution followed by washings and then olives were put in brine, where lactic acid fermentation occurred.[1] A part of each batch was kept as RO to be analyzed. Once RO and TO arrived to our laboratories, a representative sample of olives from each treatment were lyophilized for further analysis.

2.3. Polyphenol Extraction and Identification and Quantification of Polyphenols by LC−PDA−MS−QTof. Lyophilized samples of fruit (∼1 g) were extracted with 10 mL of mixture containing high-performance liquid chromatography (HPLC)-grade acetone/water/methanol (1:2:2, v/v/v) reagent. The extraction was performed twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with occasional shaking. Next, the upper phase was collected and centrifuged at 19000*g* for 10 min, and the supernatant was filtered through a Hydrophilic PTFE 0.20 μm membrane (Millex Samplicity Filter, Merck, Darmstadt, Germany) and used for analysis. The compound identification was performed using an Acquity UPLC system equipped with a PDA with a binary solvent manager (Waters Corp., Milford, MA, U.S.A.) series with a mass detector G2 QTof micro mass spectrometer (Waters, Manchester, U.K.) equipped with an electrospray ionization (ESI) source operating in negative and positive modes. The chromatographic conditions for the identification and quantification have been previously reported by Wojdyło et al.[13]

2.4. Instrumental Color. Color determinations were made on fresh RO and TO, at 25 ± 1 °C, using a Minolta colorimeter CR-300 (Osaka, Japan). This spectrophotometer uses an illuminant D_{65} and a 10° observer as references. Color data are provided as CIE $L^*a^*b^*$ coordinates, which define the color in a three-dimensional space. L^* indicates lightness, taking values within the range of 0−100, and a^* and b^* are the chromatic coordinates, green−red and blue−yellow coordinates, respectively. Parameter a^* takes positives values for reddish colors and negative values for the greenish colors, whereas b^* takes positive values for yellowish colors and negative values for bluish colors. Color analyses were run in 25 replicates for each block, which means 100 olives per treatment.

2.5. Antioxidant Activity (AA) [2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and Ferric Reducing Antioxidant Power (FRAP)] and Total Polyphenols. To obtain the extract for antioxidant capacity and TPC, 0.5 g of freeze-dried RO and TO were mixed with 10 mL of MeOH/water (80:20, v/v) + 1% HCl and the mixture was sonicated at 20 °C for 15 min and left overnight at 4 °C. Then, the extract was sonicated again for 15 min and centrifuged at 10000*g* for 10 min.

The ABTS$^+$ radical cation and FRAP methods were employed according to previous studies.[14,15] An ultraviolet−visible (UV−vis) spectrophotometer (Helios Gamma model, UVG 1002E, Mercers Row, Cambridge, U.K.) was used to obtain the absorbance. In addition, the radical scavenging activity was also evaluated using the DPPH$^•$ radical method, as described by Brand-Williams et al.,[16] with a modification in the reaction time. The decrease in absorbance was measured at 515 nm using an UV−vis spectrophotometer (Helios Gamma model, UVG 1002E, Mercers Row, Cambridge, U.K.). Calibration curves, in the range of 0.5−5.0 mmol of Trolox kg^{-1} were used for the quantification of the three methods of AA, showing good linearity ($R^2 \geq 0.998$). Results were expressed in millimoles of Trolox per kilogram of fresh weight (fw).

TPC was quantified using the Folin−Ciocalteu colorimetric method described previously.[17] The extracts of freeze-dried RO and TO (0.1 mL) were mixed with 0.2 mL of Folin−Ciocalteu reagent and 2 mL of H_2O. The absorbance of the resulting blue color solution was measured at 765 nm using an UV−vis spectrophotometer (Helios Gamma model, UVG 1002E, Mercers Row, Cambridge, U.K.). Quantification was performed with respect to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), grams per kilogram of fw.

2.6. Statistical Analysis. Statistics of both experiments (experiments A and B) were performed separately as a result of differences on irrigation strategies: in experiment A, deficit irrigation was applied during the pit-hardening stage (II), while in experiment B, it was applied during the rehydration stage (III). Three one-way analysis of variance (ANOVA) were run on data: (i) irrigation treatment before processing (RO data, factor: RDI treatment), (ii) irrigation treatment after processing (TO data, factor: RDI treatment), and (iii) Spanish-style processing (comparison of RO and TO data) using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD, U.S.A.), and means were separated by Tukey's multiple range test. Pearson's correlation coefficients were calculated to study the relationship between SI and polyphenolic profile, color parameter, antioxidant capacity, and TPC in raw and processed olives.

3. RESULTS AND DISCUSSION

3.1. Irrigation. As seen in Figure 1, different levels of water stress were reached by olive trees. In experiment A, it could be seen that A0 presented the smallest SI because it was not submitted to stress (control treatment). On the other hand, A3 (severe deficit irrigation during long time) trees presented the highest SI value. The A1 (moderate deficit irrigation) and A2 (severe deficit irrigation during short time) trees had intermediate and equivalent SI values, because the stress to which they were submitted was also intermediate. In experiment B, although B0 (control) was fully irrigated, it could be seen that its SI value was higher than that of B1 (moderate deficit irrigation during short time before harvest). This experimental fact can be justified because, even though researchers can control the applied water volume, other natural factors, such as overall weather (e.g., rain) or soil differences among areas of the same field, can also significantly affect SI. Finally, B2 presented the highest SI value because water stress was moderate but during a long time before harvest.

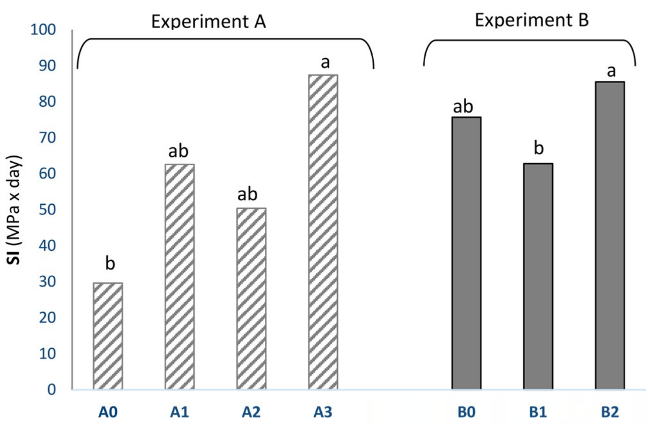

Figure 1. Water stress integral (MPa × day) applied during the irrigation season in experiments A and B. Different letters on data on each experiment indicate significant differences according to Tukey's test ($p < 0.05$).

3.2. Polyphenol Profile. A total of 17 different compounds have been identified in Manzanilla RO and TO. In Table 1, the retention indexes, λ_{max} (nm), MS (m/z), and MS/MS (m/z), used for the identification of the compounds are shown. The compounds have been classified in chemical families: (i) iridoids (10 compounds), (ii) flavonoids (3 compounds), (iii) phenylethanoid, (iv) phenethyl ester, (v) tyrosol ester of elenolic acid, and (vi) hydroxytyrosol. To make the discussion easier to follow, experiments A and B have been discussed separately.

Quantification of each identified compound is shown in Table 2. With regard to RO in experiment A, the content of the major compounds followed the order: P13 (mean of all treatments, 213 mg equiv of quercetin-3-*O*-rutinoside 100 g^{-1} of fw) > P3 (125 mg equiv of luteolin-3-*O*-rutinoside 100 g^{-1} of fw) > P5 (77.1 mg equiv of luteolin-3-*O*-rutinoside 100 g^{-1} of fw) > P9 (35.1 mg equiv of quercetin-3-*O*-rutinoside 100 g^{-1} of fw) ≈ P7 (33.5 mg equiv of quercetin-3-*O*-rutinoside 100 g^{-1} of fw). On the other hand, in experiment B, the order was as follows: P13 (mean of all treatments, 219 mg equiv of quercetin-3-*O*-rutinoside 100 g^{-1} of fw) > P3 (120 mg equiv of luteolin-3-*O*-rutinoside 100 g^{-1} of fw) > P5 (80.0 mg equiv of luteolin-3-*O*-rutinoside 100 g^{-1} of fw) > P6 (56.1 mg equiv of luteolin-3-*O*-rutinoside 100 g^{-1} of fw) ≈ P7 (52.8 mg equiv of quercetin-3-*O*-rutinoside 100 g^{-1} of fw). Not only do the cultivar and maturity stage affect phenolic composition, agronomic practices are also important parameters affecting it.[18] Phenolic compounds are metabolized as a result of the changes in both biosynthetic and catabolic pathways within the fruit. It has been observed that the activity of phenylalanine ammonia-lyase, a key enzyme that belongs to the biosynthetic pathway, was increased during water stress in the olive tree, reducing the total phenolic content at severe irrigation regimes.[19,20]

Although previous studies indicated that an increase in polyphenols is noticed when a water availability reduction is applied, our research suggested that not only is water availability affected but also the level of water stress and the phenological stage are important. Therefore, when the effect of irrigation treatments during pit hardening (experiment A) on RO is taken into account, a significant increase of P3, P10, and P17 (all of them belong to the iridoid family) was observed when moderate deficit irrigation (A1) was applied, whereas a significant decrease of P12, P14, and P16 was also perceived. The content of the rest of compounds was similar to the control treatment (A0). With regard to the short time severe deficit irrigation (A2), the contents of P2, P10, and P17 significantly increased, while the contents of P1, P8, P11, P13, and P16 were similar to the control (A0), with the rest of the compounds being reduced. Additionally, when the long time severe deficit irrigation (A3) was applied, an increment of P4 and P17 was noticed but the contents of P5, P8, P9, P14, P15, and P16 were reduced. What the studied irrigation strategies during pit hardening (A1−A3) had in common was an increase of 2″-hydroxyoleuropein (P17), probably as a result of the modification of other oleuropein derivatives, such as P5 or P10. With regard to the irrigation treatments applied just before the harvest without rehydration (experiment B), the contents of P6, P12, P14, P15, and P16 were significantly increased when the short moderate irrigation treatment (B1) was used, whereas the contents of P1, P2, P8, P10, P11, and

Table 1. Identification of Polyphenol Compounds in RO and TO

	t_R[a] (min)	λ_{max}[b] (nm)	MS[c] (m/z)	MS/MS[d] (m/z)	compound	chemical family
P1	2.82	277	315.10	153.04/183.06/220.05	hydroxytyrosol glucoside	phenylethanoid
P2	4.07	283/330	551.15	431.14/341.08/275.02	caffeoyl-6′-secologanoside	phenethyl ester
P3	4.32	213/280	389.10	371.11/345.03/209.44/165.52	oleoside	iridoids
P4	4.91	235	403.11	223.18/179/119/101.04	elenolic acid glucoside	tyrosol ester of elenolic acid
P5	5.44	280	377.14	197.07/153.09	oleuropein aglycone	iridoids
P6	6.58	339/280	609.14	301.02	quercetin-3-*O*-rutinoside	flavonoids
P7	6.95	339/280	593.15	285.03/447.08	luteolin-3-*O*-rutinoside	flavonoids
P8	7.12	329/218	623.19	491.16/315.09/377.14/195.05	verbascoside	hydroxytyrosol
P9	7.33	281/329	551.11	551.11/507.04/209.02	oleoside diglucoside	iridoids
P10	7.63	334	543.20	313.12/377.13/300.02	dihydro-oleuropein	iridoids
P11	7.87	279	701.21	377.11	oleuropein diglucoside	iridoids
P12	8.03	220/326	551.13		caffeoyl-6′-secologanoside	iridoids
P13	8.82	237/279	539.11	275.08/255.07/361.12/307.08	oleuropein	iridoids
P14	8.83	227/311	535.07	275.04/307.07/163.03	comselogoside	iridoids
P15	9.73	221/348	285.03	285.03	luteolin	flavonoids
P16	9.88	222/278/335	523.17	291.08/361.12/259.09/377.11	ligstroside	iridoids
P17	10.62	219/283	555.21	539.06/359.03/377.11/225.07/234.12/153.11	2″-hydroxyoleuropein	iridoids

[a]t_R = retention time. [b]λ_{max} = maximum wavelength. [c]MS = mass spectrometry. [d]MS/MS = tandem mass spectrometry.

P13 were reduced. Additionally, when the long time moderate deficit irrigation (B2) was applied, the contents of P3, P5, P6, P8, P13, and P17 were increased but a reduction of the contents of P2 and P9 was perceived. An increase of quercetin-3-*O*-rutinoside (P6) was observed in experiment B. Previously, some authors observed an increase of tyrosol when water deficit was applied;[21] this is why it is important to mention that hydroxytyrosol glucoside (P1), oleuropein aglycone (P5), verbacoside (P8), dihydro-oleuropein (P10), oleuropein diglucoside (P11), oleuropein (P13), lignoside (P16), and 2″-hydroxyoleuropein (P17) are formed from tyrosol.

With regard to the processing effect on the polyphenol profile, an average reduction of 90% was observed in all identified compounds in both experiments, with the exception of P9 in experiment B, which was reduced by 75%. Although compositional differences between RO and TO are well-known, this study was the first study reporting the combination between irrigation treatment and processing. Previously, it has been concluded that TO have a different qualitative and quantitative phenolic composition than RO, which could be due to the osmotic effect on phenols and other soluble constituents from RO to the lye and water and vice versa and from TO to brine and vice versa. Moreover, during the lye treatment in RO, some reactions occur involving the following compounds: (i) between NaOH and constituents that have carboxylic and hydroxyl functional groups, producing hydrophilic derivatives that are removed, and (ii) oleuropein and verbascoside that are hydrolyzed.[22] During lactic fermentation, the glycosides formed are also hydrolyzed to hydroxytyrosol. Recently, it has been clarified that different water regimes had no impact on the fermentation process and the activity of starter culture efficiency[23] because endogenous enzymes are degraded or inactivated during the NaOH treatment.[22]

With regard to the effect of irrigation in TO, in experiment A, there was an improvement of P3, P4, P9, P13, and P14 in moderate stress (A1), while an increase in the contents of P9 and P4 in short severe stress (A2) and long severe stress (A3) was only perceived, respectively. However, the contents of P13, P16, and P17 decreased in A3. In the case of experiment B, P7, P9, and P14 increased in short moderate stress (B1), while P4, P10, and P13 increased in long moderate stress (B2). A decrease of the concentration was found in P3 for B2. In a previous study performed applying deficit irrigation during all season, polyphenols increased in olive paste as the severity of the applied stress was increased, increasing, for instance, oleuropein, verbascoside, and flavonoids.[24] In our study, the highest changes in the content of polyphenols were found on the iridoid family.

After the composition of polyphenols of TO was studied and with the knowledge that there is an authorized claim for "olive oil" establishing that 5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil contribute to the protection of blood lipids from oxidative stress (European regulation 432/2012), it could be estimated (although it is important to mention that this is not an authorized claim) that 235 g of TO could provide a similar content of hydroxytyrosol and its derivatives.

Therefore, our results have demonstrated that it is also very important to study not only the total polyphenol but also their profile (each compound), taking into account the phenological stage of the fruit when the reduction of water is applied. When the stress was applied during pit hardening (experiment A), not all polyphenols increased their concentration when the stress was higher; in fact, a moderate stress level (A1) increased the content of five polyphenols. However, when the stress was applied during the rehydration phase, a higher number of polyphenols increased their concentration when the SI was higher.

3.3. AA and Total Polyphenols. The AA of 'Manzanilla' olives was evaluated using three different analytical methods: $ABTS^+$, DPPH$^•$, and FRAP (Table 3). The AA was not significantly affected ($p > 0.05$) on both RO and TO by the RDI treatments during experiment A. These results are in agreement with those obtained by Cano-Lamadrid et al. and Sánchez-Rodriguez et al.[2,10] and confirm that deficit irrigation, even severe in the hardening phase of the pit, does not affect the AA of raw or processed olives. However, Spanish-style processing affected AA. With regard to the effect of processing on antioxidant capacity, an average reduction of 73 and 75% was observed in ABTS and DPPH, respectively, while the value of FRAP was reduced in 37% in experiment A. The same effect was also found by Sánchez-Rodriguez et al.[10] in the cultivar 'Manzanilla'.

Table 2. Effect on Polyphenolic Compounds (P1 −P17) by Different Irrigation Conditions (Experiment A, A0, A1, A2, and A3; Experiment B, B1, B2, and B3) and Processing Stage (RO, Raw Olives; TO, Processed Olives)

experiment A																	
	P1[a]	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17
	(mg equiv of quercetin-3-*O*-rutinoside 100 g^{-1} of fw)									(mg equiv of luteolin-3-*O*-rutinoside 100 g^{-1} of fw)							
ANOVA[b]																	
irrigation RO	NS	*	**	*	*	*	*	*	*	**	*	*	*	*	*	*	*
irrigation TO	NS	*	*	*	NS	NS	NS	NS	**	NS	NS	NS	*	*	*	*	*
processing	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Tukey multiple range test[c]																	
irrigation RO																	
A0	3.88	4.00 b	119 b	2.94 b	84.2 a	25.5 a	36.2 a	26.4 a	43.5 a	1.26 b	3.27 ab	6.22 a	211 ab	4.52 a	9.14 a	14.8 a	6.84 c
A1	3.74	3.96 ab	164 a	2.94 b	81.5 a	29.7 a	38.2 a	31.5 a	36.0 a	6.36 a	3.83 a	4.65 b	230 a	1.75 c	8.17 ab	12.9 b	8.10 b
A2	2.96	5.27 a	91.7 c	1.38 c	68.1 c	14.8 b	26.7 b	27.4 a	29.4 b	6.49 a	2.91 ab	4.53 b	187 b	2.66 b	7.03 b	13.9 ab	9.55 a
A3	3.94	3.32 bc	124 b	3.01 a	74.8 b	27.4 a	32.9 ab	15.8 b	31.6 b	2.94 b	1.78 b	5.37 ab	224 a	2.01 bc	6.48 b	13.2 b	9.09 ab
irrigation TO																	
A0	0.68	0.08 ab	0.22 b	0.10 b	0.09	0.24	0.14	0.12	1.67 c	0.16	0.08	0.07	0.27 b	0.12 b	0.10 a	0.12 a	0.13 a
A1	0.67	0.15 a	0.33 a	0.18 a	0.17	0.20	0.09	0.12	3.14 a	0.10	0.10	0.08	0.60 a	0.20 a	0.02 b	0.11 a	0.13 a
A2	0.60	0.04 b	0.23 b	0.08 b	0.11	0.17	0.10	0.08	2.20 b	0.06	0.08	0.08	0.19 bc	0.11 b	0.02 b	0.12 a	0.17 a
A3	0.56	0.05 b	0.24 b	0.14 a	0.11	0.23	0.09	0.08	1.58 c	0.02	0.04	0.06	0.09 c	0.03 c	0.05 b	0.06 b	0.07 b
processing																	
RO	3.62 a	4.14 a	125 a	2.57 a	77.1 a	24.3 a	33.5 a	25.3 a	35.1 a	4.26 a	2.95 a	5.19 a	213 a	2.73 a	7.70 a	13.7 a	8.39 a
TO	0.62 b	0.08 b	0.25 b	0.12 b	0.12 b	0.21 b	0.10 b	0.10 b	2.14 b	0.08 b	0.07 b	0.07 b	0.28 b	0.11 b	0.04 b	0.10 b	0.12 b
experiment B																	
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17
	(mg equiv of quercetin-3-*O*-rutinoside 100 g^{-1} of fw)									(mg equiv of luteolin-3-*O*-rutinoside 100 g^{-1} of fw)							
ANOVA[b]																	
irrigation RO	NS	*	**	NS	*	*	*	*	*	*	NS	*	*	**	*	*	*
irrigation TO	NS	NS	*	*	NS	NS	*	NS	*	*	NS	NS	*	*	NS	NS	NS
processing	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Tukey multiple range test[c]																	
irrigation RO																	
B0	4.99	10.4 a	94.7 b	4.92	70.6 b	61.1 a	44.8 b	13.9 b	31.3 a	2.93 a	3.21	2.06 b	209 b	1.01 b	6.48 b	8.92 b	4.98 b
B1	5.28	6.33 b	99.9 b	3.70	80.4 ab	51.2 b	51.7 ab	12.0 b	31.0 a	1.77 b	2.50	3.84 a	219 ab	3.89 a	7.96 a	10.6 a	5.16 b
B2	5.21	7.01 b	166 a	4.73	89.0 a	55.9 ab	61.8 a	18.9 a	25.5 b	3.13 a	2.51	2.18 b	228 a	0.33 c	6.12 b	9.45 ab	8.88 a
irrigation TO																	
B0	0.77	0.19	0.88 a	0.19 b	0.18	0.39	0.20 b	0.09	7.17 ab	0.11 ab	0.15	0.14	0.45 ab	0.13 ab	0.05	0.18	0.34
B1	0.72	0.16	0.54 ab	0.30 ab	0.12	0.39	0.34 a	0.09	7.83 a	0.08 b	0.11	0.09	0.25 b	0.16 a	0.04	0.17	0.35
B2	0.70	0.23	0.39 b	0.38 a	0.12	0.40	0.18 b	0.11	6.82 b	0.18 a	0.13	0.13	0.53 a	0.08 b	0.03	0.18	0.30
processing																	
RO	5.16 a	7.91 a	120 a	4.44 a	80.0 a	56.1 a	52.8 a	14.9 a	29.2 a	2.61 a	2.74 a	2.69 a	219 a	1.74 a	6.85 a	9.64 a	6.34 a
TO	0.73	0.19 b	0.60 b	0.28 b	0.14 b	0.39 b	0.24 b	0.09 b	7.27 b	0.12 b	0.13 b	0.12 b	0.41 b	0.12 b	0.04 b	0.17 b	0.33 b

[a]P1, hydroxytyrosol glucoside; P2, caffeoyl-6′-secologanoside; P3, oleoside; P4, elenolic acid glucoside; P5, oleuropein aglycone; P6, quercetin-3-*O*-rutinoside; P7, luteolin-3-*O*-rutinoside; P8, verbascoside; P9, oleoside diglucoside; P10, dihydro-oleuropein; P11, oleuropein diglucoside; P12, caffeoyl-6′-secologanoside; P13, oleuropein; P14, comselogoside; P15, luteolin; P16, ligstroside; and

Table 2. continued

P17, 2″-hydroxyoleuropein. [b]NS, not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [c]Values (mean of three 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.

Contrarily, in experiment B, the $ABTS^+$, $DPPH^•$, and FRAP results were affected by water stress just before harvest without rehydration. The $ABTS^+$ and FRAP capacities were increased in RO when the long-time severe treatment was applied (B2), whereas the short-time treatment (B1) enlarged $DPPH^•$ capacity. Plus, both B1 and B2 also increased $DPPH^•$ capacity. In experiment B, a decrease of 66, 83, and 25% was noticed in $ABTS^+$, $DPPH^•$m and FRAP assays, respectively. Our results determined that water availability during olive growing affects the AA; thus, it is important to take into account the timing when the stress is applied because, even if similar stress integral values are reached, they can lead to different antioxidant capacities.

With regard to the content of total phenols, both the deficit irrigation and the Spanish-style processing of the olives affected their content. A positive effect of moderate deficit irrigation (A1) was noticed, increasing around 25 and 5% on TPC in RO and TO olives, respectively. Plus, both B1 and B2 also improved TPC to ≈8%, while with severe deficit irrigation (A3) and with processing, the total phenols decreased. In accordance with previous data (loss range between 78 and 80%), it is worth pointing out that antioxidant capacity and TPC were reduced after Spanish-style processing. Whereas the decrease was 70% of TPC in experiment A, in experiment B, the decrease was 80%. A loss in AA during brining has been already reported and was found to be well-correlated to polyphenol loss in other cultivars.[25] The lower decrease in some of the antioxidant capacity assays (specially FRAP) compared to TPC loss in both experiments could be explained as a result of the intermediate stage compounds, which were not polyphenols.[26]

In this study, a severe irrigation deficit for a short time (A2) and a severe irrigation deficit for a long time and in phase II (pit hardening) (A3) induced a lower content of total phenols; however, other authors[24] found a higher content of total phenols in olive paste with severe water stress. These differences may be mainly due to the genetic component and stage of development of the fruit in which the water deficit is applied.

3.4. Instrumental Color. Table 3 shows the effect of experiments A and B in RO and TO and processing on CIE $L^*a^*b^*$ coordinates.

With regard to RO and TO, experiment A did not significantly affect color coordinates. On the other hand, processed olives (TO) in experiment B ($p < 0.001$) affected lightness (L^*) and the blue−yellow coordinate, b^*; however, no significant effect was found in the green−red coordinate, a^*. The color of B2 TO was lighter and had higher yellow intensity than olives from B0 and B1 trees. The general trend showed increases of L^* and b^* as the irrigation conditions with moderate deficit became longer over time.

The Spanish-style processing significantly ($p < 0.001$) affected lightness (L^*), the green−red coordinate, a^*, and the blue−yellow coordinate, b^*, in both experiments. Thus, the coordinates L^* and b^* decreased, while the green−red coordinate a^* increased as a result of the processing from RO to TO. These results are in agreement with previous results performed with different irrigation strategies but with the same cultivar.[2]

It is well-known that the polyphenols are correlated with color. In this study, it can be see the olive color changes after Spanish-style processing. It could be explained because of the chemical and enzymatic oxidation of *o*-dyphenol compounds

Table 3. Effect on Color (L^*, a^*, and b^*), Antioxidant Capacity (ABTS$^+$, DPPH$^•$, and FRAP), and TPC by Different Irrigation Conditions (Experiment A, A0, A1, A2, and A3; Experiment B, B1, B2, and B3) and Processing Stage (RO, Raw Olives; TO, Processed Olives)

	color[a] L^*	a^*	b^*	ABTS$^+$ [b] (mmol of Trolox equiv kg^{-1} of fw)	DPPH$^•$ [b] (mmol of Trolox equiv kg^{-1} of fw)	FRAP[b] (mmol of Trolox equiv kg^{-1} of fw)	TPC[b] (g of GAE equiv kg^{-1} of fw)
experiment A							
ANOVA[c]							
irrigation RO	NS	NS	NS	NS	NS	NS	***
irrigation TO	NS	NS	NS	NS	NS	NS	***
processing	***	***	***	***	***	***	***
Tukey multiple range test[d]							
irrigation RO							
A0	59.6	−19.7	41.4	24.7	46.3	~~23.5~~	19.3 b
A1	59.4	−19.7	40.8	27.4	50.8	25.0	24.5 a
A2	60.0	−19.6	41.4	25.4	48.9	23.7	18.9 b
A3	59.1	−19.4	39.8	27.2	48.0	25.6	19.1 b
irrigation TO							
A0	54.5	0.75	36.7	6.62	12.1	15.4	6.17 ab
A1	56.9	0.66	37.9	7.49	13.1	16.0	6.44 a
A2	56.6	0.82	36.5	7.08	12.5	14.8	5.91 ab
A3	56.4	0.62	37.0	7.19	11.2	15.5	5.47 b
processing							
RO	59.5 a	−19.6 b	40.9 a	26.2 a	48.5 a	24.4 a	20.4 a
TO	56.6 b	0.71 a	37.0 b	7.09 b	12.2 b	15.5 b	6.00 b
experiment B							
	color[a] L^*	a^*	b^*	ABTS$^+$ [b] (mmol of Trolox equiv kg^{-1} of fw)	DPPH$^•$ [b] (mmol of Trolox equiv kg^{-1} of fw)	FRAP[b] (mmol of Trolox equiv kg^{-1} of fw)	TPC[b] (g of GAE equiv kg^{-1} of fw)
ANOVA[c]							
irrigation RO	NS	NS	NS	*	*	*	***
irrigation TO	*	NS	*	NS	*	NS	**
processing	***	***	***	***	***	***	***
Tukey multiple range test[d]							
irrigation RO							
B0	59.9	−19.2	41.1	27.6 ab	47.7 b	~~23.6 b~~	32.6 a
B1	58.7	−17.8	39.5	25.2 b	53.9 a	23.2 b	21.6 b
B2	60.0	−18.9	41.3	28.8 a	48.6 b	27.9 a	33.4 a
irrigation TO							
B0	54.6 b	1.43	33.3 b	8.74	7.31 c	19.1	5.46 c
B1	55.7 b	1.19	33.8 b	9.41	9.64 a	17.8	5.90 a
B2	58.0 a	0.87	36.2 a	9.34	8.62 b	19.3	5.83 b

Table 3. continued

	experiment B						
	color[d]						
processing	L^*	a^*	b^*	ABTS⁺ [b] (mmol of Trolox equiv kg^{-1} of fw)	DPPH• [b] (mmol of Trolox equiv kg^{-1} of fw)	FRAP[b] (mmol of Trolox equiv kg^{-1} of fw)	TPC[b] (g of GAE equiv kg^{-1} of fw)
RO	59.5 a	−18.6 b	40.6 a	27.2 a	50.1 a	24.9 a	29.2 a
TO	56.0 b	1.16 a	34.4 b	9.16 b	8.53 b	18.8 b	5.70 b

[a]n = 100. [b]n = 3. [c]NS, not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [d]Values 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.

(hydroxytyrosol, hydroxytyrosol-1-glucoside, caffeic acid, verbascoside, dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol, oleuropein, and caffeoyl ester of secologanoside) by polyphenol oxidase from greenish to brownish colors. This brownish color was also correlated to the oleuropein content.[27]

3.5. Pearson Correlation between SI and Studied Variables. A Pearson correlation was run between SI and studied variables (Table 4). With regard to experiment A, a positive statistical significant correlation between polyphenol compounds and SI in RO was observed with P1, P13, and P16 (0.46, 0.56, and 0.56, respectively) and a negative correlation was noticed with P8 (−0.52). On the other hand, after Spanish-style processing, a negative significant Pearson correlation between SI and several compounds was obtained (P7, P8, P10, P11, P12, and P17: −0.60, −0.61, −0.57, −0.55, −0.50, and −0.50, respectively).

Table 4. Pearson's Correlation Coefficients between SI (MPa × Day) and Different Variables: Polyphenolic Compounds (P1−P17), Color Parameters (L^*, a^*, and b^*), Antioxidant Capacity (ABTS⁺, DPPH•, and FRAP), and TPC in Both RO and TO[a]

	experiment A		experiment B	
	SI RO	SI TO	SI RO	SI TO
P1[b]	0.46*	−0.41	−0.55*	−0.08
P2	0.32	−0.12	0.16	0.69[NS]
P3	0.32	0.39	0.68*	−0.08
P4	−0.17	0.39	0.03	0.36
P5	0.21	0.39	0.2	−0.03
P6	0.06	−0.34	−0.09	0.18
P7	−0.06	−0.60**	−0.14	−0.39
P8	−0.52***	−0.61**	0.31	0.32
P9	−0.33	0.26	−0.43	−0.48
P10	0.42	−0.57***	0.69*	0.93***
P11	−0.32	−0.55**	−0.16	0.46
P12	0.41	−0.50**	−0.68***	0.58*
P13	0.56*	0.08	0.25	0.77**
P14	0.19	−0.09	−0.77***	−0.98***
P15	0.01	−0.44	−0.81**	−0.15
P16	0.56***	−0.37	−0.51*	0.35
P17	0.27	−0.50**	0.19	−0.27
L^*	−0.56**	0.79***	0.12	0.53***
a^*	0.06	0.02	−0.41	−0.10
b^*	−0.67***	0.71*	0.21	0.51*
ABTS⁺	0.61[ns]	0.52	0.35	−0.19
DPPH•	0.44	−0.45	−0.22	−0.43
FRAP	0.63**	0.47***	0.40*	0.61***
TPC	0.26	0.68[ns]	−0.23	−0.18

[a]NS, not significant at $p < 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [b]P1, hydroxytyrosol glucoside; P2, caffeoyl-6′-secologanoside; P3, oleoside; P4, elenolic acid glucoside; P5, oleuropein aglycone; P6, quercetin-3-*O*-rutinoside; P7, luteolin-3-*O*-rutinoside; P8, verbascoside; P9, oleoside diglucoside; P10, dihydro-oleuropein; P11, oleuropein diglucoside; P12, caffeoyl-6′-secologanoside; P13, oleuropein; P14, comselogoside; P15, luteolin; P16, ligstroside; and P17, 2″-hydroxyoleuropein.

In the case of experiment B, a positive relationship was found between SI and P3 and P10 (0.68 and 0.69, respectively), while a negative relationship was observed with P1, P14, P15, and P16 (−0.55, −0.77, −0.81, and −0.51, respectively). After processing, P10, P12, and P13 were positively correlated with SI (0.93, 0.58, and 0.77, respectively), while a negative significant correlation was also noticed with P14 (−0.98).

With regard to antioxidant capacity, a positive significant correlation was observed between the FRAP assay with SI during pit hardening (experiment A) and just before the harvest without rehydration (experiment B) in both RO (0.63 and 0.47, respectively) and TO (0.40 and 0.61, respectively). Thus, the FRAP assay could be considered a key antioxidant capacity assay to be correlated with SI.

When the L^* and a^* parameters are taken into account, not only was a positive relationship noticed between them and SI in TO in experiment A (0.79 and 0.53, respectively) and experiment B (0.71 and 0.51, respectively) but a negative significant relationship was also observed in RO during experiment A (−0.59 and −0.67, respectively).

From the best of our knowledge, this is the first work comparing the polyphenolic profile of RO and TO under different irrigation treatments. A total of 17 polyphenols were identified, and results showed that, after yielding green olives to Spanish-style processing, the concentration of all compounds decreased. It could be explained as a result of the osmosis effect during fermentation and brining. Our study has shown that the polyphenol content was closely related to the amount of water applied and the timing of application. In this study, polyphenols improved their profile when olives were submitted to a moderate stress during pit hardening and also when they were submitted to a moderate deficit irrigation (long time) during the rehydration phase. Therefore, it could be said that hydroSOStainable TO provide more benefits to the health of consumers as a result of the increase of some polyphenols, such as oleuropein, oleoside diglucoside, or comselogoside, among others.

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Notes

The authors declare no competing financial interest.

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7.4. Fourth Publication

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Volatile composition, Sensory Profile and Consumer Acceptability of HydroSOStainable Table Olives

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Volatile Composition, Sensory Profile and Consumer Acceptability of HydroSOStainable Table Olives

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Abstract: HydroSOStainable table olives (cultivar Manzanilla) are produced from olive trees grown under regulated deficit irrigation (RDI) strategies. Olives produced by RDI are known to have a higher content of some bioactive compounds (e.g. polyphenols), but no information about consumer acceptance (or liking) have been reported so far. In this study, the volatile composition, the sensory profile and the consumer opinion and willingness to pay (at three locations) for HydroSOStainable table olives produced from three RDI treatments and a control were studied. Volatile composition was affected by RDI, by increasing alcohols, ketones and phenolic compounds in some treatments, while others led to a decrease in esters and the content of organic acids. Descriptive sensory analysis (10 panelists) showed an increase of green-olive flavor with a decrease of bitterness in the HydroSOStainable samples. Consumers (study done with 100 consumers in 2-rural and 1-urban locations; n_{total} = 300), after being informed about the HydroSOStainable concept, preferred HydroSOStainable table olives to the conventional samples and were willing to pay a higher price for them (52% 1.35–1.75 € and 32% 1.75–2.50 € as compared to the regular price of 1.25 € for a 200 g bag). Finally, green-olive flavor, hardness, crunchiness, bitterness, sweetness and saltiness were defined as the attributes driving consumer acceptance of HydroSOStainable table olives.

Keywords: bitterness; consumer willingness to pay; descriptive sensory analysis; green-olive flavor; "Manzanilla" cultivar; pit hardening; regulated deficit irrigation

1. Introduction

Many irrigation treatments have been evaluated in different crops, including olive trees, due to an increasing interest in water-sustainable and environment-friendly products by modern consumers [1, 2]. "HydroSOStainable products" are defined for the first time by Noguera-Artiaga et al. [3] as fruits and vegetables cultivated under regulated deficit irrigation (RDI) treatments [3]. Furthermore, Corell et al. [4] have defined HydroSOStainable index for olive trees agronomic conditions. The main aim for application of these types of sustainable strategies is conservation of water (a hot topic in arid farming research) and improving the content of bioactive compounds in vegetables and fruits as a defense mechanism against water stress [5–7]. However, to date, the effects of RDI on the consumer acceptability of olives has not been evaluated.

During the last decade, several studies about the effect of RDI on table olives agronomical, chemical and functional characteristics have been published [5,8–13], but none of them included consumer insights. The use of moderate RDI (reducing water irrigation in a moderate way but without neglecting irrigation) in table olive orchards led to an enhanced antioxidant capacity and higher polyphenolic content [2,14,15]. Although in those studies, an improvement in the sensory attributes of trees growing under moderate RDI was reported by a trained sensory panel, no consumer acceptance study was conducted. Consumer studies are essential to adjust the sensory profile of food products to consumer demands and needs by adjusting irrigation treatments, to identify the main buying drivers, to develop successful marketing strategies, and to determine an acceptable price for HydroSOStainable table olives. Recently, an affective study carried out in HydroSOStainable almonds [16]; the main conclusion was that RDI strategies led to similar global acceptance than conventional treatments but being sustainable with the environment by saving irrigation water. In addition, consumers were willing to pay a higher price for HydroSOStainable almonds (~2 € kg^{-1} more), which could be an argument to convince farmers to implement these water-saving irrigation technologies. The same behavior was observed in a study with HydroSOStainable pistachios [3], in which authors concluded that consumers were willing to pay approximately 1 euro more per kg of HydroSOStainable pistachio as compared to control samples.

Consequently, the aim of the present study was to evaluate consumer insights about HydroSOStainable table olives produced using different technologies and to link consumer data with descriptive sensory analysis and the contents of the volatile compounds. For that purpose, table olives coming from three RDI treatments [moderate deficit irrigation (T1), severe deficit irrigation during short time (T2) and severe deficit irrigation during long time (T3), and a control were assayed at the field, and the following analyses were conducted: (i) volatile composition by gas-chromatography, (ii) descriptive sensory analysis by a trained panel, and (iii) affective opinion of consumers and their willingness to pay.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Olives were collected on September 2017 from a farm, Doña Ana, which is located in Dos Hermanas (Seville, Spain) (37° 25′N, 5° 95′W). Olive trees (cultivar "Manzanilla") were approximately 32-year-old. Irrigation was performed during the night by drip, using lateral pipes per row of trees and four emitters per plant, split between the two rows (each delivering 2 L h^{-1}). A pressure chamber (PMS Instrument Company, Albany, OR, USA) was used to measured stem water potential at midday (Ψ_{stem}). Water stress integral (SI), calculated as Myers [17] was used to describe the cumulative effect of the water deficit [18]. Three different irrigation treatments and a control were carried out:

- control (T0), trees were fully irrigated, to avoid any water stress;
- moderate deficit irrigation (T1), the threshold value for water stress level (Ψ_{stem}) was set up at −2 MPa during pit hardening stage;
- severe deficit irrigation (short time) (T2), the threshold value for Ψ_{stem} was set up at −3 MPa during half period of pit hardening stage; and,
- severe deficit irrigation (long time) (T3), the threshold value for Ψ_{stem} was −3 MPa until the end of the period of pit hardening stage.

Table 1 shows the average of minimum stem water potential (min Ψ_{stem}) and SI values, together with the volume of applied water in each treatment.

Table 1. Minimum midday stem water potential (min Ψ_{stem}), water stress integral (SI) and water applied as affected by the irrigation treatment.

Sample	Min Ψ_{stem} (MPa)	SI (MPa × Day)	Water Applied (mm)
	ANOVA †		
	*	**	NS
	Multiple Range Tukey Test ‡		
T0	-2.16^{a}	17.5^{b}	274.3
T1	$-3.07^{b,c}$	$45.4^{a,b}$	294.9
T2	$-2.44^{a,b}$	$31.3^{a,b}$	347.7
T3	-3.69^{c}	69.2^{a}	105.1

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

2.2. *Spanish-style Processing*

For each RDI treatment, four batches of fresh olives were processed. Each one was formed by 50 kg of raw olives that were mixed and transported to Cooperativa Nuestra Señora de las Virtudes (La Puebla de Cazalla, Seville, Spain). First, olives were submitted to lye treatment during 6–8 h with 1.3–2.6% (weight:volume) of NaOH. Then, olives were washed with water during 12 h for cleaning and they were put on 12% NaCl for fermentation (it began with 0.17 mol L^{-1} and finished with 0.09 mol L^{-1}). After 4 months of fermentation, table olives reached an equilibrium with brine (pH < 4.2, 8% NaCl, 0.8% lactic acid and residual alkalinity < 0.120 N).

2.3. *Volatile Compounds*

Volatile extraction was performed using headspace solid phase micro-extraction (HS-SPME). Analysis were carried out according to Cano-Lamadrid et al. [2]. Briefly, 5 g of olives mixed with 15 mL of ultrapure water and 1.5 g of NaCl were placed into a vial. The vial was put in a bath at 40 °C and, after equilibration, a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane fiber (2 cm, 24 ga, StableFlex) was manually exposed to the headspace during 50 min. Volatiles were desorbed from fiber into the Gas Chromatograph-Mass Spectrometry (GC-MS) for 3 min.

V+olatile compounds identification was performed in a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A. GC-MS was equipped with a Restek Rxi-1301 2016 column. Helium was used as carrier gas with same program previously reported by Cano-Lamadrid et al. [2]. Identification was based on: (i) retention indices, (ii) GC-MS retention times, and (iii) mass spectra matches in Wiley 09 MS library (Wiley, New York, NY, USA) and NIST14 (National Institute of Standards and Technology, Gaithersburg, MD, USA). Results for each of the volatile compounds were expressed as percentage of the total area.

2.4. *Sensory Analysis*

2.4.1. Descriptive Sensory Evaluation

Ten trained panelists (aged from 25–55 years) from the Food Quality and Safety research group (Miguel Hernández University of Elche, Alicante, Spain) carried out the descriptive sensory analysis of samples under study. Each panelist had more than 600 h of experience with a variety of products, mostly, vegetable or horticultural products. For the present study, the panel was trained during 3 sessions of 1 h each, where they worked on the International Olive Oil Council, IOOC [19] table olives lexicon and finally, the panel agreed on the useful lexicon for the samples: color (from yellow to green), saltiness, bitterness, sourness, sweetness, aftertaste, hardness, crunchiness and fibrousness, and off-flavors or negative attributes; if off-flavors were present panelists could choose among the options abnormal fermentation, musty, rancid, cooking effect, soapy, metallic, earthy, and winey-vinegary [19].

Odor-free disposable 100 mL plastic cups were used to serve samples to panelists at room temperature (~20 °C). Cups were half filled with table olives coded with random 3-digit numbers and covered. Distilled water and crackers were used to cleanse palates between samples. Three sessions were used for the descriptive sensory evaluation of samples (each sample was evaluated in triplicate). Panelists used a 0–10 scale (0: no intensity; and 10: extremely strong).

2.4.2. Consumer Acceptance

For affective sensory evaluation, 100 regular table olive consumers were invited from three locations: (i) L1: El Esparragal (Murcia, Spain); (ii) L2: Elche (Alicante, Spain); and, (iii) L3: Los Desamparados (Alicante, Spain). L1 and L3 were chosen to represent consumers from rural areas, while L2 was chosen to represent consumers from urban locations. Consumers were recruited by telephone from the database of SensoFood Solutions of Universidad Miguel Hernández de Elche. The eligibility criteria was that they consume, at least, three times per week table olives. Informed consent was obtained and it is available from the Principal Investigators of the project AGL2016-75794-C4-1-R, Prof. Carbonell-Barrachina. Demographic questions were added to the questionnaire. The consumer age range was 18–24 (13%), 25–35 (14%), 36–45 (19%), 45–55 (26%) and >55 (28%) with a 62:38 gender ratio (women:men). Forty-six percent of consumers participating in this study were full-time workers, 17% part-time, 17% were students and 20% were unemployed. Consumers were also asked about their interest on food labels, and 79% answered that pay attention to product labels, especially, for Spanish-products (64%), healthy products (57%) and sustainable products (25%).

The study was carried out using SensoFood Solutions individual booths (Inverso Estudio Creativo, Murcia, Spain) in all locations to isolate participants and ensure that they worked individually, with a randomized block design and using 3-digits codes for each sample. Samples were served following the same way as for descriptive sensory evaluation. Questionnaires were prepared using 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) for color, flavor, bitterness, saltiness, sourness, hardness, crunchiness, fibrousness, aftertaste and overall. Just About Right (JAR) scale (1 = low intensity, and 9 = high intensity) was also used to score intensity attributes (flavor, bitterness, saltiness, sourness and aftertaste) to later evaluate how samples could be improve using penalty analysis. Additionally, preference test was done to rank irrigation treatments under study where consumers had to order table olive samples from dislike to like and later, Friedman test was carried out to interpret data.

All panelists (descriptive test) and consumers (affective tests) gave their informed consent for inclusion before they participated in the study. Universidad Miguel Hernández de Elche automatically exempts "general taste tests", including descriptive sensory tests from needing ethical approval, based on European Union guidelines. However, the study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández de Elche (project AGL2016-75794-C4-1-R).

2.4.3. Consumer Willingness to Pay

Consumer were first informed about HydroSOStainability concept by a leaflet and answering their questions. Then, two samples of table olives were provided to them. Commercial Spanish-style "Manzanilla" table olives were purchased from Mercadona supermarket (Mercadona is one of the most popular food supermarkets in the Mediterranean area of Spain). These table olives were labeled as "conventional" as opposed to olives labeled "HydroSOStainable", with its logo (Figure 1); in this way, the same product was presented to the consumers but with and without the HydroSOStainability logo. Each sample ("conventional" or "HydroSOStainable") was presented to the consumer together with its corresponding questionnaire. Firstly, consumer evaluated "conventional" table olives green-olive flavor, saltiness, hardness and overall liking, and secondly, HydroSOStainable table olives green-olive flavor, saltiness, hardness overall liking and willingness to pay. They were given a price for conventional table olives of 1.35 € per 200 g (Mercadona price) and 4 options to pay for HydroSOStainable table olives: ≤1.35 € (distributor brand), range 1.35–1.75 € (known brand prices), range 1.75–2.50 € (known brand prices), and >2.50 € (gourmet table olives).

This study was done in the same three locations than the affective sensory evaluation but using 100 consumers in each site (some of them were the same than in the affective sensory evaluation).

(**A**) (**B**)

Figure 1. HydroSOStainable logo. (**A**): English version. (**B**): Spanish version.

2.5. Statistical Analysis

Two or three-way analysis of variance (ANOVA) followed by Tukey's multiple range test were the chosen statistical tests. To assess panel performance, a 3-way ANOVA (factor 1: irrigation treatment; factor 2: panel session; and, factor 3: panelist) was carried out in the descriptive sensory evaluation. For affective sensory data, 2-way ANOVA was used (factor 1: irrigation treatment; and, factor 2: location). Additionally, penalty analysis was carried out with JAR data from the affective test to study how samples could be improved, and partial least squares regression (PLS) was also performed to correlate consumer overall liking with the volatile compounds and descriptive sensory attributes. All statistics were performed using XLSTAT Premium 2016 (Addinsoft, New York, NY, USA). Finally, data from the JAR analysis (Penalty analysis) were graphically represented.

3. Results and Discussion

3.1. Irrigation

Table 1 summarizes the information regarding the water stress achieved by the olive trees during 2017 season, by using 2 parameters (minimum midday stem water potential (min Ψ_{stem}) and water stress integral (SI)). Statistical differences were found among three RDI treatments and control in both parameters studied, Min Ψ_{stem} and SI. In fact, T3 was the treatment presenting the highest SI value (69.2 MPa × day) as well as the highest min Ψ_{stem} (−3.69 MPa) and this strong stress was basically due to the fact that the smallest volume of water was applied (105.1 mm). T1 and T2 occupied an intermediate position, reflecting a moderate water stress level as compared to T0 (control), which trees suffered the lowest stress. T1 and T2 were not statistically different although the stress applied was different (harder for T2) because of time of application, so applying moderate stress during log time and severe stress during short time caused similar stress on trees. These results followed a similar trend to those from previous seasons (2015 and 2016), as reported by Sánchez-Rodríguez et al. [18].

3.2. Volatile Compounds

Thirty-eight volatile compounds were identified in the table olives and their content for each irrigation treatment are shown in Table 2. Esters were the predominant volatiles in control table olives (38.48%), although their content decreased as RDI was more severe. On the contrary, terpenes were the predominant chemical family on HydroSOStainable table olives (T1–T3), with T2 olives (severe deficit irrigation, short time) having the highest content (47.39%). Organic acids were also in a high proportion (>10%) in all table olives, except T2 (2.95%). Besides, T2 showed the highest percentage of ketones (14.47%), while phenolic compounds and alcohols having similar contents in T1 and T3 samples but higher than those of T0 and T2.

There are some volatile compounds that showed the same trend in all RDI table olives, such as ethyl acetate, isoamyl acetate, *cis*-3-hexen-1-ol, 1-hexanol and *γ*-terpineol, that increased when water stress was applied, and, therefore, HydroSOStainable table olives would have, at least theoretically, stronger pineapple, banana, pear, green, woody and lilac notes than control samples. On the other hand, other compounds showed a decreased content when RDI treatments were applied (2-butanol, propanoic acid, ethyl cyclohexanecarboxylate and cyclohexanecarboxylic acid, butyl ester). Apart from these general trends, T1 experienced an increase on the contents of ethanol, dimethylsulfide (green, sulfurous), acetic acid (vinegar), ethyl propionate (fruity, pineapple), n-propyl acetate (celery), propyl propionate (oily, fruity), propyl butanoate and p-cresol (green, woody). With respect to T2, dimethylsulfide, propyl butanoate, D-limonene (citrus, lemon), p-cymene (citrus), *γ*-Terpinene (herbaceous, citrus), ethyl propanoate (fruity, melon, peach) and 6-methyl-5-hepten-2-one (herbaceous, oily) as compared to the control table olives, while 2-butanol, acetic acid and p-cresol were not found on these samples. Finally, T3 olives had an increased content of ethyl heptanoate, guaiacol (woody, smoky) and cyclohexanecarboxylic acid (fatty, fruity) but a decreased content on 2-butanol, propyl propionate and p-cresol always as compared to control samples. The sensory descriptors were obtained from relevant olive related references, including GC-olfactometry studies [2,20].

A previous study with "Manzanilla" Spanish-style table olives processed in the same way than in the current research, but under different irrigation conditions also showed statistically significant differences in a high number of volatile compounds [2]. For instance, it was found that acids and straight chain hydrocarbons increased their concentration simultaneously with the stress while aldehydes and phenol compounds decreased. These results did not agree with those found in the current research but it could be due to different irrigation conditions, among other agronomic differences such as soil characteristic or climate conditions. Brahmi, et al. [21] also found differences among volatile compounds as affected by the irrigation strategies on "Koroneiki" cultivar grown under Tunisian conditions. The content of some alcohols decreased, but others increased as it was found in the present work. In the same way, it was found that some aldehydes decreased.

Table 2. Retention indexes, sensory descriptors and percentage of total area of volatile compounds found in table olives as affected by the irrigation treatment.

Compounds	Chemical Family	Ions	RI		Descriptors §	ANOVA †	Content (%)			
		m/z	Exp.	Lit.			T0	T1	T2	T3
Ethanol	Alcohol	45	659			**	$0.663^{b,\ddagger}$	1.135^{a}	0.604^{b}	$0.998^{a,b}$
Dimethylsulfide	Sulfur compound	62/47	679		Green, sulfurous	*	0.221^{c}	0.552^{b}	1.063^{a}	0.285^{c}
Ethyl acetate	Ester	45/61/70/88	703		Pineapple	**	1.243^{c}	1.856^{b}	2.319^{a}	$2.115^{a,b}$
2-Butanol	Alcohol	45	704			*	0.690^{a}	$0.430^{a,b}$	nd ^{c}	0.285^{b}
Acetic acid	Acid	45/60	724		Vinegar	***	11.86^{b}	14.11^{a}	nd ^{c}	11.03^{b}
Ethyl propionate	Ester	57	746	726	Fruity, pineapple	*	$0.953^{b,c}$	1.764^{a}	1.377^{b}	0.737^{c}
n-Propyl acetate	Ester	61/73	749	728	Celery	*	$1.105^{b,c}$	2.040^{a}	1.353^{b}	0.927^{c}
Propanoic acid	Acid	74/45	771		Dairy, acidic	*	0.925^{a}	0.614^{b}	0.217^{c}	0.238^{c}
2,4-dimethylhexane	Hydrocarbon	85/57/71	793			NS	0.580	1.135	0.773^{b}	0.523
Ethyl butanoate	Ester	71	812	802		NS	0.221	0.706	0.411	0.333
Propyl propionate	Ester	57/75	820	810	Oily, fruity	*	1.022^{b}	1.595^{a}	1.208^{b}	0.713^{c}
Butyl acetate	Ester	56/73	827	812	Fruity, greenish	NS	0.041	0.184	0.121	0.166
Ethyl lactate	Ester	45	846	813	Butter, fruity	NS	0.083	0.230	0.121	0.095
Ethyl 2-methyl butanoate	Ester	57/102/85	861	846		NS	0.124	0.368	0.242	0.190
Ethyl 3-methyl butanoate	Ester	88/57	865	859		NS	0.124	0.199	0.145	0.166
Isoamyl acetate	Ester	55/70	895	878	Banana, pear	*	0.041^{c}	0.138^{a}	0.072^{b}	0.048^{a}
cis 3-Hexen-1-ol	Alcohol	67/55/82	899	902	Green	***	0.097^{c}	0.245^{a}	0.121^{b}	0.119^{b}
1-Hexanol	Alcohol	56/69	907	912	Green, woody	**	0.069^{c}	0.153^{a}	0.097^{b}	0.143^{a}
Propyl butanoate	Ester	71/89/55	914	896		*	0.152^{c}	0.629^{a}	0.362^{b}	0.119^{c}
β-Myrcene	Terpene	93/69	997	992	Fruity, vegetable	***	0.801	1.089	1.594	1.426
Ethyl hexanoate	Ester	88	1016	1001		NS	1.229	2.086	2.126	1.949
D-Limonene	Terpene	68/93	1041	1044	Citrus, lemon	***	20.97^{b}	20.92^{b}	34.44^{a}	21.17^{b}
p-Cymene	Terpene	119/134/91	1044	1030	Citrus	**	3.148^{c}	$3.896^{b,c}$	6.449^{a}	4.705^{b}
γ-Terpinene	Terpene	93/91/136	1069	1076	Herbaceous, citrus	**	2.223^{b}	2.470^{b}	3.913^{a}	$2.733^{a,b}$
Methyl cyclohexanecarboxylate	Ester	55/87	1093	1056	Berry, creamy	NS	5.633	2.807	1.957	3.446
Ethyl heptanoate	Ester	88/115/60	1117	1095	Fruity, melon, peach	***	0.690^{b}	0.890^{b}	2.101^{a}	2.163^{a}
Guaiacol	Phenolic compound	109/124/81	1148	1114	Woody, smoky	***	0.318^{b}	0.322^{b}	0.725^{b}	18.560^{a}
Ethyl cyclohexanecarboxylate	Ester	55/83/101	1163	1170		***	25.81^{a}	8.943^{c}	10.72^{b}	2.614^{d}
p-Cresol	Phenolic compound	107	1180		Green, woody	***	2.844^{b}	12.62^{a}	nd ^{c}	0.285^{c}
2-Phenethylalcohol	Alcohol	91/107	1184	1159	Honey, rose	*	0.207	0.675	0.411	1.355
Cyclohexanecarboxylic acid	Acid	56/73/45/82	1197	1157	Fatty, fruity	**	0.801^{b}	0.123^{b}	nd ^{b}	10.91^{a}
6-Methyl-5-hepten-2-one	Ketone	55/108/69/91	1207		Herbaceous, oily	**	$3.907^{b,c}$	6.412^{b}	14.469^{a}	0.974^{c}
γ-Terpineol	Terpene	59/93/121/136	1243	1224	Lilac	*	0.400^{c}	0.660^{b}	$0.990^{a,b}$	1.972^{a}
1,4-Dimethoxy-benzene	Phenolic compound	123/138/95	1254		Fatty	**	2.968^{c}	5.093^{a}	5.217^{a}	4.111^{b}
Cyclohexanecarboxylic acid, butil ester	Acid	129/83/55/111	1266			*	6.227^{a}	1.411^{c}	2.729^{b}	1.854^{c}
4-Ethylphenol	Phenolic compound	107/122/77	1271		Alcohol, medicinal	NS	0.870	1.104	1.546	0.547
Ethyl dihydrocinnamate		104/91	1396	1390		NS	0.469	0.383	nd	nd
β-Bisabolene	Terpene	69/93	1525	1517		NS	0.262	nd	nd	nd
Σ Alcohols						*	1.726^{b}	2.638^{a}	1.233^{b}	2.900^{a}
Σ Sulfur compounds						NS	0.221	0.552	1.063	0.285
Σ Esters						**	38.48^{a}	24.44^{b}	24.64^{b}	15.78^{c}
Σ Ketones						**	$3.907^{b,c}$	6.412^{b}	14.47^{a}	0.974^{c}
Σ Terpenes						***	27.81^{c}	$29.04^{b,c}$	47.39^{a}	32.01^{b}
Σ Acids						*	19.81^{a}	16.26^{a}	2.95^{b}	24.03^{a}
Σ Phenolic compounds						***	7.000^{b}	19.14^{a}	7.488^{b}	23.50^{a}
Σ Hydrocarbons						NS	0.580	1.135	0.773	0.523

† NS = not significant at $p > 0.05$. *, ** and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values followed by the same letter within the same row were not significantly different ($p > 0.05$), according to Tukey's least significant difference test. § Cano-Lamadrid et al. [2], Angerosa et al. [20], SAFC [22].R.I.: retention index; Exp.: experimental; Lit.: literature; nd: not detected.

3.3. Descriptive Sensory Analysis

Descriptive sensory analysis by trained panel (0–10 scale) of table olives under study was carried out and results are shown in Table 3. Saltiness, sweetness and fibrousness had mean values (for all treatments under study) of 5.4, 2.2 and 0.5, respectively; no statistically significant (ANOVA, $p < 0.05$) differences were found for these attributes and mean values are reported. With respect to color, T0 olives presented the highest color intensity (6.5), while T1 had the lowest intensity (5.4), and therefore the most yellowish color. T2 and T3 showed intermediate positions and thus, they presented intermediate colors between yellow and green. As far as the green-olive flavor is concerned, T1 table olives had the highest intensity (6.9), with T3 having the lowest score (6.2), and T0 and T2 having being in the middle. Bitterness decreased its intensity (up to 3 points) as the water stress increased. The T3 olives were the sourest ones (4.5 points higher than control) and at the same time had the longest aftertaste (2.2 points higher than control), but they simultaneously had the lowest intensity of hardness and crunchiness (3.5 and 1.7, respectively). Finally, it is important to mention that no off-flavors were found in any of the table olive under study.

Previous studies had also found changes on the intensity of key sensory descriptors as an effect of irrigation regimes on table olives. For instance, Cano-Lamadrid et al. [2] and Cano-Lamadrid et al. [13] showed the effect of two RDI treatments on the descriptive sensory profile of "Manzanilla" Spanish-style table olives. In those studies, saltiness, green-olive flavor, aftertaste, bitterness and hardness were affected by irrigation. It was found that moderate stress caused an increase of ~5% on the intensity value of the green-olive flavor attribute; result which agreed well with the trend just reported on the current research. However, results on bitterness and aftertaste showed an increase in trees grown under moderate stress [2] while in the current experiment a decreased intensity of bitterness and aftertaste (as compared to the control sample) at moderate level, while an increased aftertaste intensity was observed at severe stress. With respect to bitterness, a similar result was found on "Ascolana" olives [5], in which the bitter character decreased with the irrigation regime. The same trend was also found for hardness [5], which agreed with the low hardness of the T3 samples in the present work. In the case of "Nocellara del Belice" cultivar produced following Greek style [13], an increase on green-olive aroma, sourness, sweetness and crispness were reported under moderate water stress.

3.4. Consumer Acceptance

Affective sensory evaluation was carried out at three locations, although no statistical differences were found among data obtained; thus, the mean values of nine descriptors and the corresponding overall liking of consumers at the three locations is shown in Table 4. Table olives showed a high overall acceptability by consumers (mean of 6.3 in a scale up to a maximum score of 9). The rest of attributes under study (color, 6.5; flavor, 6.4; bitterness, 6.0, saltiness, 6.1; sourness, 6.0; hardness, 6.6; crunchiness, 6.6; fibrousness, 6.5; and aftertaste, 6.2) also received high values (1–9 scale) of consumer satisfaction degree.

Consumer preference for table olives was analyzed using the Friedman test. No statistical significant differences ($p < 0.05$) were found among preferences for control (T0) and HydroSOStainable table olives (T1–T3). Thus, this experimental finding confirmed that HydroSOStainable olives were as least as preferred as those coming from fully irrigated trees (T0), but saving water and being more sustainable; this sustainability makes these olives attractive for consumption [23].

From the best of our knowledge, only one affective sensory evaluation had been previously conducted for table olives coming for RDI treatments [2]. In this study, "Manzanilla" Spanish-style table olives under moderate deficit irrigation (but with different treatments than in the current research) were the preferred ones by consumers because of their flavor, crunchiness and aftertaste.

Table 3. Descriptive sensory attributes of table olives as affected by the irrigation treatment. Scale used ranged from 0 = no intensity to 10 = extremely strong intensity.

	Appearance	Flavor							Texture		
Sample	**Color**	**Green-Olive Flavor**	**Saltiness**	**Bitterness**	**Sourness**	**Sweetness**	**Aftertaste**	**Off-Flavor**	**Hardness**	**Crunchiness**	**Fibrousness**
ANOVA †											
	**	*	NS	*	***	NS	*	NS	***	***	NS
Multiple Range Tukey Test ‡											
T0	6.5 ^{a,‡}	6.5 ^{a,b}	5.9	5.8 ^{a}	2.4 ^{b}	2.9	5.9 ^{a,b}	0.0	7.8 ^{a}	7.3 ^{a}	0.3
T1	5.4 ^{b}	6.9 ^{a}	5.0	3.8 ^{a,b}	3.0 ^{b}	2.1	5.9 ^{a,b}	0.0	6.6 ^{a}	5.6 ^{a}	0.8
T2	5.9 ^{a,b}	6.4 ^{a,b}	5.9	4.0 ^{a,b}	2.6 ^{b}	2.2	5.6 ^{b}	0.0	7.2 ^{a}	6.1 ^{a}	0.3
T3	5.7 ^{a,b}	6.2 ^{b}	4.9	2.8 ^{b}	6.9 ^{a}	1.7	8.1 ^{a}	0.0	3.5 ^{b}	1.7 ^{b}	0.4

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

Table 4. Affective sensory analysis (at 3 locations in Spain) of table olives as affected by irrigation treatment.

	Color	**Flavor**	**Bitterness**	**Saltiness**	**Sourness**	**Hardness**	**Crunchiness**	**Fibrousness**	**Aftertaste**	**Overall Liking**
ANOVA †										
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Multiple Range Tukey Test										
T0	6.2	6.6	6.3	6.2	6.3	7.0	6.7	6.5	6.6	6.5
T1	6.7	6.6	6.3	6.0	6.0	6.7	6.6	6.6	6.2	6.4
T2	6.5	6.3	5.7	6.3	5.8	6.5	6.6	6.5	6.2	6.4
T3	6.5	5.9	5.7	5.9	5.8	6.3	6.5	6.5	5.9	5.7

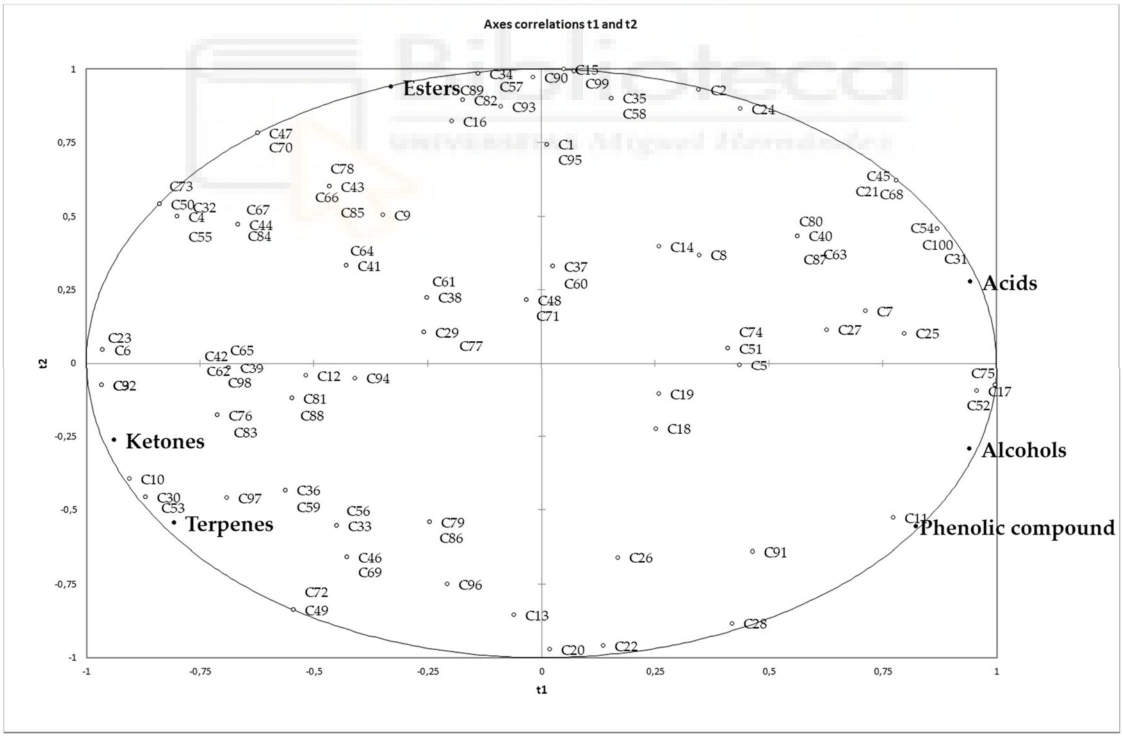
† NS = not significant at $p > 0.05$.

3.5. Driving Sensory Attributes

PLS Regression analysis was carried out to established drivers of liking for HydroSOStainable table olives (Figure 2). Two PLS maps were constructed to correlate the consumer overall liking (affective sensory analysis) with volatile compounds (total volatile contents for each chemical family) (Figure 2A) and with descriptive sensory attributes (trained panelists) (Figure 2B). Only attributes showing statistical differences among samples (ANOVA $p < 0.05$) were used to construct maps.

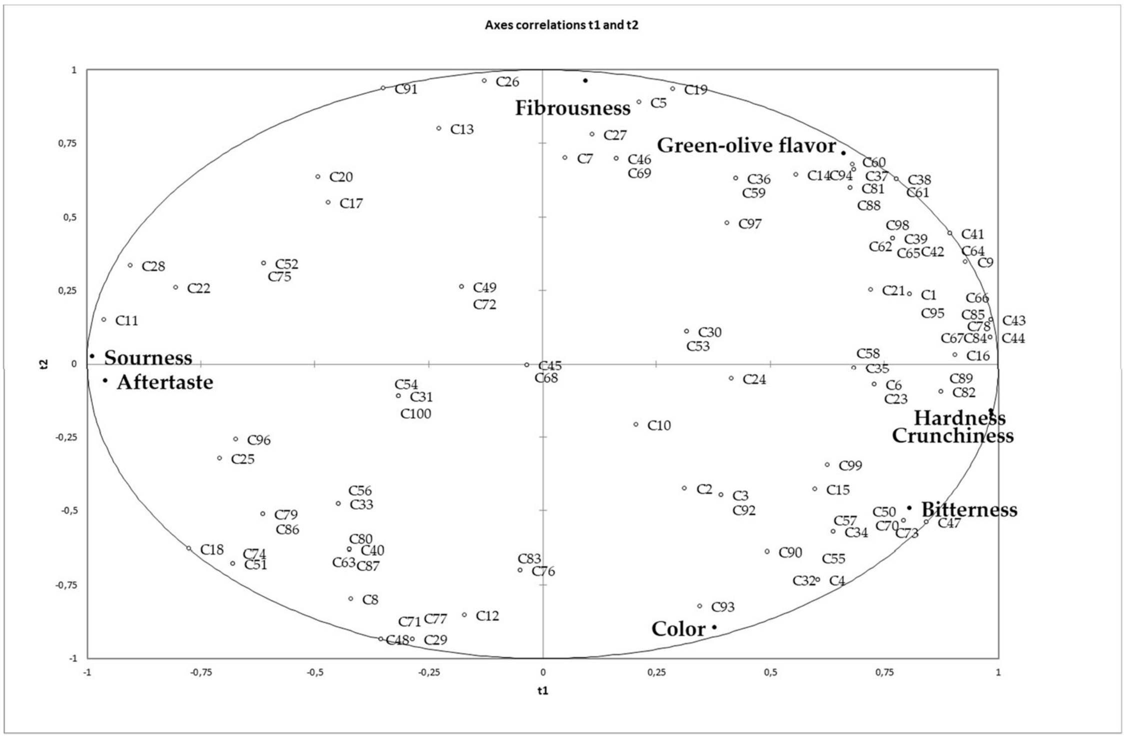
In the positive part of the x-axis (right side of the graph) volatiles associated with overall liking of consumers were acids, alcohols and phenolic compounds while in the negative part of the x-axis, ketones and terpenes can be found (Figure 2A). Although these volatile families are in opposite places on the map, consumer overall liking were not concentrate in any specific part of the map as a high dispersion on the map could be found; thus, it was not stated that no a clear relationship between overall consumer liking (affective sensory analysis) and volatile compounds was observed. Therefore, volatiles could not be considered as good driving sensory attributes for the acceptability of HydroSOStainable table olives.

Regarding map B (Figure 2B), consumer satisfaction (affective sensory analysis) was correlated with some positive attributes (descriptive sensory analysis by trained panel) of table olives such as green-olive flavor, hardness, crunchiness and bitterness, as it can be observed a high concentration of consumer overall liking in the right side of the map, where these descriptors are positioned. Consequently, these descriptors should be use as drivers to understand future consumer acceptance of HydroSOStainable table olives.



(A)

Figure 2. *Cont.*



(B)

Figure 2. Partial least squares regression (PLS) of (**A**) volatile compounds (chemical families sum) (X axis: t2) and overall consumer liking (Y axis: t1) (unfiled circles: consumer (C + number of consumer); filled circle: volatile compound); and, (**B**) descriptive sensory attributes (X axis) and overall consumer liking (Y axis) (unfiled circles: consumer (C + number of consumer; filled circle: descriptor).

3.6. Consumer Willingness to Pay

Table 5 shows the results of overall liking and satisfaction degree study done regarding consumer willingness to pay for table olives at three locations. Green-olive flavor, saltiness, hardness and consumer overall liking were evaluated as the most important attributes valued by consumers to further understanding on their perception of HydroSOStainable logo. This logo (Figure 1), caused a clear effect on consumer overall liking and green-olive flavor perception, making HydroSOStainable samples to increase their values in 1.1 and 1.3 units, respectively, as compared to the control olives. Concerning the location, for green-olive flavor attribute, consumers in L1 punctuated olives with the highest score (7.7) while L2 with the lowest (7.0), but the opposite occurred for overall liking, where L2 scored with the highest satisfaction degree (7.3). Regarding the interaction logo and location, the highest scores of the green-olive flavor attribute were found in L1 and L3 samples with the HydroSOStainability logo, and the lowest values was found in the L3 table olives without the HydroSOStainability logo. It is important to consider that L2 consumers (Elche, Alicante, Spain), corresponding to people living in an urban location, scored the highest for the overall liking without any need for the hydroSOStainability logo. No significant statistical differences were found for the effects of logo, location and their interaction on table olives saltiness and hardness.

Table 5. Overall liking and satisfaction degree on flavor, saltiness and hardness of Table Olives affected by logo effect and location.

		Green-olive Flavor	Saltiness	Hardness	Overall Liking
ANOVA Test †					
Logo effect		***	NS	NS	*
Location		***	NS	NS	*
Logo effect vs Location		***	NS	NS	*
Multiple Range Tukey Test Logo effect					
	Conventional	6.7 ^{b,‡}	6.4	6.6	6.5 ^{b}
	HydroSOStainable logo	8.0 ^{a}	7.4	7.0	7.4 ^{a}
Multiple Range Tukey Test Location					
Location	L1	7.7 ^{a}	6.6	6.9	6.9 ^{b}
	L2	7.0 ^{b}	7.1	7.2	7.3 ^{a}
	L3	7.3 ^{a,b}	7.0	6.3	6 ^{b}
Multiple Range Tukey Test Logo effect vs. Location					
Conventional	L1	7.1 ^{a,b}	5.9	6.5	6.3 ^{a,b}
	L2	7.0 ^{a,b}	6.6	7.3	7.6 ^{a}
	L3	5.9 ^{c}	6.7	5.9	5.6 ^{b}
HydroSOStainable logo	L1	8.3 ^{a}	7.2	7.3	7.5 ^{a}
	L2	6.9 ^{b}	7.7	7.0	7.1 ^{a,b}
	L3	8.7 ^{a}	7.2	6.8	7.7 ^{a}

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

Regarding willingness to pay, 88% of the participants in the study were willing to pay more than the usual price (1.35 € per 200 g) when they were informed about HydroSOStainable benefits. Concretely, 52% were willing to pay a price in the range 1.35–1.75 €, 32% 1.75-2.50 € and only 4% were willing to pay more than 2.50 €.

Previous study done with HydroSOStainable pistachios [3] also reported an increase of willingness to pay. In that case, the study was conducted in Galicia (northern Spain) and the Valencian Community (representing Mediterranean area of Spain) and consumers from Galicia willing to pay more than those from the Valencian Community; although all consumers agreed that the price for this product should be higher than for the conventional ones. A similar situation was reported by Lipan et al. [16], where Spanish and Romanian consumers were willing to pay more for HydroSOStainable almonds.

3.7. Penalty Analysis

Apart from the above described overall liking and satisfaction degree for specific sensory attributes, several JAR questions (flavor, bitterness, saltiness, sourness and aftertaste) were asked along the consumer study (affective sensory evaluation) with the purpose of analyzing the possible intensity attributes to be improved. Penalty analysis was conducted [24] an easier understanding of the relationship between JAR scores and consumer satisfaction degree scores. Figure 3 shows the proportion of consumer opinion plots against the mean penalty score. The attributes susceptible of improvement were those, which had the greatest negative impact on the sample liking for at least 20% of consumers and caused a drop of at least 1 point for liking. Results of the penalty analysis indicated that the studied deficit irrigation treatments (T1, T2 and T3) were not penalized by presenting low or high intensities of the studied attributes (Figure 2B–D). According to Spanish consumers, no improvement was necessary in these olive samples.

Previous research about overall consumer liking of HydroSOStainable almonds [16] results indicated that only the bitterness could be improved (decreasing it) when "sustained" deficit irrigation treatment was applied (deficit irrigation during whole season); however, when using RDI, HydroSOStainable almonds did not show any attribute to be improved, as it was found here for HydroSOStainable table olives, so this treatments were the best for consumer acceptance as their quality was as high as control table olives.

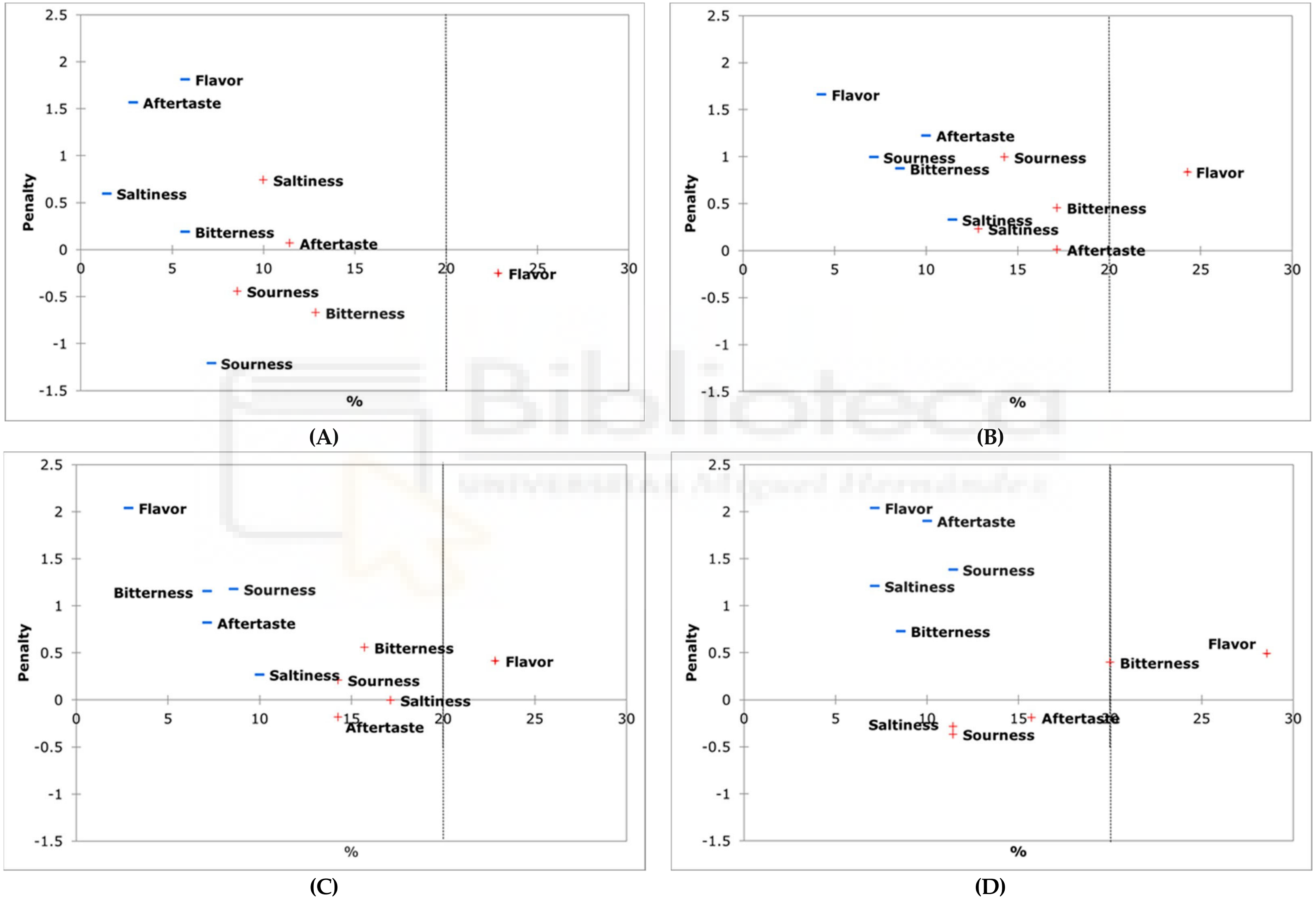


Figure 3. Penalty analysis of samples (**A**) = T0; (**B**) = T1; (**C**) = T2; (**D**) = T3. "Too low intensity" is indicated with "−" and "too high intensity" is indicated with "+".

4. Conclusions

This is the first study about consumer acceptance and willingness to pay for table olives under RDI treatments (HydroSOStainable table olives). Results indicated that RDI produced changes on volatile composition and on the intensity of several sensory descriptors. Green-olive flavor, hardness, crunchiness and bitterness seem to be the driving sensory attributes controlling consumer acceptance for HydroSOStainable table olives, although further studies are needed to fully prove this statement. Consumers preferred table olives with the HydroSOStainability logo and their satisfaction level was higher for the green-olive flavor and overall liking as compared to those of the conventional samples (without this logo). A high percentage of consumers were willing to pay a higher price for HydroSOStainable table olives. Information obtained in this research should be useful for developing the best irrigation strategy to produce table olives with the highest water saving, and the best sensory characteristics for consumers. For instance, T1 (moderate deficit irrigation where Ψ_{stem} was −2 MPa during pit hardening stage) and T2 (severe deficit irrigation during short time where Ψ_{stem} was −3 MPa during half period of pit hardening stage) strategies optimized for desirable sensory characteristics, such as green-olive flavor, hardness and crunchiness.

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7.5. Fifth Publication

Publication 5 (Open access)

Impact of gastrointestinal *in vitro* digestion and deficit irrigation on antioxidant activity and phenolic content bioaccessibility of "Manzanilla" table olives

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Research Article

Impact of Gastrointestinal *In Vitro* Digestion and Deficit Irrigation on Antioxidant Activity and Phenolic Content Bioaccessibility of "Manzanilla" Table Olives

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This was the first study investigating the polyphenol content, antioxidant potential, and polyphenol bioaccessibility after *in vitro* digestion of table olives grown using regulated deficit irrigation (RDI) treatments to save irrigation water. Two experiments were carried out: (i) experiment A, where RDI was applied during the pit hardening stage and (ii) experiment B, where RDI was applied during the rehydration stage. Only slight differences among irrigation treatments were observed in two antioxidant assays ($ABTS^{+•}$ and $DPPH^{•}$) and on TPC for the soluble fraction after *in vitro* digestion. An average of 1 g gallic acid equivalents kg^{-1} of table olives were found after digestion. Approximately, 12% of the polyphenols of table olives were bioaccessible for human absorption. Saving water techniques influence neither the final polyphenol content and antioxidant potential of table olives nor the bioaccessibility of polyphenols. The consumption of 40 g of table olives will provide 40 mg of bioaccessible polyphenols able to provide associated health benefits (~7% of the daily polyphenols intake recommendation).

1. Introduction

Table olives are a common constituent of Mediterranean diet and have beneficial effects on human health because they are antioxidant-rich foods [1]. Olives are rich in polyphenols (1-2% of its composition), and these compounds provide antioxidant, anti-inflamatory, and antitumoral properties to table olives. Olive composition can be affected by several factors such as climate, agronomic conditions, and the processing method. [2]. Nowadays, regulated deficit irrigation (RDI) strategies are being implemented on olive tree orchards with the main purpose of saving water. Additionally, moderate RDI strategies are being investigated in table olives due to their "potential" effect on enhancing the accumulation of bioactive compounds and improvement of the intensity of key sensory attributes; these special table olives are known as hydroSOStainable [3].

Studying the bioaccessibility of antioxidant and polyphenolic compounds is crucial to know their real behavior and activity *in vivo*. Bioaccessibility is defined as the tendency of compounds to be extracted from the food matrix and then, be available for intestinal cell absorption [1]. For that purpose, gastrointestinal *in vitro* digestion simulation is a model to extract compounds from the test matrix simulating human digestion, and although it is important to consider that several factors (gender, age, intestinal conditions, etc.) will affect digestion, this model is still a good alternative to avoid animal testing methods and promote animal protection [4].

In the present research, "Manzanilla" Spanish-style table olives grown under different RDI strategies (experiment A: water irrigation was reduced during the pit hardening stage; experiment B: water irrigation was reduced during the rehydration stage) were subjected to a gastrointestinal *in vitro* digestion simulation with the purpose to study how the lack of water during cultivation affects the polyphenol content, polyphenol bioaccessibility, and antioxidant activity by three methods after simulation of human digestion.

2. Materials and Methods

2.1. Experimental Conditions and Irrigation Treatments. Two irrigation experiments were carried out in this study on cultivar "Manzanilla" to evaluate the effect of water stress at two phenological stages:

2.1.1. Experiment A

(i) A0 (optimum water status): trees were fully irrigated

(ii) A1 (moderate deficit irrigation): the threshold value for water stress level (ψ_{stem}) was − 2 MPa during the pit hardening stage (day of the year (DOY) 169 to DOY 240)

(iii) A2 (severe deficit irrigation (short time)): the threshold value for ψ_{stem} was − 3 MPa during half period of the pit hardening stage (from DOY 169 to DOY 206)

(iv) A3 (severe deficit irrigation (long time)): the threshold value for ψ_{stem} was − 3 MPa until end of period of the pit hardening stage (DOY 169 to DOY 240)

2.1.2. Experiment B

(i) B0 (optimum water status): trees were fully irrigated

(ii) B1 (moderate deficit irrigation before harvest (short time)): the threshold value for ψ_{stem} was − 2 MPa at the beginning of September without the rehydration period (2 weeks before harvest)

(iii) B2 (moderate deficit irrigation (long time)): the threshold value for ψ_{stem} was − 2 MPa from mid-August without the rehydration period (four weeks before harvest)

Experiment A was carried out in a farm located in Dos Hermanas (Seville, Spain, 37°25′N, 5°95′W). "Manzanilla" olive trees were ∼30 years old and were spacing following a 7 × 4 square pattern. Irrigation was performed at night by drip with one lateral pipe per row of trees and four emitters (each delivering 2 L h^{-1}) per plant.

With respect to experiment B, the farm was located in Coria del Río (Seville, Spain, 37°17′N, 6°3′W). "Manzanilla" olive trees were ∼44 years old and were spacing following a 7 × 5 m square pattern. Also, irrigation was carried out during night by one lateral pipe per tree row and five emitters (each delivering 8 L h^{-1}) per plant. Specific agronomy characteristics could be found in [5]. Different locations were used because of land availability, although, as explained before, similar conditions characterized the field and weather.

Field characteristics of both experiments could be found in the work by Sánchez-Rodríguez and Cano-Lamadrid et al. [3]. Climatic conditions could be considered equal for both experiments because only 10 km separated the farms. A total rainfall amount of 258.94 mm was registered on experiment A from 3 September 2015 to 20 September 2016, while 254,25 mm of total rainfall amount on experiment B from 8 September 2015 to 27 September 2016. Winter minimum temperatures were around 0°C, and spring temperatures determine flowering around mid-April. Pest control, pruning, and fertilization practices were those commonly used by growers.

Stress integral (Ψ_{int}) was calculated to study the accumulative stress of olive trees produced du to reduction of water irrigation [6].

$$\Psi_{int} = \Psi - (-0.2) \times n, \quad (1)$$

where Ψ_{int} is the stress integral, Ψ is the average midday stem water potential for any interval, and n is the number of days of the interval.

Olives were collected by hand at their mature-green stage on September 2016.

2.2. "Spanish-Style" Processing. Raw olives were processed following "Spanish-Style" to table olives as previous described in [7]. Briefly, raw olives were treated with NaOH solution (1.3–2.6% weight:volume) for 6–8 h with the purpose to remove oleuropein, and then, olives were washed with water for 12–14 h to remove residual NaOH. Olives were put on 10–12% NaCl solution for fermentation until table olives-brine equilibrium was reached (pH < 4.2, 8–9% (weight:volume) NaCl, 0.7–1.0% lactic acid and residual alkalinity < 0.120 N).

2.3. Gastrointestinal In Vitro Digestion Simulation. In vitro digestion simulation was carried out following the method described in [8] on table olives of all irrigation treatments under study. Firstly, salivary solution simulation (SSS), gastric solution simulation (GSS), and intestinal solution simulation (ISS) were carried out using KCl (0.5 M), KH_2PO_4 (0.5 M), $NaHCO_3$ (1 M), NaCl (2 M), $MgCl_2(H_2O)_6$ (0.15 M), and $(NH_4)_2CO_3$ (0.5 M) following indications in [8]. Simulation of the oral phase was carried out with 10 g of table olives and simulating mastication with a-amylase (1500 U mL^{-1}) (Enzyme Commission (EC) number 3.2.1.1) and SSS. The mixture was placed in a stomacher bag and stomached for 10 minutes to simulate the mastication (Stomacher Laboratory Blender, Bioxia, Thane, India). The resulting mixture was transferred to a glass bottle. Then, the gastric phase was carried out with pepsin (2500 U mL^{-1}) (EC number 3.4.23.1) for 1 h at 37°C, 170 rpm

Table 1: Watering technique conditions of "Manzanilla" table olives of experiment A and experiment B.

	Stress integral (MPa x day)
	Experiment A
ANOVA[†]	*
A0	29.6 b[‡]
A1	62.6 ab
A2	50.4 ab
A3	87.4 a
	Experiment B
ANOVA	*
B0	75.7 ab
B1	62.9 b
B2	85.5 a

[†]*Significant at $p < 0.05$. [‡]Values followed by the same letter within the same column, and experiment were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

(Thermostatic bath with agitation BSH, Raypa, Barcelona, España), and pH 3 (adjusted with HCl, 6 M) and GSS. Finally, the intestinal step was performed with ISS, pancreatin (800 U mL^{-1}) (EC Number 232.468.9), and bile salts (160 mM) at 37°C, 170 rpm, and pH 7 (adjusted with NaOH, 1 M) for 2 hours. After the intestinal phase, liquid soluble fraction (SF) and solid residual fraction (RF) were collected. The SF was centrifuged at 10,000 rpm for 10 min at 4°C (Sigma 3–18 K; Sigma Laborzentrifugen, Germany) for later analysis.

2.4. Antioxidant Activity and Total Phenolic Content. Nondigested table olives (test matrix (TM)) and RF were extracted as described by previous authors [9]. Briefly, 2 g of fresh sample was mixed with 10 mL of MeOH/water (80 : 20 v/v) + 1% HCl. This mixture was sonicated for 15 min, and then, it was left overnight at 4°C. The next day, samples were sonicated again for 15 min and centrifuged at 10,000 rpm for 10 min at 4°C (Sigma 3–18 K; Sigma Laborzentrifugen, Germany).

Antioxidant activity of TM, SF, and RF was measured using three assays: (i) $ABTS^{+\bullet}$ as reported in [10], (ii) $DPPH^{\bullet}$ as reported in [11], and (iii) FRAP following protocol in [12]. Additionally, TPC assay was carried out as in [13]. All analyses were performed using an UV-visible spectrophotometer (Helios Gamma model, UVG 1002E). Results for antioxidant activity were quantified using calibration curves with Trolox and expressed as mmol Trolox kg^{-1} of table olives. TPC calibration curves were obtained with gallic acid, and results were expressed as gallic acid equivalents (GAE), $g\ kg^{-1}$ of table olives.

TPC bioaccessibility, expressed as the percentage of total polyphenols liberated from the TM after the gastrointestinal digestion, was calculated as previously reported by [14]:

$$\text{Bioaccessibility}(\%) = CF/CI \times 100, \quad (2)$$

where CF is the polyphenols concentration in the SF fraction and CI is the initial polyphenols concentration in undigested flesh table olives.

2.5. Statistical Analysis. One-way analysis of variance (ANOVA) was carried out to study the effect of RDI treatment, and then, Tukey's multiple range test was used to compare the means. The standard deviation (SD) of the mean is used to perform Tukey's test; therefore, the SD values were not included in tables to avoid repetition of the data and to make tables easier to understand. Statistical differences were considered significant at three levels: (i) $p < 0.05$ (*), (ii) $p < 0.01$ (**), and (iii) $p < 0.001$ (***). An XLSTAT (2016.02.27444 version, Addinsoft) was used to perform all statistical analysis.

3. Results and Discussion

3.1. Irrigation. As can be seen in Table 1, different levels of water stress were reached by olive trees. In experiment A, it could be found that A0 presented the lowest Ψ_{int} because it was not submitted to stress (control treatment). The A1 (moderate deficit irrigation) and A2 (severe deficit irrigation during short time) trees had intermediate and equivalent Ψ_{int} values, while A3 (severe deficit irrigation during long time) trees presented the highest Ψ_{int} value. In experiment B, although B0 (control) was fully irrigated, it could be seen that its Ψ_{int} value was higher than that of B1 (moderate deficit irrigation during short time before harvest). This experimental fact can be justified because even though researchers can control the applied water volume, other natural factors such as overall weather (e.g., rain) or soil differences among areas of the same field can also significantly affect Ψ_{int}. Finally, B2 presented the highest Ψ_{int} value due to water stress was moderate but during a long time before harvest.

3.2. Antioxidant Activity and Total Phenolic Content. Results of antioxidant activity and TPC of table olives under study are shown in Table 2. In experiment A, no statistical differences were found among irrigation treatments in the antioxidant activity of the TM. Regarding $ABTS^{+\bullet}$ assay, in the SF and total (SF + RF) fractions, it was found that the higher the stress, the higher the antioxidant potential. With respect to the percentage of variation (% var), after gastrointestinal *in vitro* digestion, for $ABTS^{+\bullet}$, it was found a decrease of ∼76% and the smallest decrease was found for A3 table olives (those with the highest water stress). Regarding $DPPH^{\bullet}$, in the SF and total fractions, the highest value was found on A1 at a moderate stress level, while olives from the A3 treatment (highest water stress) showed a decrease on $DPPH^{\bullet}$ in comparison to control (A0). The $DPPH^{\bullet}$ % var was higher than that of $ABTS^{+\bullet}$, with a ∼95% decrease. FRAP assay did not show statistical differences in any fraction under study, and the % var was ∼92%. Concerning TPC, only SF showed statistical differences between irrigation treatments, and a small increase (2.8%) was found between control and three RDI table olives. A decrease of ∼82% was found with respect to the variation percentage.

Regarding experiment B, TM showed statistical differences on $DPPH^{\bullet}$ and FRAP assays, as well as on TPC. For $DPPH^{\bullet}$ and TPC, both RDI treatments showed higher values than control, being the highest B1, while in FRAP assay, B1

Table 2: Antioxidant activity (ABTS+•, DPPH, and FRAP) and Total Phenolic Content (TPC) of "Manzanilla" table olives before and after gastrointestinal *in vitro* simulation digestion of experiment A and experiment B.

	Antioxidant activity (mmol trolox eq kg-1)															TPC (g GAE kg^{-1})				
	ABTS+•					DPPH•					FRAP									
	TM	SF	RF	Total	% var	TM	SF	RF	Total	% var	TM	SF	RF	Total	% var	TM	SF	RF	Total	% var
	Experiment A																			
ANOVA†	NS	**	NS	*		NS	***	NS	*		NS	NS	NS	NS		NS	**	NS	NS	
A0	6.61	1.34 b‡	0.11	1.45 b	− 78.1	12.1	0.51 ab	0.08	0.59 ab	− 95.1	15.4	1.06	0.20	1.25	− 91.9	6.17	0.70 b	0.42	1.13	− 81.7
A1	7.48	1.56 ab	0.13	1.69 ab	− 77.4	13.0	a	0.26	0.87 a	− 93.3	16.0	0.75	0.22	0.97	− 93.9	6.46	0.72 a	0.33	1.05	− 83.7
A2	7.07	1.43 ab	0.11	1.53 b	− 78.4	12.5	bc	0.09	0.47 ab	− 96.2	14.8	0.89	0.23	1.12	− 92.4	5.90	0.72 a	0.28	0.99	− 83.2
A3	7.19	1.71 a	0.25	1.98 a	− 72.5	11.2	c	0.00	0.19 b	− 98.3	15.5	0.95	0.22	1.17	− 92.5	5.47	0.72 a	0.30	1.02	− 81.4
	Experiment B																			
ANOVA	NS	NS	NS	NS		*	NS	NS	NS		**	NS	NS	NS		**	NS	NS	NS	
B0						31 c	0.47	0.00	0.47	− 93.6	19.1 a	0.99	0.22	1.21	− 93.7	5.4 c	0.72	0.23	0.95	− 82.6
B1						64 a	0.34	0.00	0.34	− 96.5	17.8 b	1.12	0.24	1.36	− 92.4	5.9 a	0.72	0.34	1.05	− 82.2
B2						62 b	0.31	0.00	0.31	− 96.4	19.3 a	1.12	0.24	1.36	− 93.0	5.8. b	0.72	0.24	0.96	− 83.5

†NS � not significant at $p < 0.05$; *, **, and ***significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡Values followed by the same letter within the same column were not significantly different ($p < 0.05$), according to Tukey's least significant difference test. *Note.* TM: test matrix; SF: soluble fraction, liquid; RF: residual fraction, solid; Total: SF + RF; % var (% variation): percentage of variation between the initial values and the values obtained after digestion.

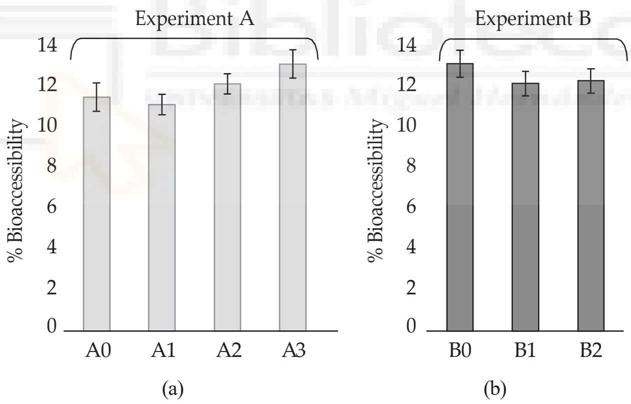

Figure 1: Total phenol content bioaccessibility after gastrointestinal *in vitro* digestion simulation of "Manzanilla" table olives of experiment A (a) (▢) and experiment B (b) (▣). Columns followed by the same letter within the same experiment were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

was the treatment that presented the lowest value. No statistical differences between treatments were found after gastrointestinal *in vitro* digestion simulation for this experiment. For $ABTS^{+\bullet}$, it was found an average of 1.4 mmol Trolox kg^{-1} of total fraction and a % var of ∼− 85%. For $DPPH^{\bullet}$ values showed an average of 0.4 mmol Trolox kg^{-1} for total fraction with a % var of ∼− 95%. With respect to FRAP assay, total fraction showed an average of 1.31 mmol Trolox kg^{-1} and a % var of ∼− 93%. Finally, average TPC was 0.99 g GAE kg^{-1} and TPC had a % var of ∼− 83%.

Although no statistical differences between irrigation treatments were found on RF, some assays showed higher values than control, such as $DPPH^{\bullet}$ (∼0.4 mmol Trolox kg^{-1}) and TPC (∼0.3 g GAE kg^{-1}). Those values could be relevant because they might indicate that there are still antioxidant and polyphenolic compounds that could be metabolized by the colon microflora, and thus, it points to a potential increase in bioavailability. Therefore, RF values found on table olives coming from deficit irrigation strategies indicated that stress caused on plant could affect the final bioavailability of antioxidants and polyphenols increasing the content of these compounds available for human absorption [15].

Figure 1 represents the percentage of bioaccessibility of TPC after the gastrointestinal in *vitro* digestion simulation. In both experiments, no statistically significant differences were found among irrigation treatments. In experiment A, A0 presented 11.5%, A1, 11.1%, A2, 12.1%, and A3, 13.1% of TPC bioaccessibility. In experiment B, control (B0) was

13.2% bioaccessible, while B1 and B2 treatments showed 12.2 and 12.3% of TPC bioaccessibility, respectively.

The decrease on antioxidant capacity and phenolic compounds content may be related to their degradation. A high proportion of table olive polyphenols are very susceptible to chemical degradation when they are submitted to the acidic conditions of digestion [15]. It was formerly described the polyphenolic profile of hydroSOStainable table olives of the current research [3], and it was found an increase of oleuropein, comselogoside, and verbascoside on A1 treatment as compared with control, as well as an increase of elenoic acid glucoside, oleuropein, and comselogoside regarding RDI treatments of experiment B. Beside this increases, it was previously reported that only 25% of oleuropein (one of the most important polyphenols in table olives) was stable during digestion, as well as only 20% of comselogoside and elenoic acid derivatives [15]. Flavonoids, verbascoside, and hydroxytyrosol derivatives also presented a small TPC bioaccessibility in a research conducted with three table olives cultivars [14]. Flavonoids are not very soluble in either organic or aqueous solvents and are usually present in foods in combination with sugars in the form of glycosides [16], so it is possible that higher concentrations that were found on RDI table olives on some flavonoids such as luteolin [3] would probably not have been extracted from the olive pulp during the in vitro digestion simulation.

Similar results, to the ones reported in the current study, have been previously reported in "Cornezuelo" table olives [15]. For instance, it was found a high decrease of $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ after gastrointestinal digestion simulation and also a decrease of 75% of TPC.

Several factors can influence the phenolic bioaccessibility. The digestion process could produce changes on polyphenol composition modifying their original profile. The food matrix where polyphenols are found could also influence the extraction [16]. It has been previously reported that the fermentation process suffered during table olives preparation and the olive cultivar could influence the final polyphenol extraction [14].

There is lack of information about the how the stress suffered by plants is related to the later metabolism of the compounds by human digestive apparatus. In the present research, the water stress suffered by olive trees due to irrigation strategies did not provide significant differences among treatments on final TPC and bioaccessibility in both experiments. It has been demonstrated that it is possible to save irrigation water without having significant effect on the final polyphenolic compounds available for human absorption. Daily intake of polyphenolic compounds is very important because of their associated health benefits. Higher total consumption of 600 mg per day would provide a protective effect against chronic diseases [17]. Consequently, eating 10 hydroSOStainable table olives per day (40 mg of bioaccessible polyphenols) would contribute to a ~7% of this total polyphenol intake recommendation.

4. Conclusions

This is the first study investigating the effect of gastrointestinal *in vitro* digestion simulation on antioxidant potential and total polyphenol content, and its bioaccessibility of table olives submitted to regulated deficit irrigation strategies. Water stress was applied during two growing stages, experiment A: the pit hardening stage and experiment B: during the rehydration phase. Results showed that, in experiment B, antioxidants and polyphenols were not affected by the stress after gastrointestinal *in vitro* digestion simulation, while in experiment A, regarding TPC, $ABTS^{+\bullet}$, and $DPPH^{\bullet}$, slight differences were found among treatments. In general, a total of ~1 g GAE kg^{-1} was extracted after digestion, indicating that the bioaccessibility of the TPC of both control and hydroSOStainable table olives was ~12%. Therefore, the daily intake of 10 hydroSOStainable table olives entails the intake of 40 mg of "bioaccessible" polyphenols and involve the daily of ~7% of polyphenols intake recommendation for protective effect against chronic diseases.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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7.6. Sixth Publication

Publication 6 (Open access)

Quality attributes and Fatty Acid, Volatile and Sensory Profiles of “Arbequina” *hydroSOStainable* Olive Oil

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Article

Quality Attributes and Fatty Acid, Volatile and Sensory Profiles of "Arbequina" *hydroSOStainable* Olive Oil

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Abstract: The use of deficit irrigation techniques on olive orchards is the main trend aiming to optimize water savings while improving functional and sensory characteristics of oils from trees under deficit irrigation techniques. The brand *hydroSOStainable* has been defined for crops produced under water restriction conditions. *HydroSOStainable* olive oils obtained under two new regulated deficit irrigation and one sustained deficit irrigation treatments in "Arbequina" olive trees were evaluated by analyzing quality parameters, antioxidant activity, total phenol content, fatty acid profile, volatile compounds, and sensory descriptors. Results showed that some of these irrigation strategies improved the phenol content at "moderate" stress levels, slightly enriched the fatty acid profile (~3.5% increased oleic acid and simultaneously decreased saturated fatty acids), and increased some key volatile compounds and also several key sensory attributes. Therefore, *hydroSOStainable* olive oil may be more attractive to consumers as it is environmentally friendly, has a higher content of several bioactive compounds, and has improved sensory characteristics as compared to control (fully irrigated) oils.

Keywords: total phenol content; oleic acid; regulated deficit irrigation; sustained deficit irrigation; antioxidants; fatty acids

1. Introduction

Olive trees were extended all over the Mediterranean countries by eastern civilization. Local wild trees were protected by families and tribes; thus, during many years, those olive trees that were well-adapted to environmental conditions were selected for cultivation. Consequently, olive trees are nowadays a traditional crop located in the Mediterranean basin, where originally wild olive trees existed. During the last decades, the demand for olive oil experienced a global increase; hence, it was necessary to increase its production using new intensification agronomic techniques [1]; one of these techniques was irrigation. This intensification produced an increase in tree growth and yield without affecting the quality of olive oil [1].

The three best-known olive tree varieties for super-high-density systems are "Arbequina", "Arbosana", and "Koroneiki". These cultivars have fast entry into production, tend to yield good annual productions, start bearing at an early age, and have excellent oil quality characteristics [2].

In Spain, olive orchards are nowadays one of the main irrigated crops (818,505 ha), only exceeded by cereals (889,411 ha) [3]. Water scarcity is one of the main issues all over the world, and it has a clear effect on agriculture. Regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI) are some of the techniques that are being developed to confront this problem. RDI decreases the use of water during some specific growing states of olives, while SDI decreases the water applied in a uniform way during all the growing season [4].

Regarding olive oil quality, the European Union and the International Olive Council have regulations to classify olive oil according to their quality [5,6]. With respect to nutritional and functional quality, it is well known that the lipid profile is one of the main contributors due to the high proportion of monounsaturated fatty acids (MUFAs) of this specific oil. Also, polyphenols have an important role, and, in fact, olive oil is the only food that has an authorized health claim [7]: "*Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress*." Volatile compounds are essential to olive oil quality, with both main and minor compounds having important roles on flavor. The odor-active compounds are responsible for the oil aroma, while the minor ones, even when they are below the olfactory threshold, can be used as quality markers as they can be essential to understand degradation or formation reactions dealing with odor-active substances [8].

HydroSOStainable products have been defined as fruits and vegetables cultivated under controlled deficit irrigation treatments, which give them differentiating characteristics that make them unique and environmentally friendly [9]. *HydroSOStainable* products provide special characteristics to the final commercial commodities, which are richer in some bioactive compounds and have a higher intensity of key sensory attributes, making them attractive for consumers [9,10]. Several products from olive trees under RDI are being studied as *hydroSOStainable*; for instance, "Manzanilla" table olives have been studied [11–13]. Arbequina olive oil under water deficit techniques have been previously studied [14–18], but there is not a clear trend on the effect of irrigation on its quality. At this time, there is not a systematic body of knowledge considering agronomic practices, phenological stage during RDI, climatic constraints, etc. to have a full understanding of the effects of RDI on "Arbequina" oil quality, and further studies looking for the best water saving technique are necessary.

Therefore, the aim of this work was to study the effect of (i) two new regulated deficit irrigation (RDI) treatments applied during phase II (pit hardening phase) [19] and (ii) one sustained deficit irrigation (SDI) treatment on "Arbequina" olive oil composition and properties. olive oil quality parameters (free acidity, peroxide value, and UV absorption characteristics), antioxidant, total phenol content, fatty acid profile, volatile compounds, and descriptive sensory analysis were carried out.

2. Results

2.1. Irrigation

Four irrigation treatments were applied to olive trees with different types of stress following crop water status by measuring midday stem water potential. Results of the applied water, stress integral (SI), minimum stem water potential (min Ψ_{stem}), yield, and mill oil yield are shown in Table 1. Not statistically significant differences in min Ψ_{stem} and SI were found. Such lack of results was likely related with a wide variability of data, and min Ψ_{stem} in control trees (T0) was low due to irrigation problems (for a few weeks in July). SI described better the levels of stress reached in the irrigation treatments. SI showed a tendency ($p < 0.1$). Control trees (T0) reached lower levels of stress than deficit irrigation treatments. While Confederation RDI (T2) had the highest stress (182 MPa × day) because of the reduced volume of water applied. Although Optimal RDI (T1) received a higher water volume than the Confederation SDI (T3), T1 water stress was higher because water deprivation was applied during stage II. The treatments did not affect significantly ($p < 0.05$) neither the yield, expressed in kilograms per hectare, nor the oil yield.

Table 1. Watering technique conditions, oil technological parameters, antioxidant activity ($ABTS^+$ and $DPPH^·$ methods), and total phenol content (TPC) of "Arbequina" olive oil.

	ANOVA†	T0	T1	T2	T3
Watering Technique Conditions					
Applied water (mm)		468	197	160	162
Stress integral (MPa × day)	NS†	53.4	152	182	132
Min ψ_{stem} (MPa)	NS	−3.80	−4.00	−4.68	−4.04
Yield (kg ha^{-1})	NS	7287	6902	6316	6764
Oil Yield (% dry weight)	NS	28.0	30.4	30.1	33.0
Olive Oil Quality Parameters					
Acidity index (%)	NS	0.31	0.37	0.24	0.31
Peroxide value (meq O_2 kg^{-1})	NS	9.29	8.07	9.36	10.1
K_{232}	NS	2.15	1.91	2.14	2.02
K_{270}	NS	0.10	0.10	0.11	0.10
ΔK	NS	−0.03	−0.02	−0.02	−0.02
Antioxidant Activity and Total Phenol Content					
$ABTS^+$ (mmol Trolox eq L^{-1})	NS	0.113	0.098	0.114	0.151
DPPH (mmol Trolox eq L^{-1})	NS	0.233	0.223	0.265	0.282
TPC (mg GAE L^{-1})	*	259.8 a‡	126.8 b	267.3 a	181.5 ab

† NS = not significant at $p < 0.05$; *, significant at $p < 0.05$. ‡ Values of olive oil quality parameters, antioxidant activity, and total phenolic content (TPC) (mean of 12 replications per irrigation treatments) followed by the same letter, within the same row, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test. Note: Acidity index: Threshold value for extra virgin olive oil (EVOO) is ≤0.8%; peroxide value: threshold value for EVOO is ≤20 meq O_2 kg^{-1}; K_{232}: threshold value for EVOO is ≤2.5; K_{270}: threshold value for EVOO is ≤0.22; ΔK: threshold value for EVOO is ≤0.01; (EEC Regulation 2568/91). T0: control (100% ETc); T1: Optimal RDI (RDI during stage II); T2: Confederation RDI (RDI during stage II using water limitation of Guadalquivir hydrographic confederation); T3: Confederation SDI (SDI using water limitation of Guadalquivir hydrographic confederation). $ABTS^+$: azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; $DPPH^·$ 2,2-diphenyl-1-pirylhydrazyl.

2.2. *Analytical Parameters for Olive Oil Grading*

Analytical parameters for olive oil grading are used to determine oil commercial quality. Following European Regulation [5], olive oil could be cataloged as extra virgin olive oil (EVOO), virgin olive oil (VOO), or lampante olive oil, which needs to be refined before consumption. EVOO has the highest quality. Results of olive oil grading are shown in Table 1. Acidity index, peroxide value, and UV absorption characteristics were under the limit established by the EU legislation; thus, it could be concluded that all oils evaluated in this study met the criteria to be categorized as EVOOs.

2.3. *Antioxidant Activity ($ABTS^+$ and $DPPH^·$ Methods) and Total Polyphenols*

Results of antioxidant activity (AA), measured by two methods ($ABTS^+$ and $DPPH^·$), and total phenolic content (TPC) are shown in Table 1. No statistical differences between irrigation treatments were found regarding both AA methods, although a different trend was observed for TPC. Treatment 2 showed the highest value of TPC, while T1 had the smallest one. The correlation between TPC and stress level was studied, and Figure 1 shows that this correlation produced a quadratic relationship in which it could be seen that TPV increased as the minimum midday stem water potential decreased until −4 MPa; at this stress level, phenols start to decrease.

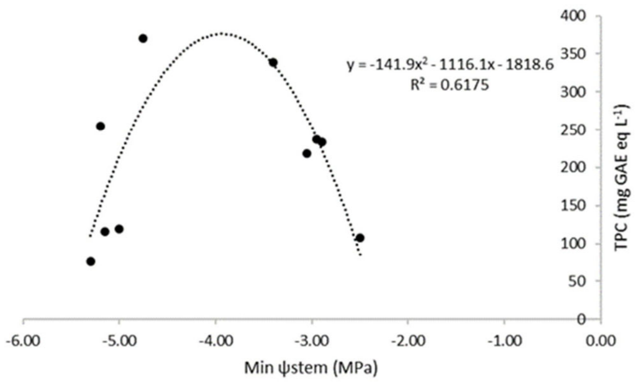

Figure 1. Quadratic correlation between total phenolic content (TPC (mg GAE eq L^{-1})) and minimum midday stem water potential (Min Ψ_{stem} (MPa)). Data shown in this figure are the mean of 12 replications per irrigation treatment.

2.4. Fatty Acids

Fatty acids are one of the most important parameters to be analyzed in olive oil, and, in this study, 22 fatty acid methyl esters (FAMEs) were identified (Table 2), providing a very detailed characterization of the composition of the oils. Ten saturated fatty acids (SFAs) were found, with palmitic and stearic acids being the predominant ones. Regarding monounsaturated fatty acids (MUFAs), eight compounds were found, among which, oleic acid was the major one; also, the compounds C18:1 *cis*-11 and C16:1 *cis*-9 (palmitoleic acid) had important concentrations. Concerning polyunsaturated fatty acids (PUFAs), five compounds were found, standing out linoleic acid.

Table 2. Fatty acid profiles of "Arbequina" olive oil as affected by the irrigation treatment.

	Compound	Concentration (g 100 g^{-1} Olive Oil)				
		ANOVA†	T0	T1	T2	T3
1	Tetradecanoic acid (Myristic acid)	NS	0.025	0.024	0.022	0.025
2	Pentadecanoic acid	NS	0.016	0.018	0.019	0.017
3	Hexadecanoic acid (Palmitic acid)	*	19.93 a‡	19.06 b	18.96 b	19.05 b
4	*cis*-6-Hexadecenoic acid (Sapienic acid)	NS	0.207	0.206	0.196	0.202
5	*cis*-9-Hexadecenoic acid (Palmitoleic acid)	NS	3.624	3.254	3.292	3.071
6	*cis*-11-Hexadecenoic acid	NS	0.030	0.025	0.023	0.021
7	Heptadecanoic acid (Margaric acid)	NS	0.135	0.150	0.161	0.154
8	*cis-9*-Heptadecenoic acid	*	0.279 b	0.310 a	0.324 a	0.312 ab
9	Octadecanoic acid (Stearic acid)	*	1.880 b	1.970 a	2.039 a	2.052 a
10	*trans*-9-Octadecenoic acid (Eleaidic acid)	NS	0.013	0.020	0.015	0.016
11	*cis*-9-Octadecenoic acid (Oleic acid)	**	47.38 b	50.13 a	51.29 a	51.00 a
12	*cis*-11-Octadecenoic acid	NS	7.026	6.514	6.537	6.419
13	9,12-Octadecadienoic acid (Linoleaidic acid)	NS	0.032	0.031	0.027	0.029
14	9,12-Octadecadienoic acid (Linoleic acid)	NS	17.55	15.90	14.76	15.38
15	Eicosanoic acid (Arachidic acid)	NS	0.512	0.499	0.497	0.506
16	6,9,12-Octadecatrienoic acid (γ-linolenic acid)	NS	0.007	0.010	0.010	0.008
17	*cis*-11-Eicosenoic acid (Gondoic acid)	NS	0.333	0.341	0.339	0.341
18	9,12,15-Octadecatrienoic acid (α-linolenic acid	NS	0.902	0.866	0.799	0.794
19	Heneicosanoic acid	NS	0.014	0.015	0.014	0.015
20	Docosanoic acid (Behenic acid)	NS	0.159	0.155	0.154	0.161
21	Tricosanoic acid	NS	0.043	0.040	0.040	0.035
22	Tetracosanoic acid (Lignoceric acid)	*	0.101 a	0.091 b	0.090 b	0.091 b
	Σ SFAs	NS	22.29	22.00	21.98	22.09
	Σ MUFAs	**	59.78 b	61.66 a	62.81 a	62.17 a
	Σ PUFAs	NS	17.61	15.96	14.82	15.43
	Atherogenic index, AI	NS	0.326	0.311	0.303	0.308
	Thrombogenic index, TI	NS	0.520	0.513	0.515	0.517

† NS = not significant at $p < 0.05$; *, **, significant at <0.05 and 0.01, respectively. ‡ Values (mean of 12 replications per irrigation treatment) followed by the same letter, within the same row, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test. Note: SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids.

Irrigation treatments induced some differences among the oil composition. In general, the highest content of SFAs was found in control oils, while the smallest one was found in the Confederation SDI (T3) oils. Palmitic acid, the predominant SFA, showed this same pattern (19.93 and 19.05 g

100 g^{-1} olive oil for T0 and T3, respectively), as well as lignoceric acid. Regarding the MUFAs content, the Optimal RDI (T1) oil showed a lower value than all deficit irrigation treatments. Oleic acid was affected by the irrigation treatments, having control (47.38 g 100 g^{-1} olive oil) oils the smallest content in comparison with Optimus RDI (50.13 g 100 g^{-1} olive oil), Confederation RDI (51.29 g 100 g^{-1} olive oil), and Confederation SDI (51.00 g 100 g^{-1} olive oil). In the case of *cis*-9-heptadecenoic acid, all the oils under deficit irrigation increased their concentration in comparison to the control. Finally, and as a general finding, PUFAs were not affected by the irrigation strategies.

Results of the atherogenic index (AI) and thrombogenic index (TI) are also shown in Table 2. The oils under study had the smallest indexes compared to different oils [20], which means that olive oil is one of the most healthy oils as reflected by their low AI and TI indexes. Low AI values represent low possibilities of atheroma formation (the possibility of lipid adhesion to cells of the immune circulatory system); besides, low TI values are associated with low chances of formation of clots in the blood vessels [20].

2.5. Volatile Compounds

Volatile profile and composition of oils under study are shown in Table 3. Alcohols were the main chemical family found in all oils, and an increase of concentration was found in all stressed olive trees as compared to the control one. Confederation SDI (T3) was the oil with the highest alcohol content mainly due to the high contents of several compounds, including ethanol (149 mg L^{-1} olive oil), 3-methylbutan-1-ol (15.7 mg L^{-1}), 2-methylbutan-1-ol (31.1 mg L^{-1}), pentan-1-ol (12.0 mg L^{-1}), (Z)-pent-2-en-1-ol (22.7 mg L^{-1}), (Z)-hex-3-en-1-ol (303 mg L^{-1}), and (E)-hex-2-en-1-ol (727 mg L^{-1} olive oil). Optimal RDI (T1) and Confederation RDI (T2) oils also showed an increase in the content of several alcohols (3-methylbutan-1-ol, 2-methylbutan-1-ol, pentan-1-ol, (Z)-hex-3-en-1-ol and (E)-hex-2-en-1-ol) as compared to the control treatment. Regarding aldehydes, the smallest concentration was found in T1 due to a decrease of pentanal, hexanal, (E)-hex-2-enal, and nonanal (0.01, 38.3, 161, and 5.86 mg L^{-1}, respectively). Similarly, 2-methylbutanal and heptanal experienced a decrease in treatment T3. In general, the ketones content decreased in T1 mainly due to a reduction in the content of pentan-2-one; however, its content increased in oils T2 and T3, while pentan-3-one increased in all stressed olive trees. An intensification of the esters contents was found in T1 and T2 oils, mainly as a result of the increased contents of (Z)-hex-3-enyl acetate and hexyl acetate. Finally, a decrease in hydrocarbons was found in T1 and T3 oils, always as compared to the control, due to significant decreases in the contents of 4,8-dimethylnona-1,7-diene and (E)-4,8-dimethylnona-1,3,7-triene in the T1 oils and of (E)-β-ocimene in the T3 ones. To summarize, the highest total volatile compound contents were those of the Confederation SDI (T3) and Confederation RDI (T2) oil. Therefore, it can be concluded that *hydroSOStainable* olive oils had higher contents of volatile compounds than oil from fully irrigated trees.

Table 3. Volatile profile (polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber) of "Arbequina" olive oils as affected by irrigation treatment.

	RI[¥]	Compound	Sensory Descriptor	Concentration (mg L^{-1} Olive Oil)				
				ANOVA[†]	T0	T1	T2	T3
V1	<500	Ethanol	Alcohol, apple, sweet	*	56.3 b[‡]	51.0 b	54.7 b	149 a
V2	568	Ethyl acetate	Aromatic, bitter, fruity	*	11.0 b	13.7 b	0.00 c	41.4 a
V3	609	Pentanal	Nutty, fruity, vanilla	*	12.8 a	0.01 c	9.68 b	11.2 ab
V4	659	2-Methylbutanal	Apple, fruity, ripe	**	7.00 b	8.93 b	17.1 a	0.01 c
V5	677	Pent-1-en-3-ol	Butter, fruity, green	*	19.7 c	17.0 c	32.5 a	26.0 b
V6	684	Pentan-2-one	Fruity, apple, pineapple	**	30.8 b	26.9 c	41.4 a	36.3 ab
V7	697	Pentan-3-one	Bitter, green, mustard	*	30.1 c	34.1 bc	39.7 b	49.2 a
V8	726	3-Methylbutan-1-ol	Sweet, woody, yeast	***	10.2 c	12.1 b	11.3 b	15.7 a
V9	730	2-Methylbutan-1-ol	Winey, spicy	*	14.0 c	20.6 b	21.3 b	31.1 a
V10	757	Pentan-1-ol	Balsamic, fruity, pungent	*	5.52 c	8.07 b	9.30 b	12.0 a
V11	762	(*Z*)-Pent-2-en-1-ol	Almond, banana, fruity	**	10.5 b	13.2 b	12.3 b	22.7 a
V12	799	Hexanal	Apple, banana, grass, green	***	63.1 b	38.3 c	65.9 b	87.3 a
V13	848	(*E*)-Hex-2-enal	Almond, apple, astringent	***	373 a	161 c	237 b	187 bc
V14	851	(*Z*)-Hex-3-en-1-ol	Apple, banana, fresh, grass	***	198 b	285 ab	279 ab	303 a
V15	861	(*E*)-Hex-2-en-1-ol	Apple, flowers, fruity, grass	*	237 b	362 ab	360 ab	727 a
V16	863	Hexan-1-ol	Banana, fruity, soft, tomato	NS	388	397	345	368
V17	890	Heptan-2-one	Banana, cinnamon, fruity	NS	4.51	1.22	3.07	0.00
V18	898	2-propenylcyclopentane		NS	9.01	4.47	14.2	8.27
V19	904	Heptanal		*	10.0 ab	12.2 ab	16.6 a	8.48 b
V20	935	3-Ethylocta-1,5-diene (isomer 1)		*	25.4 ab	19.8 b	28.5 a	28.6 a
V21	942	3-Ethylocta-1,5-diene (isomer 2)		*	26.7 ab	18.6 b	28.5 a	28.3 a
V22	998	4,8-dimethylnona-1,7-diene		**	45.8 a	27.7 b	48.7 a	42.3 a
V23	1007	(*Z*)-Hex-3-enyl acetate	Green, banana	***	229 b	377 a	357 a	236 b
V24	1016	Hexyl acetate	Green, fruity, sweet	*	70.7 c	112 a	116 a	103 b
V25	1019	(*Z*)-Hex-2-enyl acetate	Apple, banana, grape	***	8.41 a	8.35 a	8.85 a	0.87 b
V26	1053	(*E*)-β-Ocimene	Sweet, herbal	*	22.7 a	10.3 ab	8.96 ab	6.61 b
V27	1098	Methyl benzoate	Fruity	**	5.65 a	0.21 b	0.01 b	0.87 b
V28	1107	Nonanal	Apple, coconut, grape	*	12.7 a	5.86 b	10.6 ab	8.51 ab
V29	1120	(*E*)-4,8-Dimethylnona-1,3,7-triene	-	*	14.4 a	9.25 b	13.2 ab	12.9 ab
V30	1208	Methylcyclodecane	-	NS	17.3	7.85	12.7	11.8
		Σ Alcohols		***	938 b	1165 ab	1124 ab	1654 a
		Σ Aldehydes		***	478 a	226 b	356 ab	302 ab
		Σ Ketones		**	143 ab	112 b	167 a	162 a
		Σ Esters		***	324 b	511 a	482 a	382 b
		Σ Hydrocarbons		**	82.9 a	47.3 c	70.8 ab	61.8 b
		Σ Volatile compounds		*	1966 b	2061 b	2200 ab	2562 a

[†] NS = not significant at $p < 0.05$; *, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [‡] Values (mean of 12 replications per irrigation treatment) followed by the same letter, within the same row, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test. [¥] Retention index.

2.6. Descriptive Sensory Analysis

After the official panel determined the commercial quality of all oils under study as EVOO, (average of 4.0 on fruity attribute), the "Food quality and safety" panel conducted descriptive sensory analysis. The lexicon and reference materials used and the sensory profiles of the studied oils are summarized in Table 4. Regarding the positive attributes of flavor, all olive oils under deficit irrigation shared a lower intensity of both green-herbs note and sourness in comparison with the control oil but increased intensities of almond and walnut notes and sweetness. In the Optimal RDI (T1), a decrease in intensity was found for most of the attributes (fruity-olive, fruity-green, floral, green-grass, and bitter). Concerning the Confederation RDI (T2) oil, fruity-olive, fruity-green, and green-herbs increased, and woody note decreased. Finally, the Confederation SDI (T3) oils also increased the intensity of the fruity-olive and woody notes but decreased that of the green-herbs note. No negative attributes (defects) were found in any of the oils under study. Concerning mouthfeel descriptors, astringency increased in T2 and T3, which could be correlated with increased polyphenol content, and, lastly, viscosity also showed an increase in the T2 and T3 oils.

In general, it can be stated that deficit irrigation during phase II of the phenological stage of "Arbequina" affected some attributes, such as fruity, green, and nuts, and the intensity of these attributes reached the highest values in the Confederation RDI (T2) oil, which experienced the highest stress. Therefore, it could be concluded that *hydroSOStainable* olive oil had a higher intensity of several key attributes than the control.

Table 4. Descriptive sensory profiles of "Arbequina" olive oil as affected by the irrigation treatment.

	Descriptor	References	ANOVA[†]	T0	T1	T2	T3
		Flavor (positive attributes)					
D1	Fruity-olive	Canned Ripe Olives, Pitted Black = 2.3 Hacendado, Manzanilla Green olives = 5.3	***	3.9 ab[‡]	3.3 b	4.2 a	4.3 a
D2	Fruity-green (under-ripe olive)	Canned Ripe Olives, Pitted Black = 1.0 Hacendado, Manzanilla Green olives = 2.7	*	2.6 ab	2.2 b	3.0 a	2.6 ab
D3	Fruity-ripe (ripe olive)	Canned, Ripe Olives, Pitted Black = 1.0 Hacendado, Manzanilla Green olives = 3.7	NS	1.50	1.75	1.63	1.75
D4	Floral	Pompadour, Chamomile Herbal Tea = 5.0 Carrefour, White Grape Juice (diluted 1:1) = 4.7	*	1.3 a	0.8 b	1.2 a	1.3 a
D5	Green-artichoke	Hacendado, Artichoke Hearts = 3.0	NS	0.8	0.5	0.6	0.7
D6	Green-avocado	Under-ripe Fresh Avocado = 5.3	NS	0.5	0.5	0.5	0.6
D7	Green-banana	Under-ripe Green Banana = 4.0	NS	0.40	0.38	0.34	0.31
D8	Green-herbs	Verdifresh Arugula (organic, washed) = 5.7	*	2.2 a	1.3 b	1.6 b	1.6 b
D9	Green-grass	*Cis*-3-Hexen-1-ol 1000 ppm= 10.0	*	1.3 ab	0.8 b	1.5 a	0.9 b
D10	Green-peppery	Hacendado, Green-Peppercorns (dried) = 2.0	NS	0.6	0.5	0.6	0.5
D11	Apple	Fuji Apple = 5.0	NS	0.1	0.	0.21	0.4
D12	Buttery	Under-ripe Fresh Avocado = 4.0	NS	0.9	0.7	0.7	0.9
D13	Almond	Hacendado, almonds = 5.0	*	0.3 b	0.4 a	0.4 a	0.5 a
D14	Walnut	Hacendado, walnuts = 6.0	*	0.2 b	0.5 a	0.4 a	0.4 a
D15	Woody	Hacendado, walnuts = 3.0	*	0.4 ab	0.5 a	0.4 b	0.6 a
D16	Piney	Hacendado, pine nuts = 3.5	NS	0.4	0.5	0.5	0.5
D17	Sweet	1% sucrose solution = 3.0	*	0.8 b	1.4 a	1.3 a	1.4 a
D18	Sour	0.05% citric solution = 2.5	**	0.8 a	0.4 b	0.6 b	0.6 b
D19	Bitter	0,01% caffeine solution = 1.0	**	0.8 a	0.5 b	0.7 a	0.9 a
		Flavor (negative attributes)					
D20	Oxidized	La Masía, 100% sunflower oil [a] = 4.0	NS	0.00	0.00	0.00	0.00
D21	Painty	Hacendado, Green-Peppercorns (dried) = 3.3	NS	0.00	0.00	0.00	0.00
D22	Rancid	International olive council standard = 9.2	NS	0.00	0.00	0.00	0.00
D23	Musty	International olive council standard = 4.65	NS	0.00	0.00	0.00	0.00
D24	Muddy	International olive council standard = 7.9	NS	0.00	0.00	0.00	0.00
		Mouthfeel					
D25	Astringent	0,10% alum solution = 4.0	***	0.9 b	0.7 b	1.9 a	1.2 ab
D26	Pungent	Verdifresh Arugula (organic, washed) = 5.0	NS	2.7	2.5	2.6	2.9
D27	Viscosity	Hacendado, condensed milk = 10.0	***	3.9 b	3.3 b	4.2 a	4.2 a

[†] NS = not significant at $p < 0.05$; *, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. [‡] Values (mean of 12 replications per irrigation treatment) followed by the same letter, within the same row, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test.

2.7. Pearson Correlation

In order to study the correlation between the accumulative stress in the trees and all the studied functional and sensory parameters, Pearson correlation was done with SI, and significant results are compiled in Table 5. Regarding fatty acids, a positive correlation was found between the SI and C17:1 *cis* and a negative correlation with linoleic and the total content of SFAs, meaning that the higher the stress, the better the fatty acid profile (an increase of MUFAs and decrease of SFAs). Regarding volatile compounds, a negative correlation between the SI and the aldehydes was found, but the correlation was positive for the total ester content and six compounds of this chemical family (2-methylbutanal, 2-methylbuan-1-ol, (*Z*)-hex-3-en-1-ol, (*Z*)-hex-3-enyl acetate, hexyl acetate, and (*Z*)-hex-2-enyl acetate), which are associated with increased intensity of key aroma notes, such as apple, fruity, sweet, fresh, green, and grass. Finally, green-herbs and sour showed a negative correlation with the SI, although almond, walnut, sweet, and astringency were positively correlated with the SI.

Table 5. Pearson correlation between Stress Integral (SI) and fatty acids, volatile compounds, and descriptive sensory analysis attributes.

	SI
Fatty Acids	
C17:1 *cis*	0.546†*
Linoleic (C18:2 *cis*)	−0.568*
SFAs	−0.562*
Volatile Compounds	
2-Methylbutanal	0.657**
2-Methylbutan-1-ol	0.559*
(Z)-Hex-3-en-1-ol	0.670**
(Z)-Hex-3-enyl acetate	0.778**
Hexyl acetate	0.729**
(Z)-Hex-2-enyl acetate	0.602*
Σ Aldehydes	−0.706**
Σ Esters	0.871***
Descriptive Sensory Analysis	
Green-herbs	−0.841***
Almond	0.834***
Walnut	0.811***
Sweet	0.881***
Sour	−0.849***
Astringent	0.603*

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

3. Discussion

Olive oil classification (EVOO) was not affected by RDI/SDI, in agreement with results from previous studies about water deficit irrigation on olive trees [14,18,21,22]. Description of water stress was not clear with all parameters measured and could affect some of the relationship proposed. Min Ψ_{stem} was affected for variability within treatments and defined timely water stress, but presented a good agreement with some oil features. Although this measurement was not the most accurate for described irrigation treatments, it could be useful in order to describe oil features because of the reported extreme conditions. On the contrary, SI, though was also limited in comparison to irrigation treatments, it presented clear trends but did not influence the oil features. In the literature on irrigation, both indicators presented a good agreement with some yield components, such as fruit drop [23]. From our knowledge, there are very few works with the presented relationship between these water status parameter and oil features, probably because of these problems of variability.

Similar concentrations of antioxidants and TPC were reported by Sarolic et al. [24], Servili, et al. [25], and Tuberoso et al. [26] on different cultivars and also in "Arbequina" by Gomez Del Campo et al. [14] or Roodaki et al. [27], among others. In the study by Gomez Del Campo et al. [14], highest values of TPC were found when "Arbequina" olive trees were irrigated with 30% of control during the pit hardening stage, and the other irrigation treatments, even being more intense, did not show higher values. These results could be considered similar to those found in the current research, where a nonlinear relationship was found between phenolic compounds and the intensity of the water deficit. There is a previous hypothesis proposed by Horner et al. [28]: water stress in the tree can produce an increase in free phenylalanine (phenolic compounds precursor) and, therefore, phenols synthesis could be more sensitive when moderate water stress is applied.

There is contradictory information on the effect of deficit irrigation treatments on the fatty acid profile of olive oil. When stress was applied before the pit hardening stage and at the beginning of the rehydration stage, no clear effect was found neither in the study by Gucci et al. [29] nor in that of Caruso et al. [21], both with the "Frantoio" trees; these latter authors did not find a response to stress of fatty acids with a 46–48% deficit irrigation and 2–6% complementary irrigation. On the other hand, Dag et al. [30] and García et al. [22] found when studying "Koroneiki" and "Arbequina" cultivars, respectively, an increase of linoleic acid and a decrease of oleic acid, as the water stress increased during all the season. Results found by García et al. [22] (30% RDI and 60% RDI treatments before pit hardening stage) and García et al. [18] (SDI treatment with 2–3 irrigation events per week and ca. 35% of water savings and low-frequency irrigation with recovery irrigation every 3–5 weeks and ca. 35% water savings) on "Arbequina" orchard showed similar fatty acid concentrations to those of the current work; although the water stress behaved in a different way, which could be due, apart from agronomic practices, soil characteristics, climate conditions, etc., because deficit irrigation treatments were performed in a different way. Garcia et al. [22] found an increase of linolenic acid and MUFAs and a decrease of oleic acid and PUFAs in 30% RDI oils, and intermediate values for 60% RDI, while García et al. [18] found an increase of oleic acid, a decrease of linoleic acid, and MUFAs and SFAs were not affected. Therefore, it is difficult to reach a clear conclusion, considering that from the beginning of pit hardening to the end of fruit maturation, many types of enzymes contribute to synthesis of fatty acids in the olives. Irrigation has a high impact on fruit physiology, as well as the timing and the stress level [22,29]; therefore, it could be said that changes in fatty acid profiles of the studied oils in the current work could be due to the stress, but also due to the time when the stress was applied.

The synthesis of volatile compounds in olives arises during the oil accumulation phase because the main compounds (hexanal, hexyl acetate, (*Z*)-hex-3-en-1-al, (*Z*)-hex-3-en-1-ol, (*E*)-hex-2-en-1-al, (*E*)-hex-2-en-1-ol, (*Z*)-hex-3-enyl acetate, and (*Z*)-hex-2-enyl acetate) are formed through the lipoxygenase (LPO) pathway from linoleic and linolenic acids. Alcohols, esters, and ketones are also formed by fatty acid metabolism [8]. In this study, it was noticed that the alcohol concentration of all deficit irrigation oils was higher than that of the control oil. This fact may be related to an increase of LPO pathway as a result of water stress [22,25,31]. García et al. [22] found similar results to those shown in the current study; alcohols increased when water stress was applied to "Arbequina" cultivar. Other studies with different olive varieties also reported an increase of volatiles after applying water stress. It was found that 6C "green volatile" compounds, *trans*-3-hexen-1-ol, and hexyl acetate augmented when stress was applied on "Koroneiki" cultivar [31]. Similar changes on aldehydes and alcohols were reported by Servili et al. [25] on "Leccino" cultivar under water stress, as well as an increase in 2-hexen-1-ol on "Frantoio" olive oil [21]. Changes in polyphenols, volatiles, and fatty acids are directly correlated with changes in sensory descriptors of olive oil [8,32–36]. With respect to other cultivars under water stress, it was found that for "Leccino" and "Koroneiki" olive oils, under water stress had an increase in the pungent and bitter descriptors on oils with higher phenolic concentrations [25,31], but, with respect to "Arbequina" olive oil, it was found that deficit irrigation did not affect sensory quality [22], although Gomez Del Campo et al. [14] reported that bitterness could change when the irrigation is applied in July or August and also with the intensity of the stress. In the current study, bitterness and astringency scores only decreased in the T1 oil, where the lower concentration of polyphenols, aldehydes, and ketones was found.

4. Materials and Methods

4.1. Experimental Design and Sample Processing

Experiments were performed in 11-year-old "Arbequina" olive trees located at Carmona (37.49° N, −5.67° W, Seville, Spain). The orchard has a super-high density (4.0 m × 1.5 m), is 360 m^2, and has 60 trees organized in 3 lines (30 m). The design was done with randomized blocks with 4 repetitions per treatment. Harvesting was done with a mechanical harvester, like at super-intensive farming. The trees from the inside row (20) of each orchard were harvested for olive oil production. Harvest was carried out when olives had 1.9 maturity index [37]. Each block was collected in one day, and the average yield was 7117 kg ha^{-1}. Afterward, olive oil was elaborated in an olive mill model Frantoino Bio (Toscana Enologica Mori, Florence, Italy) at 40–50 kg h^{-1}, with oil extraction 2 phases technique. Each sample milled was 100 kg of olives per plot (4 per irrigation treatment). Firstly, the olives were cleaned and washed, then they were transferred to the milling, which was held in a mill mixer (<28 °C, 20 min), with 1% (*w:w*) talc and 2% (*w:w*) water, for the extraction of water flow meter 5 L h^{-1}.

Stem water potential at midday (ψ) was determined using a pressure chamber (PMS Instrument Company, Albany, OR, USA) in 4 trees per irrigation treatment, weekly during the experiment (March 24 to October 20, 2017). Water stress integral (SI) was calculated (Equation (1)) [38] to describe the accumulative effect of deficit irrigation strategies, from the beginning of pit hardening (9 June 2017) to harvest (30 October 2017) (143 days):

$$SI = |\Sigma(\psi - (-0.2)) \times n| \quad (1)$$

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

Table 1 shows the average of minimum stem water potential (min ψ_{stem}) and SI values, besides the applied water in each treatment, yield and oil yield.

Following the pressure chamber technique and the threshold values of midday stem water potential before and after the pit hardening period, 4 irrigation treatments were carried out:

- Control (T0): trees were watered to supply the 100% crop evapotranspiration (ETc).
- Optimal RDI (T1): trees were under non-limited water conditions during stage I and III while regulated deficit irrigation was applied during stage II (58% of reduction of total water irrigation amount).
- Confederation RDI (T2): the same way was followed as in T1 but with the limitation of water dotation of Guadalquivir hydrographic confederation (66% of reduction of total water irrigation amount).
- Confederation SDI (T3): sustained deficit irrigation with the water amount allowed by the Guadalquivir hydrographic confederation (66% of reduction of total water irrigation amount).

4.2. Analytical Parameters for Olive Oil Grading

Chemical parameters defined under EU Regulation [5] to classify the quality of olive oil were analyzed: free acidity (% of oleic acid), peroxide value (mEq O_2 kg^{-1} oil), and UV absorption characteristics (K_{232}, K_{270}, and ΔK) were analyzed following the procedure described by European Union Commission [5]. UV absorption indexes were measured using cyclohexane, in a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK) and 10 mm quartz cuvettes.

4.3. Antioxidant Activity ($ABTS^+$ and $DPPH^·$ Methods) and Total Polyphenols

Measurement of antioxidants (AA) and total polyphenols (TPC) was done with an extract prepared as previously described by Tuberoso et al. [39] with some modifications. Briefly, 3 g of olive oil was mixed with 5 mL of methanol/water (80/20, *v*/*v*). The mixture was shaken for 2 min, and the hydrophilic phase was filtered with a GD/X 0.45 µm cellulose acetate septa (25 mm, Sartorius, Madrid, Spain). This procedure was repeated twice with the lipophilic phases, and all the hydrophilic extracts were evaporated in a rotary evaporator at 35 °C. Finally, the residue was dissolved in 1.5 mL of methanol.

$DPPH^·$ radical (2,2-diphenyl-1-pirylhydrazyl) and $ABTS^+$ (azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) methods were used to evaluate the antioxidant activity (AA) of the olive oils. The $DPPH^·$ was done as described by Brand-Williams et al. [40], and the $ABTS^+$ as described by Re et al. [41] using a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E; Helios, Cambridge, UK). Calibration curves (3.5–5.0 mmol Trolox L^{-1}) with good linearity ($R^2 \geq 0.999$) were used for the quantification of the AA by both methods. Analyses were run in triplicate, and the results were expressed as mmol Trolox L^{-1} of olive oil.

Total phenolic content (TPC) was quantified using Folin-Ciocalteu reagent, as described by Gao et al. [42]. Absorbance was measured using the same extract and spectrophotometer as in AA. Gallic acid was used to prepare calibration curves. This analysis was run in triplicate, and the results were expressed as gallic acid equivalents (GAE) L^{-1} of olive oil. Gallic acid was used to facilitate comparison with previous studies.

4.4. Fatty Acids

Fatty acid methyl esters (FAMEs) were prepared following ISO-12966-2 [43]. The internal standard was added (C13:0; 0.04 mg mL^{-1}) to calculate the fatty acids concentration. Gas chromatography (C-17A; Shimadzu Corporation, Kyoto, Japan) connected to a flame ionization detector (FID) was used to inject oils after transmethylation following ISO-12966-4 [44] with some modifications. The capillary column used was CPSil-88 (100 m × 0.25 mm ID. 0.2 µm film thickness; J&W 112-88A7; Agilent Technologies, Santa Clara, CA, USA), which is appropriate for olive oil fatty acids separation. Detector temperature was 260 °C, and oils were injected with a 1:20 split ratio. The oven temperature was 175 °C for 10 min, then raised to 220 °C (3 °C min^{-1}) and kept at 220 °C for 5 min. The carrier gas was helium, and detector gases were hydrogen (30 mL min^{-1}) and air (350 mL min^{-1}), and helium (30 mL min^{-1}) was used as a make-up gas. Standard solutions (FAME 37 MIX, Supelco; Bellefonte, PA, USA), were injected under the same conditions as oils for the identification of compounds.

Additionally, atherogenic index (AI) and thromogenic index (TI) were calculated as indicated in Equations (2) and (3) [20]:

$$AI = (4 \times C14{:}0 + C16{:}0) / [\Sigma PUFA\ (n - 3) + \Sigma PUFA\ (n - 6) + \Sigma MUFA] \quad (2)$$

where C14:0 is myristic acid, C16:0 is palmitic acid, PUFA means polyunsaturated fatty acids, and MUFA is monounsaturated fatty acids.

$$TI = (C14{:}0 + C16{:}0 + C18{:}0) / [0.5 \times \Sigma MUFA + 0.5 \times \Sigma PUFA\ (\mathrm{n} - 6) + 3 \times \Sigma PUFA\ (n - 3) + (n - 3) / (n - 6)] \quad (3)$$

where C18:0 is stearic acid.

4.5. Headspace Solid-Phase Microextraction (HS-SPME)

For HS-SPME extraction, 5 mL of olive oil was added into a 15 mL glass vial with the addition of 2 µL of carvacrol (325.6 mg carvacrol in 1 L of olive oil) as an internal standard. One gram of NaCl salt was added, and the vial was hermetically sealed with polytetrafluorethilenesilicone septa and maintained in a water bath at 40 °C during equilibration (15 min) and extraction (40 min) and was partially submerged such that the liquid phase of the oils was below the water level. All the experiments were performed under constant stirring (500 rpm) with a magnetic stirrer. After sampling, the SPME fiber was inserted into the injector (250 °C) of the GC-MS for 7 min, where the extracted volatiles were thermally desorbed directly into the GC column. Polydimethylsiloxane/divinylbenzene (65 µm PDMS/DVB) fiber, obtained from Supelco Company (Bellefonte, PA, USA), was used previously conditioned according to the manufacturer instructions [24].

4.6. Gas Chromatography and Mass Spectrometry (GC-MS)

An Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7890A equipped with the mass selective detector, model 5977E, and capillary column HP-5MS (5%-phenyl)-methylpolysiloxane (Agilent J & W; Santa Clara, CA, USA) GC column, 30 m, 0.25 mm i.d., coating thickness 0.25 µm was used. The flow rate of the helium carrier gas was 1.5 mL min^{-1}. The injector was operated in split mode (2:1 split ratio) at 260 °C. The column was maintained at 40 °C for 3 min, heated to 100 °C at a rate of 5 °C min^{-1}, heated to 260 °C at a rate of 3 °C min^{-1}, and held to 260 °C for 3 min. MS conditions were as follows: source temperature 230 °C; quadrupole temperature 150 °C; transfer line temperature 270 °C; acquisition mode electron impact (EI 70 eV) by 3 scans s−1, and mass range m/z 29–350. The analyses were carried out in triplicate. The individual peaks were identified by comparison of their retention indices (relative to C9–C25 *n*-alkanes for HP-5MS) to those of authentic samples and literature as well as by comparing their mass spectra with the Wiley v9-MS library (Wiley, New York, NY, USA) and NIST14 (National Institute of Standards and Technology; Gaithersburg, MD, USA) mass spectral database [24].

4.7. Descriptive Sensory Analysis

Four olive oils of each irrigation treatment were analyzed by an accredited sensory panel with the purpose to determine olive oils commercial quality as described by the European regulation [5]. With that objective, oils were sent to the Laboratorio Agroalimentario de Granada (Granada, Spain) (ENAC number: 276/LE 507).

Additionally, 8 panelists from the Research Group "Food Quality and Safety" (Universidad Miguel Hernández; Alicante, Spain) analyzed the same oils to fully understand how deficit irrigation techniques affected the olive oil sensory characteristics. This panel had more than 600 h of training in sensory analysis, especially of fruit and vegetables, and it consisted of 4 males and 4 females aged from 25 to 55 years old.

The panelists did three orientation days in order to determine the scales of each attribute and the reference product. A previous lexicon developed by this panel was used [45] following International Olive Council (IOC) [46]. The scale ranged from 0 to 10, and the reference products were adapted to the Spanish market.

The descriptive sensory analysis used in this study was mandatory from the European normative to accurate oil quality [5,46], as well as other attributes to provide more detailed information about how deficit irrigation affected sensory descriptors. These descriptors were divided into 3 categories: (i) Flavor (positive attributes): fruity-olive, fruity-green, fruity-ripe, floral, green-artichoke, green-avocado, green-banana, green-herbs, green-grass, green-peppery, apple, buttery, almond, walnut, woody, piney, sweet, sour, and bitter; (ii) Flavor (negative attributes): oxidized, painty, rancid, musty, and muddy; (iii) Mouthfeel: astringent, pungent, and viscosity. Definition for all attributes and reference products with their punctuation are shown in Table 4.

4.8. Statistical Analyses

One-way analysis of variance (ANOVA) and Tukey's multiple range test were performed to compare experimental data and determine significant differences among irrigation treatments ($p < 0.05$). The standard deviation (SD) of the mean is used to perform Tukey´s test; therefore, the SD values were not included in Tables to avoid repetition of the data and to make Tables easier to understand.

A three-way ANOVA was used (factor 1: irrigation treatment; factor 2: session; factor 3: panelist) to study the effect of these three factors on the composition, quality, and functionality of the olive oils under study and to check panel consistency. Pearson correlation was also done to correlate all data with water stress integral. XLSTAT (Version 2016.02.27444, Addinsoft, Paris, France) was used to perform all statistical analysis.

5. Conclusions

It can be concluded that *hydroSOStainable* olive oils had: (i) complied with criteria to be classified as EVOO, (ii) some of them improved contents of total phenolic compounds at moderate stress levels, (iii) an enriched fatty acid profile (~3.5% increased contents of oleic acid and decreased contents of SFAs), (iv) higher contents of several volatile compounds, and (v) higher intensities of key sensory attributes, which may make them more attractive to consumers. Finally, the Confederation RDI (T2) is the recommended irrigation treatment because it (i) saved 66% of irrigation water, (ii) led to high simultaneous contents of phenolic compounds and slightly increased the monounsaturated fatty acids, and (iii) had a balanced sensory profile.

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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 the absence of light, and it is planned to store them until the end of 2019.

7.7. Seventh Publication

Publication 7 (Literal transcription)

"Arbequina" Olive Oil Composition Is Affected by the Application of Regulated Deficit Irrigation during Pit Hardening Stage

Sánchez-Rodríguez, L., Kranjac, M., Marijanovic´, Z., Jerkovic´, I., Pérez-López, D., Carbonell-Barrachina, Á.A., Hernández, F., Sendra, E.

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"Arbequina" Olive Oil Composition Is Affected by the Application of Regulated Deficit Irrigation during Pit Hardening Stage

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Abstract Three new regulated deficit irrigation (RDI) treatments were applied to "Arbequina" olive orchards during pit hardening. Oil quality was determined by measuring analytical parameters for olive oil grading, antioxidant activity, total phenol content, fatty acid profile, volatile compounds profile, and sensory analysis. Oils from RDI were classified as "extra virgin olive oil" and their quality was improved due to their higher antioxidant potential (ABTS+ [increased ~75%] and DPPH˙ [increased ~25%] assays) and phenols (increased ~53%) than control. Concentration of total volatile compounds decreased (~27%) but RDI olive oils showed a more balanced profile (alcohols, aldehydes, and esters). Monounsaturated fatty acid content increased (~5%) and atherogenic and thrombogenic indexes decreased (~8.5%) in RDI olive oil. Regarding sensory analysis, RDI provided more balanced oils with higher fruit aroma than control. Other benefits of RDI olive oil, when compared with oil from full irrigated orchards are reduced use of water and improved functional and sensory quality.

Supporting information Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Keywords Water stress · Fatty acids · Volatile compounds · Antioxidants · Sensory analysis

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Introduction

Virgin olive oil is one of the most appreciated products around the world, not only due to its nutritional properties and sensory characteristics, but also because previous studies have proven that its regular consumption could help in preventing some health diseases (Aparicio and Harwood, 2013). The health benefits and sensory quality (e.g., flavor and taste) depend on several factors such as cultivar, growing conditions, ripening state at harvest, extraction method, etc. (Vazquez-Araujo et al., 2015). "Arbequina" variety is one of the most cultivated in Spain; 22–27% of Spanish olive oil comes from "Arbequina" olive trees. This olive cultivar is characterized by having one of the highest olive fruits productivity and oil yield (Aparicio and Harwood, 2013).

The aroma of virgin olive oil is attributed to certain volatile compounds, and some of them are crucial and have been directly correlated with olive oil sensory quality (Kalua et al., 2007). Regarding nutritional properties, its fatty acid profile is of major relevance given its high content in monounsaturated fatty acids (MUFA). Antioxidants and phenolic compounds also play an important role in olive oil health-related properties (Aparicio and Harwood, 2013); for instance, 5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil contribute to the protection of blood lipids from oxidative stress (European Union, 2012). Physico-chemical composition of both olive fruits and olive oil, are affected by pre- and post-harvest factors, such as irrigation (Mele et al., 2018). Previous studies have reported that water status of olive trees can affect volatile and phenolic profiles as well as the intensity of key sensory attributes. Some volatile compounds that showed an inverse relationship with water stress were hexanal, *trans*-hex-2-enal and hexan-1-ol, while a positive relationship was found between phenolic concentration and water stress (Servili et al., 2007). Regarding sensory quality, the use of low volumes of irrigation water led to high values of the intensity of key sensory attributes; in this way, medium irrigation levels could produce oils with proper sensory characteristics and highly appealing for consumers (Campestre et al., 2017).

Nowadays, crops that traditionally did not need irrigation are being irrigated because of the intensification of agriculture; however, water availability is reduced day by day, especially that meant for agriculture. Consequently, the application of regulated deficit irrigation (RDI) has been widely used to irrigate trees reducing the use of water but without jeopardizing fruit yield and fruit quality (Fereres and Goldhamer, 1990). In this way, "*HydroSOStainable*" olive oil is the product obtained from olive trees cultivated under RDI. This oil differs clearly from others because it requires less irrigation water, has higher contents of certain bioactive compounds and intensifies key sensory attributes, making the commercial product more attractive for consumers (Sanchez-Rodriguez et al., 2019).

Considering all the above information, it is essential to optimize the application of the RDI treatments to maximize fruit yield and to increase functionality and sensory quality of the commercial olive oil. Consequently, three new irrigation treatments were carried out in "Arbequina" olive trees, by manipulating the mid-day stem water potential during pit hardening stage (day of the year [DOY] 135–239) (from −1.2 MPa to −3 MPa for moderate stress and a total water reduction for severe stress during the same stage). Analytical parameters for olive oil grading (acidity, peroxide value, and UV absorption characteristics), antioxidant activity (AA), total phenol content, fatty acid profile, volatile compounds profile and sensory quality were analyzed.

Materials and Methods

Experimental Design and Sample Processing

The experiment was performed in an olive orchard (cultivar "Arbequina") near Ciudad Real, Spain (39º N, 3º,56' W; altitude 640 m) in 2017 season. Olive plantlets were planted in 1999 with a tree spacing of 7 m × 4.76 m (300 trees ha^{-1}). Canopy volume of the average tree on the DOY 180 was 16.71 m^3, what means 5012 m^3 ha^{-1} and a percentage of soil cover by the trees of about the 30%. Irrigation was performed daily using a drip irrigation system with four self-compensating emitters (each delivering 8 L h^{-1}) per tree and irrigation water with an electrical conductivity of 2.6–2.9 dS cm^{-1}. The distance between drippers was 1 m, and the distance from trunk to drippers was 0.5 m.

The soil at the experimental site was an alkaline (pH 8.1) shallow soil with a discontinuous petrocalcic horizon located at 0.75 m (Petrocalcic Palexeralfs), with a clay loam texture, low electrical conductivity (0.2 dS/m), 1.05% of organic matter, 0.12% of nitrogen, 17 10^{-4} mol kg^{-1} of potassium levels and high cationic exchange capacity (0.186 mol kg^{-1}). The soil volumetric water content for the first 0.3 m depth is 22.8% at field capacity (soil matric potential −0.03 MPa) and 12.1% at permanent wilting point (soil matric potential −1.5 MPa), and from 0.3 to 0.75 m it was 43.0 and 21.1%, respectively.

The orchard was managed under no tillage conditions; weeds were controlled with postemergence herbicides. Pest control and fertilization practices were those usually followed by local growers. Before irrigation treatments started, with the Spring rain, in the first fortnight of May, orchard was fertilized with 500 g per tree of potassium sulphate added in the irrigation system.

Meteorological data (daily air temperature, crop reference evapotranspiration and mean daily air vapor pressure deficit) of a near station of the orchard (100 m) were recorded (Fig. 1).

The experimental plot consisted of 11 rows *per* five columns of olives trees. The two rows and columns in the outer part of the field were maintained as line borders and fruits from these trees were not harvested. Thus, measurements were made in representative olive trees and their fruits from the inner rows and columns (9 × 3). The experimental design consisted of randomized blocks with four repetitions. Around the experimental orchard, two rows and columns made of border of the orchard.

Stem water potential at mid-day (Ψ) was determined weekly using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA). Leaves near to the main trunk were covered with aluminum foil at least 1 hour before measurements were taken. Water potential was measured at

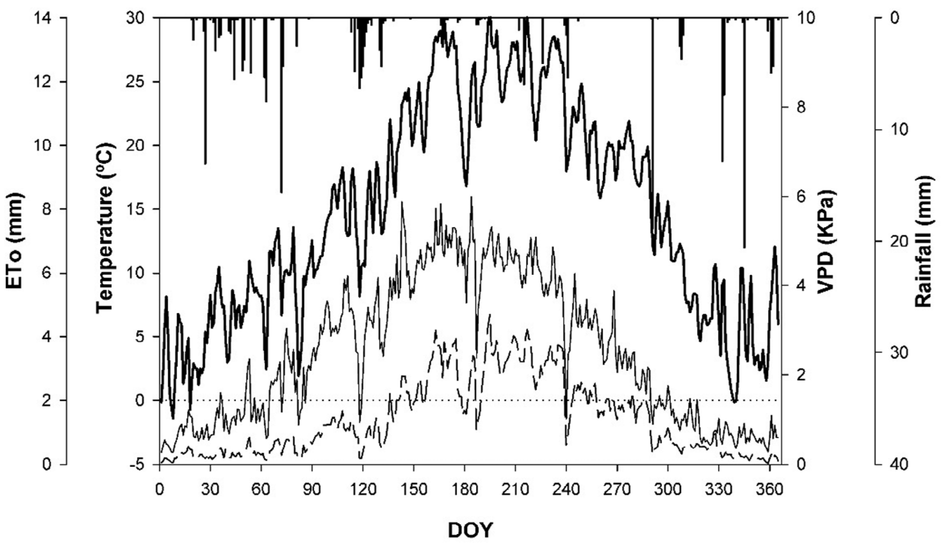

Fig. 1 Daily air temperature (Tm, solid thick line), daily crop reference evapotranspiration (ETo, thin line), mean daily air vapor pressure deficit (VPD, dashed thin line), daily rainfall (vertical bars)

mid-day in eight trees *per* treatment (two for each experimental plot).

In order to describe the accumulative effect of the different irrigation strategies, the water stress integral (SI) (equation 1) (as defined by Myers, 1988) was calculated from the Ψ data:

$$SI = \left| \sum (\Psi - (-0.2)) \times n \right| \quad (1)$$

where SI is the stress integral, Ψ is the average of mid-day stem water potential for any interval, and *n* is the number of the days in the interval.

Leaf samples from the 27 olives trees of each experimental plot were collected in July. Collection was made according the protocol of Fernandez-Escobar (2017). Leaf of each experimental plot were mixed, conserved in cold conditions until to be sent to a laboratory for its analysis of nutrient contents.

Harvesting was made on the DOY 317 (the 13 of November of 2017), 175 days after full bloom. Yield was measured in five trees of each experimental plot. Twenty five kilograms of the total production were taken as representative of each specific treatment. The oil from the olives under study was extracted with a mini-mill (Toscana Ecologica Mori 50, Tavernelle Val di Pesa, Italy) at 40–50 kg h^{-1}, with 2 extraction phases. After cleaned and washed, olives were held in a mill mixer at <28 ºC during 20 min for extraction with flowmeter 5 L h^{-1}. Oil extraction was made (as soon as possible) the next day of harvest. Two complete days (morning and evening) were necessary for the oil extraction of the all samples. Fruit samples were conserved outdoors; there was not frost temperatures, but the chill temperatures of November were optimal to conserve samples. After extraction, olive oil samples were stored at 4 ºC in the absence of light until analysis were done.

Four irrigation treatments were applied:

1. Control treatment (T0) served to determine potential yield. Control plants (T0 treatment) were irrigated at 100% of crop irrigation requirements *(ETc)* of the previous week.
2. Treatment 1 (T1) reduced irrigation water to produce water stress during pit hardening, maintaining Ψ at −2 MPa during this phase.
3. Treatment 2 (T2) reduced irrigation water in a more severe way to maintain Ψ at −3 MPa during the same phase.
4. Finally, treatment 3 (T3) was the one producing the strongest water stress conditions because no irrigation was performed during the pit hardening stage.

The irrigation protocol for stressed treatments was derived from the methodology proposed by Moriana et al. (2012) using the stem water potential. The Ψ threshold for T1 and T2 treatments before starting pit massive hardening was −1.2 MPa, while the threshold for T1, T2, and T3 after pit hardening was −1.4 MPa. Both thresholds were established in Moriana et al. (2012) as those corresponding to a well irrigated olive orchard.

Analytical Parameters for Olive Oil Grading

Acidity, peroxide value and UV absorption characteristics (K_{232}, K_{270}, and ΔK) were analyzed following the procedure described by European Union Commission Regulation (EEC 2568/91, 1991).

Rancimat (Metrohm, model 743, Switzerland) was used to evaluate oxidative stability of olives oil under study. It was carried out with 3 g of oil, at 120 ºC with air flow rate of 20 L h^{-1}. Results were expressed as induction time (h).

Antioxidant Activity and Total Phenolic Content

Extraction of antioxidant and polyphenol compounds was performed using 3 g of olive oil, adding 5 mL of methanol: water (80:20) and shaking during 1 min. The hydrophilic layer was filtered using a GD/X 0.45 μm cellulose acetate septa and kept in a flask. This procedure was repeated twice (Tuberoso et al., 2007). Hydrophilic layers were mixed and used as extract to evaluate two AA methods: (1) $DPPH^{\cdot}$ (Brand-Williams et al., 1995) and (2) $ABTS^{+}$ (Re et al., 1999), and total phenol content, total phenolic content (TPC) (Gao et al., 2000). All analyses were performed using an UV–visible spectrophotometer (Helios Gamma model, UVG 1002E). Analyses were run in triplicate and results were expressed as mmol Trolox L^{-1} olive oil for AA and gallic acid equivalents (GAE) L^{-1} olive oil for TPC; gallic acid was used to facilitate comparison with previous studies.

Volatile Compounds

An aliquot of 5 mL of olive oil was used for HS-SPME extraction. Carvacrol, at a concentration of 325 mg L^{-1} olive oil, was used as internal standard. Besides, 1 g of NaCl salt was added to the mixture to promote volatilization of compounds and it was hermetically closed and maintained in a water bath at 40 ºC during 15 min of the equilibration and 40 min of the extraction at a constant stirring speed of 500 rpm. Two fibers were used: divinylbenzene/carboxen/polydimethylsiloxane (50/30 μm DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) and polydimethylsiloxane/divinylbenzene (65 μm PDMS/DVB, Supelco). The gas chromatograph used was an Agilent (Palo Alto, CA, USA) 7890A model, equipped with mass selective detector, model 5977E; and capillary column HP-5MS (5% phenyl) methylpolysiloxane Agilent J & W GC column (30 m × 0.25 mm internal diameter × coating thickness 0.25 μm). The flow rate of the He carrier gas was 1.5 mL min^{-1}. The injector was operated in split mode (2:1 split ratio) at 260 ºC. The column was maintained at 40 ºC for 3 min; heated to 100 ºC at a rate of 5 ºC min^{-1}; then, heated to 260 ºC at a rate of 3 ºC min^{-1}; and, finally held at 260 ºC for 3 min. MS conditions were as follows: source temperature 230 ºC; quadrupole temperature 150 ºC; transfer line temperature 270 ºC; acquisition mode electron impact (EI 70 eV) by 3 scans s^{-1} and mass range *m/z* 29–350. The analyses were carried out in triplicate. The individual peaks were identified by comparison of their retention indexes to those of authentic standards and literature as well as by comparing their mass spectra with the Wiley 09 MS library (Wiley, New York, NY, USA) and NIST14 (Gaithersburg, MD, USA) mass spectral database.

Fatty Acids

Determination of fatty acid profile was done using a *trans*-methylation technique (ISO-12966-2, 2017) followed by separation of the fatty acid methyl esters (FAME) in a gas chromatograph Shimadzu C17A (Shimadzu Corporation Kyoto, Japan) connected to a flame ionization detector as described the ISO-12966-4 (2015) with slight modifications. FAME were prepared with the addition of 13:0 fatty acid as internal standard (0.04 mg mL^{-1}). A CPSil-88 capillary column was used (100 m of length × 0.25 mm internal diameter × 0.2 μm film thickness; J&W 112-88A7; Agilent Technologies). Injector temperature was set at 250 ºC, while detector temperature was 260 ºC. Samples were injected with a 1:20 split ratio. Oven temperature was initially 175 ºC and was held for 10 min; then, raised to 220 ºC at 3 ºC min^{-1}, and held at 220 ºC for 5 min. Carrier gas was He (316 KPa) and detector gases were H_2 (30 mL min^{-1}), air (350 mL min^{-1}) and He (30 mL min^{-1}) as make-up gas. Comparison with retention time of standards (37 FAME mix from Supelco) was used for fatty acid identification.

Additionally, atherogenic index (AI) (equation 2) and thrombogenic index (TI) (equation 3) were calculated as previous reported by Ulbricht and Southgate (1991):

$$AI = \frac{4 \times 14:0 + 16:0}{\sum PUFA(n-3) + \sum PUFA(n-6) + \sum MUFA} \quad (2)$$

where, 14:0 is myristic acid, 16:0 is palmitic acid, PUFA means polyunsaturated fatty acids and MUFA

$$TI = \frac{14:0 + 16:0 + 18:0}{0.5 \times \sum MUFA + 0.5 \times \sum PUFA(n-6) + 3 \times \sum PUFA(n-3) + (n-3)/(n-6)} \quad (3)$$

where 18:0 is stearic acid.

Descriptive Sensory Analysis

The *Laboratorio Agroalimentario de Granada* [official panel accredited by ENAC (nº 276/LE 507)] analyzed four samples of each irrigation treatment to grade the samples according to their commercial quality, as established by European Regulation (EEC 2568/91, 1991).

Additionally, the same samples were evaluated by eight trained panelists (25–55 years old) of the "Food Quality and Safety" Research Group (Universidad Miguel Hernández, UMH). Evaluation was performed following attributes and definitions initially proposed by the International Olive Oil Council (IOC, 2007) and European Regulation (EEC 2568/91, 1991) and later slightly modified by Vazquez-Araujo et al. (2015), These later modifications were performed to split the attribute "fruity" into more detailed attributes, having more reference products, and using a scale from 0 (representing "no intensity") to 10 (representing "extremely strong intensity") (Table 5). This slightly modified sensory protocol will improve the knowledge of specific descriptors whose presence/intensity maybe affected by the water stress produced in the olive trees leading *hydroSOStainable* olive oils; these specific descriptors could be important to define consumers' perception and acceptability.

Statistical Analyses

One-way analysis of variance (ANOVA) and Tukey's multiple range test were performed to compare experimental data and determine significant differences among irrigation treatments ($P < 0.05$). The SD of the mean is used to perform Tukey's test; therefore, the SD values were not included in tables to avoid repetition of the data and to make tables easier to understand. Additionally, Pearson's correlation coefficients were calculated to determine correlations between water SI and variables, as well as to study correlations among volatile compounds and sensory descriptors. XLSTAT (2016.02.27444 version, Addinsoft) was used to perform all statistical analysis.

Results and Discussion

Meteorological Data, Nutritional Tree Status, and Yield

Meteorological conditions are represented in the Fig. 1. 2017 season was characterized by a warm spring that produced a bloom 50 days earlier than the average year. Rest of conditions were similar to the average year.

No deficiency was found for all nutrients and in all treatments (Table 1). There were significant differences among treatments in the tree status of Barium (Ba), Sulfur (S), Sodium (Na), and Calcium (Ca). Only in the case of Ca, explanation can be given for the irrigation treatments, since Ca moves through xylem, a closure of stomata aperture due to water stress can reduce the uptake of Ca.

Yield was apparently proportional to irrigation water applied; however, this trend was not statistically significant (Table 2). Differences in yield were associated to canopy volume since there were no differences in canopy productivity among treatments (Table 2), neither in fruit fresh weight (Table 2). It was previously reported that yield was not affected by light water stress, Ψ of -3.5 MPa, after fruit set; however, it is reduced with an intense water stress, Ψ of -5 MPa, after fruit set (Ahumada-Orellana et al., 2017).

Irrigation

Different levels of water stress were applied to olive trees during pit hardening state to save irrigation water. Pit hardening occurred between DOY 185 and 239 (43–94 days after full bloom). Fig. 2 shows water SI of the three RDI (T1–T3) and control (T0) treatments. As can be seen, stress started during pit hardening period, with T0 trees having the lowest stress and T3 the strongest one, while T1 and T2 presented intermediate SI.

Analytical Parameters for Olive Oil Grading

Acidity, peroxide value, and UV absorption characteristics (K_{232}, K_{270}, and ΔK) of the olive oils obtained from trees submitted to four different irrigation treatments under evaluation are shown in Table 2. Those data fulfill the requirements of all samples to be classified as extra virgin olive oils (EVOO) as established by the European Union commission Regulation (European Union, 2016/2095). No statistical differences were found among samples; thus, it could be stated that RDI strategies did not affect commercial classification of the final olive oils and the *hydroSOStainable* olive oils under study meet the highest quality standards that were evaluated corresponding to the commercial category of EVOO. A previous study with same cultivar but carried out in a different location (Sevilla, Spain) also studied deficit irrigation during the pit hardening stage (Sanchez-Rodriguez et al., 2019) and similar results were obtained for analytical parameters for olive oil grading. No statistical differences were obtained among olive oils, and all of them meet the highest quality standards.

With respect to Rancimat test, results are also shown in Table 2. The higher the water stress is, the higher the olive oil stability is. Previous studies had reported that olive oil stability is correlated with antioxidant and polyphenolic content (Martínez-Nieto et al., 2010), so the studied samples of olives oil with increased polyphenols and antioxidants will be more stable in the time than others.

Table 1 "Arbequina" olive tree status as affected by the irrigation treatment

Parameter	ANOVA[a]	T0	T1	T2	T3
Nitrogen, N (%)	NS	1.65[b]	1.56	1.62	1.55
Phosphorus, P (%)	NS	0.16	0.16	0.16	0.16
Potassium, K (%)	NS	0.82	0.73	0.69	0.68
Calcium, Ca(%)	**	1.43 a	1.39 a	1.40 a	1.26 b
Magnesium, Mg (%)	NS	0.25	0.24	0.24	0.22
Sodium. Na (%)	***	$8.5 \cdot 10^{-3}$ a	$5.8 \cdot 10^{-3}$ b	$4.8 \cdot 10^{-3}$ bc	$3.0 \cdot 10^{-3}$ c
Sulfur, S (%)	*	0.17 a	0.16 ab	0.16 ab	0.15 b
Manganese, Mn (ppm)	NS	42.1	39.5	35.7	36.1
Zinc, Zn (ppm)	NS	12.4	11.8	12.5	11.9
Copper, Cu (ppm)	NS	21.9	11.9	12.3	12.1
Boron, B (ppm)	NS	30.7	29.2	28.3	26.6
Aluminum, Al (ppm)	NS	37.6	41.2	42.9	44.4
Iron, Fe (ppm)	NS	66.6	65.2	70.5	75.2
Strontium, Sr. (ppm)	NS	235	255	242	229
Barium, Ba (ppm)	*	7.42 ab	7.75 ab	8.49 a	6.83 b
Cobalt, Co (ppm)	NS	0.06	0.08	0.05	0.09
Cadmium, Cd (ppm)	NS	0.02	0.02	0.01	0.02
Nickel, Ni (ppm)	NS	0.54	0.46	0.45	0.35
Lead, Pb (ppm)	NS	0.12	0.17	0.14	0.17

[a] NS = not significant at $P < 0.05$; *, ***, significant at $P < 0.05$ and $P < 0.001$, respectively.

[b] Values (mean of 12 replications per irrigation treatment) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

T0: treatment 0 (control; full irrigated); T1: treatment 1 (RDI during pit hardening; −2 MPa); T2: treatment 2 (RDI during pit hardening; −3 MPa); T3: treatment 3 (no irrigation during pit hardening).

Table 2 Analytical parameters for oil grading, antioxidant activity ($ABTS^+$ and $DPPH^·$) and total phenolic content (TPC) of "Arbequina" olive oils as affected by the irrigation treatment

Parameter	ANOVA[a]	T0	T1	T2	T3
Yield					
Yield (kg olive ha^{-1})	NS	10,267[b]	9171	8246	8303
Productivity (kg olive m^{-3} canopy volume)	NS	2.5	2.1	2.0	2.2
Fruit fresh weight (g)	NS	1.3	1.2	1.1	1.2
Olive oil quality parameters[c]					
Acidity (%)	NS	0.33	0.22	0.17	0.19
Peroxide value (meq O_2 kg^{-1})	NS	7.86	7.63	8.07	6.94
K_{232}	NS	1.67	1.75	1.67	1.52
K_{270}	NS	0.12	0.15	0.13	0.13
ΔK	NS	−0.01	0.01	−0.01	0.00
Rancimat test (h)	**	9.39 c	12.5 b	12.9 b	14.4 a
Antioxidant activity and total phenol content					
$ABTS^+$ (mmol Trolox eq L^{-1})	***	0.131 c	0.265 a	0.188 b	0.233 a
$DPPH^·$ (mmol Trolox eq L^{-1})	*	0.352 b	0.441 a	0.413 a	0.449 a
TPC (mg GAE eq L^{-1})	***	442 c	646 b	581 b	803 a

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

[b] Values (mean of 12 replications per irrigation treatment) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

[c] Maximum level of acidity for EVOO is 0.8; maximum peroxide value for EVOO is 20; maximum value of K232 for EVOO is 2.5; maximum value of K270 for EVOO is 0.22; maximum value of ΔK for EVOO is 0.01 (European Union, 2016/2095).

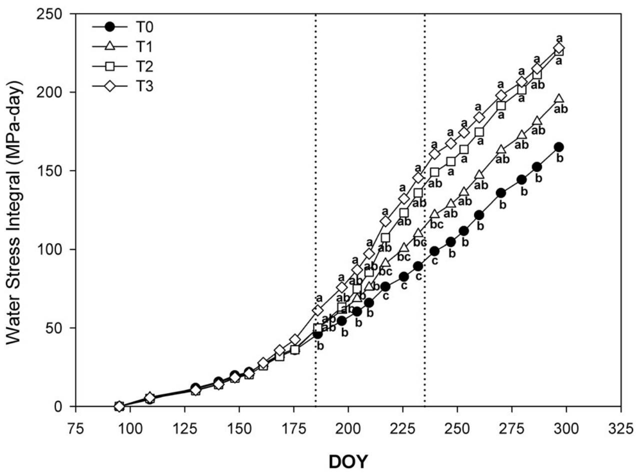

Fig. 2 Water stress integral (SI) in T0 (•), T1 (Δ), T2 (□), and T3 (◇). Different letters on data points at each date indicate significant differences according to Tukey's test ($P < 0.05$). Broken vertical lines indicate begin and end of pit massive hardening

Antioxidant Activity and Total Phenolic Content

Results of both AA methods and TPC are shown in Table 2. $ABTS^+$ and $DPPH^·$ assays are commonly used to evaluate the AA in olive oil (Baiano et al., 2009; Giuffrè et al., 2017). Concerning data of the $ABTS^+$ assay, the three oils with RDI (T1–T3) showed higher AA values than those of the control treatment, although T1 and T3 showed higher values than T2. For the $DPPH^·$ assay, the three oils with RDI exhibited higher capacity than the control ones, and a similar trend was observed for the TPC values (the highest values were for T3 while T1 and T2 showed similar concentrations, but higher than control).

Similar results were found in a recent study performed with "Arbequina" olive oil under water stress during the whole season, where the polyphenol profile and the AA improved with the water deficit (Sena-Moreno et al., 2018). In this way, there are previous evidences that tree water status is inversely correlated with polyphenols content in olive oil (Servili et al., 2007). A recent study has demonstrated that timing of deficit irrigation also influences the polyphenols synthesis (Gucci et al., 2019), who concluded that, water deficit was carried out before pit hardening, polyphenol synthesis was increased. Another study with water stress during pit hardening stage in "Arbequina" cultivar but in a different location (Sanchez-Rodriguez et al., 2019) found a quadratic correlation and polyphenols decreased when stress was higher than −4.00 MPa. In the present work, deficit irrigation during the pit hardening period, also increased TPC concentration, and, as expected, the higher the water stress, the higher the antioxidant capacity and the TPC because stress did not overcrom −4.00 MPa; these results agreed with the hypothesis of Gucci et al. (2019): early stages of fruit growth influence not only the cell division but also the phenolic concentration. In brief, *hydroSOStainable* olive oils (T1–T3) presented higher antioxidant capacity and TPC than conventional oils (T0 oils); therefore, this improvement could be linked, at least in part, to the polyphenolic health claim for olive oil (5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil contribute to the protection of blood lipids from oxidative stress [European Union, 2012]). However, to fully support this statement it would be necessary to quantify the content of hydroxytyrosol in *hydroSOStainable* olive oils.

Volatile Compounds

The headspace volatile compounds were isolated using two adsorption fibers. The recovered amount of compounds in the DVB/CAR/PDMS fiber were lower than that in the PDMS/DVB fiber; thus, experimental results indicated that PDMS/DVB fiber was the most suitable to capture olive oil volatiles, and, only results from this fiber are presented (Table 3). DVB/CAR/PDMS data are available as Table S1.

Identification and quantification of volatile compounds in the olive oil samples is shown in Table 3. (*E*)-Hex-2-en-1-ol (V17) was the most abundant compound in control oil but decreased as water stress increased, although it was also the main compound in the T1 oil. (*Z*)-Hex-3-en-1-ol (V16) and hexan-1-ol (V18) also decreased as the irrigation water was reduced, reaching hexan-1-ol values close to zero in T2 and T3. However, (*E*)-hex-2-enal (V15) increased its concentration with RDI and was the most abundant compound in T2 oils. Previous research reported negative correlation between water stress and the contents of the compounds V15 and V18 in "Arbequina" cultivar (García et al., 2017); however, a positive correlation with V15 has been observed in the current study. This difference could be due to the irrigation schedule followed, considering that the majority of studies did not apply RDI during pit hardening period but during the whole plant season.

Although, the previously discussed compounds were the predominant ones in the headspace of *hydroSOStainable* olive oils, other minor compounds were also affected by the watering strategies and can influence the oil aroma as well. For example, ethyl acetate (V3), 3-methylbutanal (V4), and 2-methylbutanal (V5) were not present in the control and appeared in samples of the RDI treatments. Otherwise, 6-methylhepta-1,5-diene (V23), 4,8-dimethylnona-1,7-diene (V24), and hexyl acetate (V26) in T1 were found at the same concentration as in the control, and in T2 and T3 exhibited a small decrease. In T2 treatment, (*Z*)-hex-3-enyl acetate (V25) was at the same concentration as the control. Results of current study

Table 3 Volatile profiles (PDMS/DVB fiber) of "Arbequina" olive oils as affected by the irrigation treatment

	RI	Compound	Sensory descriptor[a]	Concentration (mg L^{-1} olive oil)				
				ANOVA[b]	T0	T1	T2	T3
V1	<500	Ethanol	Alcohol, apple, sweet	*	92.3 b	248 a	108 b	109 b
V2	568	Acetic acid	Sour	**	0.01 b	6.80 a	0.01 b	0.01 b
V3	609	Ethyl acetate	Aromatic, bitter, fruity	**	0.01 b	32.4 a	0.17 b	9.52 ab
V4	648	3-Methylbutanal	Fruity, peach, sour	**	0.01 b	14.3 a	12.2 a	13.9 a
V5	659	2-Methylbutanal	Apple, fruity, ripe	**	0.01 b	12.3 a	13.5 a	11.2 a
V6	677	Pent-1-en-3-ol	Butter, fruity, green	**	30.3 ab	38.6 a	33.5 a	20.4 b
V7	684	Pentan-2-one	Fruity, apple, pineapple	***	56.5 a	31.6 b	32.0 b	20.5 c
V8	697	Pentan-3-one	Bitter, green, mustard	***	54.5 a	55.2 a	46.2 b	28.9 c
V9	716	Methyl butanoate		NS	0.00	0.00	0.59	0.00
V10	726	3-Methylbutan-1-ol	Sweet, woody, yeast	*	16.0 b	19.3 a	15.9 b	9.63 c
V11	730	2-Methylbutan-1-ol	Winey, spicy	**	14.7 ab	22.7 a	15.0 ab	10.6 b
V12	757	Pentan-1-ol	Balsamic, fruity, pungent	NS	5.38	6.46	8.09	5.76
V13	762	(*Z*)-Pent-2-en-1-ol	Almond, banana, fruity	*	33.7 ab	39.4 a	28.2 ab	16.5 b
V14	799	Hexanal	Apple, banana, grass, green	**	43.7 ab	45.7 a	49.2 a	33.7 b
V15	848	(*E*)-Hex-2-enal	Almond, apple, astringent	***	373 c	521 b	821 a	537 b
V16	851	(*Z*)-Hex-3-en-1-ol	Apple, banana, fresh, grass	***	349 a	102 b	0.00 c	0.00 c
V17	861	(*E*)-Hex-2-en-1-ol	Apple, flowers, fruity, grass	***	971 a	728 b	541 c	322 d
V18	863	Hexan-1-ol	Banana, fruity, soft, tomato	***	647 a	423 ab	322 ab	291 b
V19	899	Prop-2-enyl cyclopentane		NS	34.3	26.7	24.0	20.2
V20	922	Methyl hexanoate		NS	0.00	0.00	0.27	0.00
V21	935	3-Ethylocta-1,5-diene[c]		***	112 a	99.8 ab	83.2 ab	68.4 b
V22	942	3-Ethylocta-1,5-diene[c]		***	97.5 a	86.8 ab	71.0 ab	61.0 b
V23	995	6-Methylhepta-1,5-diene		*	50.5 a	51.0 a	44.2 ab	35.9 b
V24	998	4,8-Dimethylnona-1,7-diene		**	158 a	149 a	124 ab	97.7 b
V25	1007	(*Z*)-Hex-3-enyl acetate	Green, banana	**	33.6 ab	16.9 b	49.4 a	10.7 b
V26	1013	Hexyl acetate	Green, fruity, sweet	***	20.4 a	8.05 b	21.6 a	5.44 b
			ΣAlcohols	***	2160 a	1627 ab	1071 b	785 c
			ΣAldehydes	**	416 b	593 ab	896 a	596 ab
			ΣKetones	*	355 a	300 ab	256 ab	199 b
			ΣEsters	**	53.9 ab	57.3 ab	72.0 a	25.6 b
			ΣHydrocarbons	**	208 a	200 a	169 ab	134 b
			ΣAcids	*	0.01 b	6.80 a	0.01 b	0.01 b
			ΣTotal volatiles	***	3194 a	2785 ab	2469 b	1740 c

[a] SAFC 2014.

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

[c] Correct isomer was not identified.

Values (mean of 12 replications per irrigation treatment) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

agreed with the previously reported by Stefanoudaki et al. (2009) (*cv*. "Koroneiki") and Servili et al. (2007) (*cv*. "Leccino") in relation to V26, that decreased with water stress.

Alcohols were the most abundant chemical family in olive oils of all treatments, and its highest concentration was found in T0, reaching more than 1500 mg L^{-1}. The control- (T0) and the T1-treatment had the highest contents of ketones, while T2 the largest amount of aldehydes and esters. Previous studies also found an increase in aldehyde concentration when water stress was applied (Servili et al., 2007; Stefanoudaki et al., 2009). Within *hydroSOStainable* olive oils, T1 and T2 showed the highest total content of volatile contents; although, their total content was lower than that of the control samples (T0). However, T2 showed a profile with the highest contents of aldehydes and esters, but the second lowest alcohol content. It was also found several changes on volatile composition on a recent study about RDI during pit hardening carried out in Sevilla

Table 4 Fatty acid profiles of "Arbequina" olive oils from Ciudad Real as affected by the irrigation treatment

Compound		Concentration (g 100 g^{-1} olive oil)				
		ANOVA[a]	T0	T1	T2	T3
1	14:0 (Myristic acid)	NS	0.02[b]	0.02	0.02	0.02
2	15:0	NS	0.01	0.01	0.01	0.01
3	16:0 (Palmitic acid)	*	15.77 a	14.27 b	14.88 ab	14.82 ab
4	16:1 *cis*-10	NS	0.16	0.15	0.17	0.17
5	16:1 *cis*-9 (Palmitoleic acid)	NS	1.56	1.29	1.36	1.42
6	16:1 *cis*-11	NS	0.01	0.01	0.01	0.01
7	17:0 (Margaric acid)	NS	0.14	0.15	0.17	0,17
8	17:1 *cis*-9	*	0.26 b	0.28 ab	0.32 a	0.30 ab
9	18:0 (Stearic acid)	NS	2.26	2.21	2.36	2.39
10	18:1 *trans*-9 (Eleaidic acid)	NS	0.01	0.01	0.01	0.01
11	18:1 *cis*-9 (Oleic acid)	**	61.85 b	65.67 a	64.99 a	64.75 a
12	18:1 *trans*-11	NS	4.99	4.59	4.49	4.52
13	18:2 *cis*-9 *trans*-12 (Linoleaidic acid)	NS	0.02	0.02	0.01	0.01
14	18:2 *cis*-6 *cis*-9 *cis*-12 (Linoleic acid)	**	10.66 a	9.11 b	9.12 b	9.16 b
15	20:0 (Araquidic acid)	NS	0.53	0.52	0.51	0.53
16	18:3 n6 *cis*-6 *cis*-9 *cis*-12 (γ-Linolenic acid)	NS	0.01	0.01	0.01	0.01
17	20:1 n9 *cis*-11 (Gondoic acid)	NS	0.39	0.37	0.34	0.36
18	18:3 n3 *cis*-9 *cis*-12 *cis*-15	NS	0.62	0.57	0.57	0.59
19	21:0	NS	0.01	0.01	0.01	0.01
20	22:0 (Behenic acid)	NS	0.16	0.16	0.15	0.16
21	23:0	NS	0.03	0.03	0.02	0.03
22	24:0 (Lignoceric acid)	*	0.09 a	0.08 ab	0.07 b	0.08 ab
ΣSFA		**	19.08 a	17.38 b	17.01 b	18.18 b
ΣMUFA		***	68.33 b	72.35 a	71.92 a	71.02 b
ΣPUFA		*	11.56 a	9.917 b	10.10 ab	10.23 b
AI		*	0.23 a	0.19 b	0.21 ab	0.21 ab
TI		**	0.43 a	0.39 b	0.41 ab	0.41 ab

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

[b] Values (mean of 12 replications per irrigation treatment) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI, Atherogenic index; TI, Thrombogenic index.

(Spain) (Sanchez-Rodriguez et al., 2019). Compound behavior was quite different, as it was found an increase on alcohols as a result of water stress, and a decrease on aldehydes, contrary to the effect found on the current research, so location had a high impact on volatile composition on olive oil.

The main formation pathways of the key volatile compounds in virgin olive oil was previously studied by Kalua et al. (2007). Mostly, lipoxygenase pathway is considered responsible of olive oil aroma formation through oxidation of linoleic and linolenic acids. The most abundant compounds, such as (*E*)-hex-2-en-1-ol or (*E*)-hex-2-enal are formed by this route throughout different enzymes. Along this pathway, compounds are transformed to aldehydes, then to alcohols and finally to esters. As explained before, in this study, alcohols decreased their concentration as water stress was more intense and simultaneously aldehydes increased. Thus, it can be assumed that the stress in the plant decreased the formation and/or activity of alcohol dehydrogenase and consequently less alcohols were formed in the olive oil; however, further research is needed to fully prove this hypothesis. Stefanoudaki et al. (2009) found similar results when submitted "Koroneiki" cultivar trees to stress by non-irrigation; these authors also concluded that the lipoxygenase pathway could be affected by water stress.

Fatty Acids

Twenty-four fatty acids were identified in *hydroSOStainable* olive oil samples (Table 4). Regarding

Table 5 Descriptive sensory profiles of "Arbequina" olive oils as affected by the irrigation treatment

Descriptor		References	ANOVA[a]	T0	T1	T2	T3
Flavor (positive attributes)							
D1	Fruity-olive	Canned Ripe Olives, Pitted Black = 2.3 Hacendado, Manzanilla Green olives = 5.3	***	3.88 b[b]	4.44 a	3.96 b	4.53 a
D2	Fruity-green (under-ripe olive)	Canned Ripe Olives, Pitted Black = 1.0 Hacendado, Manzanilla Green olives = 2.7	**	2.36 b	3.19 a	2.71 ab	2.90 a
D3	Fruity-ripe (ripe olive)	Canned Ripe Olives, Pitted Black = 1.0 Hacendado, Manzanilla Green olives = 3.7	NS	1.38	1.34	1.34	1.21
D4	Floral	Pompadour, Chamomile Herbal Tea = 5.0 Carrefour, White Grape Juice (diluted 1:1) = 4.7	*	1.16 b	1.56 a	1.31 ab	1.63 a
D5	Green-artichoke	Hacendado, Artichoke Hearts = 3.0	*	0.48 b	0.63 ab	0.65 ab	0.74 a
D6	Green-avocado	Under-ripe Fresh Avocado = 5.3	NS	0.65	0.63	0.59	0.46
D7	Green-banana	Under-ripe Green Banana = 4.0	NS	0.21	0.28	0.34	0.28
D8	Green-herbs	Verdifresh Arugula (organic, washed) = 5.7	*	1.44 b	1.81 ab	1.44 b	2.13 a
D9	Green-grass	*Cis*-3-Hexen-1-ol 1000 ppm = 10.0	**	1.00 b	1.44 ab	1.30 ab	1.68 a
D10	Green-peppery	Hacendado, Green-Peppercorns (dried) = 2.0	NS	1.13	1.06	0.89	0.89
D11	Apple	Fuji Apple = 5.0	*	0.24 b	0.08 c	0.46 a	0.29 b
D12	Buttery	Under-ripe Fresh Avocado = 4.0	NS	0.63	0.75	0.75	0.75
D13	Almond	Hacendado, almonds = 5.0	NS	0.35	0.29	0.25	0.45
D14	Walnut	Hacendado, walnuts = 6.0	NS	0.30	0.30	0.38	0.44
D15	Woody	Hacendado, walnuts = 3.0	*	0.50 ab	0.44 ab	0.75 a	0.38 b
D16	Piney	Hacendado, pine nuts = 3.5	NS	0.69	0.51	0.38	0.50
D17	Sweet	1% sucrose solution = 3.0	NS	1.06	1.00	1.13	0.94
D18	Sour	0.05% citric solution = 2.5	NS	0.69	0.69	0.75	0.75
D19	Salty	0.25% NaCl solution = 1.0	NS	1.10	1.18	1.19	1.16
D20	Bitter	0,01% caffeine solution = 1.0	NS	0.00	0.00	0.00	0.00
Flavor (negative attributes)							
D21	Oxidized	La Masía, 100% sunflower oil = 4.0	NS	0.00	0.00	0.00	0.00
D22	Painty	Hacendado, Green-Peppercorns (dried) = 3.3	NS	0.00	0.00	0.00	0.00
D23	Rancid	International olive council standard = 9.2	NS	0.00	0.00	0.00	0.00
D24	Musty	International olive council standard = 4.65	NS	0.00	0.00	0.00	0.00
D25	Muddy	International olive council standard = 7.9	NS	0.00	0.00	0.00	0.00
Mouthfeel							
D26	Astringent	0,10% alum solution = 4.0	*	2.44 b	2.81 a	2.56 ab	2.56 ab

(Continues)

Table 5 Continued

Descriptor		References	ANOVA[a]	T0	T1	T2	T3
D27	Pungent	Verdifresh Arugula (organic, washed) = 5.0	NS	2.94	2.88	3.00	2.75
D28	Viscosity	Hacendado, condensed milk = 10.0	***	3.88 b	4.44 a	3.96 b	4.53 a

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

[b] Values (mean of 12 replications per irrigation treatment) followed by the same letter, within the same row, were not significantly different ($P < 0.05$), according to Tukey's least significant difference test.

MUFA, oleic acid was the main compound and its content in T1, T2, and T3 oils was significantly higher than that in the control oil. Something similar was observed for 17:1 *cis*-9, which had the highest content in T2. With respect to saturated fatty acids (SFA), palmitic and lignoceric acids showed an important decrease of their concentration in RDI olive oils as compared to the control sample. Linoleic acid (polyunsaturated fatty acid [PUFA]) experienced a small decrease of concentration in all RDI treatments, and, also 20:4 n6 *cis*-5 *cis*-8 *cis*-11 *cis*-14, in T2 and T3 samples.

From a general point of view, it can be stated that major fatty acids in the profile were MUFA followed by SFA and, finally, PUFA. The contents of all saturated and unsaturated fatty acids were affected by RDI. Actually, T1 and T2 showed higher concentration of MUFA than control and T3, and, regarding SFA, the concentration was smaller in all RDI treatments (T1–T3) as compared to the control. Regarding PUFA, the control oil showed the highest content but this content was statistically equivalent to that of T2. Similar concentrations of fatty acids were previous reported on "Arbequina" cultivar (Aparicio and Harwood, 2013; García et al., 2017).

The AI expresses the relationship between atherogenic and anti-atherogenic fatty acids, which means that the lower the AI, the lower is the possibility of lipid adhesion to cells of the immune circulatory system (atheroma formation) (Ulbricht and Southgate, 1991). In a similar way, TI is the ratio between pro-thrombogenic and antithrombogenic fatty acids; hence, the lower the index, the lower the possibility of formation of clots in the blood vessels is indicated (Ulbricht and Southgate, 1991). Experimental values showed that T1 presented statistically the lowest indexes, although also T2 and T3 showed lower indexes than control (Table 3). Similar values of AI and TI were previously found in other olive oils (Ulbricht and Southgate, 1991) although, from our best knowledge, this is the first time that these indexes are calculated in olive oil under RDI.

Several studies reported olive oil fatty acid composition on "Arbequina" cultivar affected by different RDI strategies. This is the case of García et al. (2017) who reported that 30% RDI in an "Arbequina" orchard induces an increase in the oleic/linoleic ratio. The same effect was observed by Gomez del Campo and García (2013) using different irrigation strategies in "Arbequina" orchards. However, the opposite effect (reduction in oleic/linoleic ratio with increasing water stress) was found in cv. "Koroneiki" (Stefanoudaki et al., 2009), indicating that the effect of different water regimes on olive oil fatty acid composition could be cultivar dependent. Regarding the "Frantoio" cultivar, the application of RDI before pit hardening had no clear effects on fatty acid composition (Gucci et al., 2019). A recent study about RDI during pit hardening carried out in Sevilla (Spain) (Sanchez-Rodriguez et al., 2019) also found an increased on MUFA due to water stress during this stage, but no statistical differences were found on SFA, PUFA, AI, and TI, so location is a very

Table 6 Pearson correlation between stress integral (SI) and antioxidant activity and total phenol content (TPC), fatty acids, volatile compounds, and descriptive sensory analysis

	SI
Antioxidant Activity and Total Phenol Content	
ABTS+	0.487*
TPC	0.762***
Fatty Acids	
17:1 *cis*	0.498*
18:2 *cis*	−0.795***
ΣAGS	−0.599*
Volatile Compounds	
3-Methylbutanal	0.410*
(*Z*)-Hex-3-en-1-ol	0.692***
(*E*)-hex-2-en-1-ol	−0.682**
Hexan-1-ol	−0.630*
ΣAlcohols	−0.698***
Descriptive Sensory analysis	
Fruity-olive	0.450*
Green-artichoke	0.525*
Floral	0.628**
Green-herbs	0.585**
Green-grass	0.697***

*, **, ***, significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 7 Pearson correlation among volatile compounds and sensory descriptors (only significant correlations are shown in this table)

	Fruity-olive	Fruity-green	Floral	Green-artichoke	Green-grass	Apple	Woody
Ethyl acetate	0.540**	0.680***	0.580***	NS	NS	−0.660***	NS
Pentan-2-one	0.532**	NS	NS	−0.627***	NS	NS	NS
3-Methylbutan-1-ol	NS	NS	NS	NS	NS	NS	0.534**
(*E*)-Hex-2-enal	NS	NS	NS	NS	NS	0.512**	0.442*
(*E*)-Hex-2-en-1-ol	0.558***	NS	−0.588***	−0.816***	0.608***	NS	NS
Hexan-1-ol	0.478*	0.496*	NS	−0.741***	NS	NS	NS
Hexyl acetate	0.627***	0.484*	−0.645***	0.512*	NS	NS	NS

*, **, ***, significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

important factor to consider. Thus, applying RDI during pit hardening could be a better option for the improvement of olive oil quality. As a summary of this section, it can be concluded that *hydroSOStainable* olive oil presented better fatty acid profile than the control as it had higher contents of MUFA, lower of SFA, and also lower values of the indexes AI and TI.

Descriptive Sensory Analysis

An official and certified panel determined the commercial grade of the oils, being: (1) T0: EVOO with a fruity average of 4.0; (2) T1: EVOO with 4.5 of fruity; (3) T2: EVOO with 4.0 of fruity; and, (4) T3: EVOO with 4.5 of fruity.

Sensory characteristics of *hydroSOStainable* olive oil were assessed by a more detailed descriptive sensory analysis, providing essential data to estimate whether consumers would buy and like the oils under analysis. Therefore, 28 sensory attributes were evaluated and the results are shown in Table 5.

Although previous studies reported that stressed olive trees produced oils with unbalanced bitter and pungency, and also low fruity aroma and woody characteristics (Stefanoudaki et al., 2009), it could be seen that RDI of "Arbequina" during pit hardening did not had these negative effects on the olive oils under study. T1 and T3 were evaluated as more fruity (general and under-ripe olive), floral, green (artichoke, herbs and grass) and, also presented a balance between astringency and pungency. These two samples (T1 and T3) also had higher viscosity than those of control and T1. Besides, T1 oils were also evaluated with better scores than the control in attributes such as floral, green-artichoke, apple, and woody, although these attributes showed relatively low scores in all samples.

This section can be summarized stating that the RDI treatments studied in this work increased the intensity of positive sensory attributes of the olive oil; *hydroSOStainable* olive oils were supposed to be more attractive for consumers because their flavor was more balanced and intense than that of olive oil without deficit irrigation. However, consumer studies must be carried out in future studies to support this hypothesis.

Pearson Correlations

As SI gives information about stress that trees had accumulated during all season, Pearson's correlation between SI and variables under study was performed to study how it affects olive oil composition. Significant results are shown in Table 6. Regarding AA, $ABTS^+$ assay showed a positive correlation with SI, as well as TPC, although the latest had higher significance than the former, that mean that higher stress produced higher phenolic and antioxidant potential. With respect to fatty acids, 17:1 *cis* showed a positive correlation while the opposite was found for 18:2 *cis* and ΣAGS. Same behavior was previously found in "Arbequina" cultivar with similar irrigation treatments (water stress during pit hardening period) but in a different location (Sanchez-Rodriguez et al., 2019). For volatile compounds, 3-methylbutanal and (*Z*)-hex-3-en-1-ol were found a positive correlation while negative correlations for (*E*)-hex-2-en-1-ol, hexan-1-ol and ΣAlcohols, which means that the two first would be increased with high water stress levels but the fourth later would suffer a decreased in their concentration. These results could be correlated with an increase in fruity, apple, fresh and grass sensory descriptors but also with a decrease on apple, fruity, grass, banana and tomato, so it indicated that an equilibrium between profit and loss could be found. When sensory descriptors were correlated with SI, only positive correlation were found. For instance, fruity-olive, green-artichoke, floral, green-herbs and green-grass would show higher intensities as higher water stress. Different correlations were found in study done by Sanchez-Rodriguez et al. (2019) as green-herbs showed negative correlation. This could be due to different location and small differences between irrigation treatments.

In order to study the correlation among sensory descriptors and volatile composition of olive oils, Pearson correlations were carried out. Significant results (bold letter) are shown in Table 7. Ethyl acetate was positively correlated with fruity-olive, fruity-green, and floral, but negatively with apple attribute. Pentan-2-one was correlated positively with fruity-olive and negatively with green-artichoke. The compound 3-methylbutan-1-ol only showed a positive correlation with woody sensory attribute. (*E*)-Hex-2-enal had been correlated positively with apple and woody. A positive correlation was found between (*E*)-Hex-2-en-1-ol and fruity-olive and green-grass while a negative one between this compound and floral and green-artichoke. Hexan-1-ol was correlated with fruity-olive and fruity-green in a positive way, while it was negatively correlated with green-artichoke. Finally, Hexyl acetate was positively correlated with fruity-olive, fruity-green and green-artichoke but in a negative way with floral attribute.

Conclusions

The commercial classification of the oils under study was not affected by the reduced irrigation, and all investigated oils were classified as "extra virgin olive oil". Regarding antioxidant capacity and phenolic content, RDI olive oils (branded as *hydroSOStainable*) yielded higher capacity and content than the control oils. Besides, RDI oils showed equilibrated profiles of volatile compounds, with high contents of aldehydes and esters. All tested RDI treatments decreased the content of saturated fatty acids while simultaneously increased that of monounsaturated compounds, and, also improved AI and TI indexes (leading to lower values). *HydroSOStainable* olive oils had the fruitiest flavor and were, in general, more balanced and had higher intensities of key sensory attributes as compared to the control oils. As a general conclusion, it can be stated that applying RDI during pit hardening improved the functional and sensory quality of the "Arbequina" olive oil, and the commercial *hydroSOStainable* olive oils (European Union 2016/2095) were characterized by proper profiles of volatile compounds, fatty acids, and sensory attributes and high antioxidant capacity due to their phenolic content.

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Conflict of Interest The authors declare that they have no conflict of interest.

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8.

8.1. "Manzanilla" raw and table olives

8.1.1. Experiment A

Results of Experiment A are presented on first publication (irrigation, morphological analysis, mineral analysis, antioxidant activity, total phenolic content, organic acids and sugars), third publication (polyphenolic profile), fourth publication (volatile compounds and sensory analysis) and fifth publication (bioaccessibility).

8.1.1.1. Irrigation

Four irrigation treatments were tested applying different levels and length of water stress during pit hardening stage, on 2015 and 2016 seasons. A0 showed the lowest minimum stem water potential and stress integral while A3 showed the highest levels of stress. Levels of stress varied mainly depending on tree load, in 2015 the load of the trees was 15 % of the 2016 load, this could explain the difference between the values of the two seasons studied.

8.1.1.2. Morphological analysis

Morphological analysis for raw and table olives of Experiment A showed that A2 olives had the highest fruit weight, while A3 had the lowest weight. Previous studies reported similar results showing that strong RDI treatments decreased the weight of olive fruits, but moderate treatments yielded larger fruits (Cano-Lamadrid et al., 2015; Martorana et al., 2016; Cano-Lamadrid et al., 2017). Fruit/pit ratio showed a trend on RO (the higher the stress during pit hardening stage, the higher the ratio) but this trend was not observed on table olives where A3 showed the smallest fruit/pit ratio.

The shape of olives changed with RDI; RDI treatments showed a tendency to lead to rounder olives than control; this can be an advantage of RDI (during pit hardening stage) on fruit shape because consumers usually prefer round 'Manzanilla' olives (Cano-Lamadrid et al., 2017).

Texture tests showed that A3 RO had the hardest peel (PT) and flesh (MTT) which is logical due to the highest DMC of A3 fruits. Texture of table olives is a main attribute for consumer's acceptance and for the industry; thus, from a texture point of view, A3 olives are very interesting because they had strong peel and hard flesh.

Color results showed differences in lightness (L*) and the green-red coordinate (a*). A3 olives were the lightest and greenest ones. These results agreed with those of a previous study with the same olive variety (but different irrigation treatments) in which the most severe treatment yielded the lightest and greenest olives (Cano-Lamadrid et al., 2017).

After processing RO to TO following Spanish-Style process, all morphological parameters under study decreased due to osmotic dehydration suffered during the exposure to sodium chloride (NaCl) (Albarracín et al., 2011) and the solubilization of components from the olive flesh to the surrounding fermentation liquid.

8.1.1.3. Mineral analysis

Calcium (Ca), Potassium (K), Magnesium (Mg), Zinc (Zn) and Cupper (Cu) were found on both raw and table olives. RDI applied during pit hardening stage did not affect mineral content although, after processing, Ca and K contents decreased. These two minerals are highly soluble and decrease during the washing steps, where they can be eluted to the acidic surrounding media (Ünal and Nergiz, 2003).

8.1.1.4. Antioxidant activity (AA) and total phenol content (TPC)

High AA and TPC concentrations were found in ‘Manzanilla’ RO but, during processing to table olives, the TPC and AA decreased. The Spanish-style green olives experienced loss of AA by a 72 %, 80 %, and 38 % for $ABTS^{+}$, $DPPH^{\bullet}$, and FRAP, respectively, and 70 % for TPC. The Spanish-style processing of green olives affects the loss of TPC depending on the ripening stage of fresh olives, the debittering method employed, and the fermentation type used. During fermentation, the contents of some phenols (hydroxytyrosol, tyrosol, and oleoside-11-methyl ester) decrease because of diffusion to the preservation liquid due to its acidic pH. Another important change during fermentation is the conversion of oleoside-11-methyl ester to elenoic acid, which is rapidly hydrolyzed due to the acidic conditions (Boskou et al., 2015).

Regarding irrigation treatments, no significant statistical differences were reported for raw or table olives. Other authors reported similar results also in table olives, when applying other RDI treatments (Cano-Lamadrid et al., 2017).

8.1.1.5. Organic acids and sugars

Concentrations of organic acids and sugars were not significantly affected by any RDI treatment on either raw or table olives. Major change in the profiles of organic acids and sugars were due to processing. Citric acid, tartaric acid, malic acid, succinic acid, sucrose, glucose and fructose were present on RO; while phytic acid, lactic acid, acetic acid, maltoheptaose, mannitol and glycerol appeared in table olives. Previous studies also identified these compounds in different table olive varieties (López-López et al., 2009; Cano-Lamadrid et al., 2017).

Lactic acid bacteria metabolize sugars in RO yielding CO_2, lactic acid and other organic acids (Hurtado et al., 2012). Malic and citric acids play an important role in oil accumulation during the Krebs cycle. Similar results on organic acids were found in a study that identified citric, malic and succinic acids in different varieties of RO (Nergiz and Ergönül, 2009). Sucrose, glucose and fructose are naturally present in the olive flesh because of transport by phloem from mature leaves and by formation by photosynthesis and they are very important for fruit growth and lipid biosynthesis. These three sugars were also identified in other studies as the main sugars in RO (Tekaya et al., 2018).

8.1.1.6. Polyphenolic profile

Polyphenolic profile of RO and TO of experiment A were studied. In both of them, 17 compounds (10 irioids, 3 flavonoids, phenylethanoid, phenethyl ester, tyrosol ester of elenoic acid and hydroxytyrosol) were identified, Spanish-style processing dramatically decreased their content in TO. In fact, all compounds were reduced by a ~ 90 % of their concentration due to processing to from RO to TO. Water irrigation strategies did not have any effect on the fermentation process of olives (Perpetuini et al., 2018). Spanish-style processing affected the polyphenol composition due to osmotic mechanism, lye treatment (NaOH react with compounds with carboxylic and hydroxyl functional groups producing hydrophilic derivatives that are removed and oleuropein and verbascoside are hydrolysed), and lactic fermentation that transform glycosides to hydroxytyrosol (Ramírez et al., 2016).

In RO, all irrigation strategies increased 2”-hydroxyoleuropein, maybe due to a transformation of other polyphenols such as oleuropein aglycone. In A1 treatment oleoside and dihydro-oleuropein increased, whereas caffeoyl-6’-secologanoside, comselogoside and ligstroside decreased. Regarding A2, also dihydro-oleuropein and caffeoyl-6’-secologanoside increased their concentration. A3 treatment also increased the concentration of elenoic acid glucoside but experience a decreased in 6 polyphenols (oleuropein aglycone, verbascoside, oleoside diglucoside, comselogoside, luteolin and ligstroside). These results agree with previous studies showing that total phenolic content decreased under severe deficit irrigation regimes because of the increase of activity of phenylalanine ammonia-lyase as hard stress in the olive oil tree (Patumi et al., 1999; Tovar et al., 2002).

With regard to TO, A1 showed an increase of oleoside, elenoic acid glucoside, oleoside diglucoside and oleuropein, A2 increased oleoside diglucoside and finally, A3 increased elenoic acid glucoside but decreased oleuropein, ligstroside and 2”-hydroxyoleuropein. Such observations are consistent with previous observation on RO: the higher the water stress the lower the phenolic content (Patumi et al., 1999; Tovar et al., 2002).The highest increase of concentration could be found when a moderate stress was applied (A1). In a previous study carried out with sustained deficit irrigation, it was found that the higher the water stress, the higher the concentration of oleuropein, verbascoside and flavonoids (Sena-Moreno et al., 2018).

8.1.1.7. Pearson correlation between SI, and polyphenols, antioxidant activity, total phenol content and color.

After studying several parameters, correlations between the stress integral and some of these parameters were performed to better understand their interaction. With respect to polyphenols, in RO it was found a positive correlation between SI and hydroxytyrosol glucoside, oleuropein and ligstroside and the correlation was negative with verbascoside. On the other hand, for TO, only negative correlations were found: luteolin-3-*O*-rutinoside, verbascoside, dihydro-oleuropein, oleuropein diglucoside and 2”-hydroxyoleuropein.

A positive correlation was found between FRAP assay and SI in both RO (0.63) and TO (0.40). Thus, FRAP assay could be considered as key to determine antioxidant potential on RDI olives as it showed a good correlation with the SI. FRAP assay, which means Ferric Reducing Ability of Plasma, works in an acid medium and it consist on the ability of an antioxidant to reduce the ferric complex Fe^{+3} on the presence of 2,4,6-Tipyridyl-s-triazine (TPTZ) to the ferric complex Fe^{+2}-TPTZ (Benzie and Strain, 1996). Previous studies with hydroSOStainable almonds presented a positive correlation between FRAP and SI, as in the current research (Lipan et al., 2020). FRAP assay can react with non-enzymatic antioxidants as ascorbic or uric acid, but it does not react with antioxidants with SH groups as glutathione, lipoic acid or some amino acids (Cao and Prior, 1998).

Finally, TO $L*$ and $a*$ color parameters were positively correlated with SI and negatively in the case of RO.

8.1.1.8. Volatile composition

Alcohols, sulfur compounds, esters, ketones, terpenes, acids, phenolic compounds and hydrocarbons were the volatile compounds identified and quantified on TO under study. The highest concentration was found for esters on control olives (38.48 %), and their concentration was decreased on hydroSOStainable TO, being terpenes the predominant volatile compounds on TO coming from water scarcity strategies.

Some general trends were seen on volatile compounds. For instance, it was found that, as water stress increased, also increased concentrations of ethyl acetate, isoamyl acetate, cis 3-hexen-1-ol, 1-hexanol and γ-terpineol in TO. Contents of 2-butanol, propanoic acid, ethyl cycloheanecarboxylate and cyclohexanecarboxilic acid, butyl ester decreased as water stress increased.

Previous research on volatile profile of TO from RDI is scarce: changes on the volatile profile of “Koroneiki” (Brahmi et al., 2013) and “Manzanilla” olives under different RDI strategies (Cano-Lamadrid et al., 2015) have been reported.

8.1.1.9. Sensory analysis

Descriptive sensory analysis

Attributes evaluated for the descriptive sensory analysis of TO of Experiment A were: color, green-olive flavor, saltiness, bitterness, sourness, sweetness, aftertaste, off-flavor, hardness, crunchiness and fibrousness. No off-flavor was detected; and, saltiness, sweetness and fibrousness did not shown statistical significant differences among samples. Whit respect to color, A2 and A3 showed an intermediate position between yellow and green, whereas A0 were the most intense green olives and A1 the most yellow ones. A0 and A2 TO had intermediate green-olive flavor intensity whereas A1 had the most intense green-olive flavor and A3 the least one. As water stress increased, bitterness decreased its intensity. A3 TO showed the sourest flavor and the longest aftertaste, but also the lowest hardness and crunchiness intensities.

Previous studies of descriptive sensory analysis of “Manzanilla” Spanish-Style TO reported that intensities of saltiness, green-olive flavor, aftertaste, bitterness and hardness were affected by irrigation constrains. Different results were obtained in the current study for parameters such as bitterness and aftertaste that were highest when moderate stress was applied, and similar results were obtained for green-olive flavor which intensity increased with water stress (Cano-Lamadrid et al., 2015; Cano-Lamadrid et al., 2017). In "Ascolana" TO also bitterness decreased when irrigation water was decreased (Marsilio et al., 2006). It was also reported that green-olive aroma, sourness and sweetness intensities decreased due to water stress on "Nocellara del Belice" Greek-style TO (Martorana et al., 2017).

Affective sensory analysis

The TO under study did not shown statistically significant differences on affective results at any of the 3 locations used. Overall, TO from all irrigation treatments were given high acceptability scores by consumers. For instance, a mean of 6.3 in a scale up to a maximum score of 9 was obtained for overall liking. Color (6.5) flavor (6.4), bitterness (6.0), saltiness (6.1), sourness (6.0), hardness (6.6) crunchiness (6.6), fibrousness (6.5) and aftertaste (6.2) were highly valued by consumers.

A previous study conducted on the same variety reported that consumers preferred TO coming from deficit irrigation strategies due to their flavor, crunchiness and aftertaste (Cano-Lamadrid et al., 2015).

Driving sensory attributes

Consumers overall liking were correlated to volatile compounds (total volatile content for each chemical family) and descriptive sensory attributes respectively by two PLS (partial least squares regression) maps to established drivers of liking for hydroSOStainable TO.

The first PLS map (consumers overall liking and volatile compounds) did not show a clear trend, so volatiles are not good drivers to determine the sensory consumer liking of hydroSOStainable TO.

On the other hand, the second map positively correlated some sensory descriptors to the consumer liking. Therefore, green-olive flavor, hardness, crunchiness, bitterness, sweetness and saltiness could be used as drivers to estimate future consumers' acceptance of hydroSOStainable TO.

Consumer willingness to pay

Consumer willingness to pay for hydroSOStainable TO as well as the hydroSOStainable logo effect on consumer decisions was studied at three locations and using green-olive flavor, saltiness and hardness sensory attributes, as well as overall liking. For saltiness and hardness no significant differences were found at any location, so it could be said that hydroSOStainable logo did affect the perception of these attributes. On the other hand, green-olive flavor and consumer overall liking were affected by the logo; scores increased by 1.3 and 1.1 units, respectively, in comparison with control olives. Regarding the location of the study: L1 gave the highest score for green-olive flavor (7.7) and L2 the lowest (7.0) but the opposite occurred for overall liking, where L2 scored the highest (7.3). Concerning the statistical interaction between logo and location, the highest scores for green-olive flavor were given at L1 and L3 with hydroSOS logo, and the lowest at L3 to TO without hydroSOS logo. Regarding overall liking: also, L1 and L3 showed preference for TO marked as hydroSOStainable. However, it is important to mention that, for L2 location (Elche, Alicante, Spain), corresponding to people living in a city, the highest value for overall liking was given to TO labeled as conventional. Therefore, it could indicate that people living in a city are not aware of water scarcity on agriculture.

Consumers were asked about their willingness to pay for hydroSOStainable TO: 88 % of them were willing to pay more than the conventional price (1.35 € per 200 g) after being informed about hydroSOStainable benefits. Furthermore, 52 % were willing to pay a price from 1.35 to 1.75 €, 32 % from 1.75 to 2.50 € and 4 % more than 2.50 € per 200 g. Previous studies on hydroSOStainable pistachios and almonds also reported increased willingness to pay more for those products than for the conventional ones (Noguera-Artiaga et al., 2016; Lipan et al., 2019).

Penalty analysis

During the affective sensory analysis, some JAR questions were included for several attributes such as flavor, bitterness, saltiness, sourness and aftertaste. With this information, a penalty analysis was conducted aiming to understand the relationship between JAR scores and consumer satisfaction degree. Results are in agreement with the previously presented on affective sensory analysis, as they indicated that consumers did not marked any attribute as susceptible to change due to RDI treatments (A1, A2 and A3). Accordingly, Spanish consumers of three different locations did not point to the need of improvement on olives quality. In a previous study carried out with RDI almonds (Lipan et al., 2019), it was also found that no improvements were indicated by consumers on RDI almonds, as occurred for TO in the current study.

8.1.1.10. Antioxidant Activity, Total Phenol Content and its bioaccessibility after Gastrointestinal in vitro digestion

In this experiment, TO did not show statistical differences due to RDI in the test matrix (TO before in vitro digestion), but some differences were found after the digestion. No statistically significant differences were found for FRAP assay, that showed a high variability percentage (% var) of ~ 92 %. $ABTS^{+}$ activity increased with increased water stress for SF and total fraction (SF+RF). For $DPPH^{\cdot}$, A1 showed the highest activity while A3 the smallest for SF and total fraction. As TPC is concerned, a small increase of concentration in hydroSOStainable olives was found for SF. Regarding the percentage of variability, $ABTS^{+}$, $DPPH^{\cdot}$ and TPC showed decreases of ~ 76 %, ~ 95 % and ~ 82 % respectively.

TPC bioaccessibility was calculated and an 11.5 % was found on A0, 11.1 % on A1, 12.1 % on A2 and 13.1 % on A3. Although slight differences were found among samples, they were not statistically significant.

The acidic conditions of the gastrointestinal digestion could degrade antioxidants and polyphenols, so it could be the main cause of their dramatic decrease after in vitro digestion as compared with the test matrix (Fernández-Poyatos et al., 2019). As previously reported, polyphenolic profile of this TO was studied (Sánchez-Rodríguez et al., 2019) and an increase in oleuropein, comselogoside and verbascoside concentrations were found in A1 sample in comparison with A0 but only 25 % of oleuropein and 20 % of comselogoside are stable during digestion (Fernández-Poyatos et al., 2019). It was also previously reported a low TPC bioaccessibility in some table olives varieties of verbascoside, flavonoids and hydroxytyrosol derivatives (D'Antuono et al., 2018).

Similar results were previous reported in "Cornezuelo" table olives, as it was found a decrease in antioxidant potential after gastrointestinal in vitro digestion in $ABTS^{+}$ and $DPPH^{\cdot}$ and in TPC (Fernández-Poyatos et al., 2019).

8.1.2. Experiment B

Results of Experiment B are shown on publications: second (irrigation, morphological analysis, antioxidant activity, total phenolic content, fatty acids, organic acids and sugars), third (polyphenolic profile) and fifth (bioaccessibility).

8.1.2.1. Irrigation

Two RDI treatments applied during rehydration stage and a control were studied on 2015 and 2016 seasons. During 2015, min ψ_{stem} and SI were smaller than in 2016. For the period of 2015, although non-statistical differences were found due to the high variability of data, moderate deficit irrigation during long time (B2) showed the highest min ψ_{stem} and the SI was the same than control. Regarding 2016 season, also B2 showed the highest min ψ_{stem} and, in this case, SI showed statistical significant differences. In summary, B2 was the most stressed treatment while B1 was the least one. It has to be considered that even though researchers could control the applied irrigation, other natural factors due to real on-field conditions (rain, overall weather, soil differences among areas of the same field, among others) significantly affect SI and modified the targeted values; thus, statistical differences were found between both seasons, being 2016 significantly more stressed than 2015.

8.1.2.2. Morphological and physical analyses

The only physical parameter of RO affected by RDI was one of the texture ones (MTT). In both seasons, RDI decreased the hardness of the pulp of RO. In TO, in 2015 season, fruit weight was significantly affected by RDI treatments, with B1 and B0 having the smallest and highest values, respectively. Regarding longitudinal diameter, B1 TO were longer than the others. With respect to color, a* and b* parameters showed statistical differences; B2 provided the greenest and less yellow TO. During 2016 season, the only morphological parameter affected by RDI treatments was MTT, showing that B2 TO had the hardest pulp. If both seasons are compared, it could be seen that the highest stress (2016) produced the smallest olives, affecting weight and diameters.

Fruit/pit ratio is one of the most important quality factors for table olive production and also for olive oil extraction (Gucci et al., 2009). In the current study, not statistically significant differences were found for fruit/pit ratio. This result agreed with that by Gucci et al. (2019), who studied the effect of RDI applied before pit hardening period and during rehydration phase.

In Experiment B (RDI during rehydration stage), different behavior on morphological parameters was found when compared with Experiment A (RDI during pit hardening stage). Experiment A showed that the higher the stress the higher the differences between RDI and control olives, but in Experiment B, as the stress was higher, the lower were the differences between both types of olives. Therefore, the timing of application of RDI is a highly relevant variable for olives quality.

8.1.2.3. Antioxidant activity (AA) and total phenol content (TPC)

Regarding RO, in $ABTS^{+}$ assay, B2 performed as the control, and in FRAP assay, B2 had the highest values in both studied seasons, although for $DPPH^{\bullet}$ radical, the highest values were found for B1. Treatment 2 showed the same TPC than control, whereas B1 had the lowest content. In general, B1 olives had the lowest AA and TPC, as compared to B0 and B2 fruits. If both seasons are compared, 2015 showed the highest AA regarding FRAP assay, whereas $DPPH^{\bullet}$ and TPC showed highest values on 2016 season. Previous studies with hydroSOStainable pistachio found significant differences among samples in FRAP and $DPPH^{\bullet}$ assay (a decrease in concentration was found with severe RDI treatment), while $ABTS^{+}$ did not showed significant differences (Noguera-Artiaga et al., 2020).

In relation to TO, AA was affected by irrigation in both seasons; in the $DPPH^{\bullet}$ assay, B1 and B2 presented higher antioxidant power than control; while in the FRAP assay, B2 had the same concentration than control. In TO, also both RDI treatments yielded higher concentrations of TPC than B0. No statistical differences were found on AA of TO between seasons.

When RDI was applied during stage II (Experiment A), no statistical differences were found among irrigation treatments on AA and TPC. Consequently, the timing of RDI application is very important regarding antioxidants and TPC. Stage III of fruit growth corresponds to the maturation and oil accumulation period, and when the stress was applied in that period, phenol content and antioxidant power increased. Gucci et al. (2019), in a study on the effect of irrigation time on the polyphenolic compounds of olive oil in the "Frantoio" variety, reported the highest increase in the concentration of polyphenols when RDI was applied before the hardening period of the pit. Differences with the current work could be due to agronomic conditions, variety, water stress conditions, etc.

8.1.2.4. *Fatty acids*

During 2015 season no statistical differences were found among irrigation treatments on RO, whereas in 2016, the percentages of stearic and oleic acids slightly changed. Stearic acid increased in B2 (3.40 %) and oleic increased in B1 (70.7 %) as compared to 2.90 % and 68.8 % in the control treatment (B0). This observation may be related to the fact that during 2016, the midday stem water potential (Ψ_{stem}) values were smaller than in 2015, and the stress integral values were larger. The stress in the trees was higher in 2016 than in 2015; thus, only a high water stress (applied during rehydration stage) modified the fatty acid profile.

Regarding TO, when the trees were under a low stress (2015), no differences were found on the fatty acid profile (following the same trend reported for RO) although total SFA slightly decreased in B2. However, in 2016 season, palmitic acid percentage decreased in both RDI treatments, and oleic acid concentration increased in B2 (71.4 %) as compared to 70.1 % in the control olives. Therefore, these results showed a slight trend to an enhanced functional quality of TO under water stress, as SFA decreased while simultaneously MUFA increased on the total fatty acid profile.

Other studies were done on olive trees with different RDI treatments (Cano-Lamadrid et al., 2015; Cano-Lamadrid et al., 2017) in which the RDI was conducted during pit hardening stage (non-critical stage), and no differences were found on antioxidant activity or total phenol content, although MUFA percentage increased with high stress and PUFA with moderate stress. Thus, similar results were found when moderate long time deficit irrigation was applied during stage III because MUFA content increased and SFA content decreased, leading to an improvement of the fatty acid profile.

Several previous studies proved that timing of olive tree irrigation influenced fruit tissues evolution (Rapoport et al., 2004; Gucci et al., 2009) although little information is provided about timing. Gucci et al. (2019) did not found a clear trend on “Frantoio” olive oil fatty acid composition after applying RDI before stage II and during III.

8.1.2.5. *Organic acids and sugars*

Main organic acids found in RO were citric, tartaric, malic, and succinic acids while main sugars were sucrose, glucose, and fructose. Both seasons under study yielded similar contents of organic acids and sugars, so it could be said that differences between the stress levels in both seasons did not affect the organic acids and sugars profiles. Only tartaric and succinic acids decreased their concentration when both RDI treatments were applied.

Regarding TO, phytic, lactic and acetic acids were found as major organic acids; maltoheptaose and mannitol as major sugars, and glycerol as a polyalcohol in the sugar profile. No statistical differences were found due to irrigation treatments and seasons. Same profiles of organic acids and sugars were previously seen in Experiment A. The differences among the organic acid and sugar profiles in raw and TO were the consequence of the transformation of RO into TO via fermentation during the Spanish-style process as previous reported on Experiment A.

For further information about the effect of the water stress in the parameters studied, correlations were done among all parameters (for both RO and TO) and SI including both seasons. Negative correlations among SI and (i) fruit weight, (ii) pit weight, and (iii) equatorial diameter. Although non-statistically significant, regarding morphological characteristics (Table 2), it was observed that the higher the stress applied during stage III, the higher the decrease in fruit and pit weight. Thus, the relation fruit/pit was maintained, and similar fruit quality was obtained. As it was expected, equatorial diameter was also negatively correlated with SI, and it was definitely linked to the weight loss. Regarding fatty acids, positive correlations among SI and (i) MUFAs for RO and TO, (ii) linolenic acid, and (iii) (MUFA + PUFA)/SFA percentages only for TO; only TO data are represented in such figures. The higher the stress applied during stage III, the higher the concentration of linolenic acid in TO, also the sum PUFA + MUFAs slightly increased due to water stress. The higher the stress applied, the higher the content on MUFA in RO and TO olives.

8.1.2.6. Polyphenol profile

Polyphenol profile of RO and TO of experiment B was determined and 17 polyphenols were identified and quantified. As occurred in experiment A, RO experienced a high decrease of concentration when submitted to Spanish-style processing to TO. For RO, the major polyphenol was oleuropein, followed by oleoside, oleuropein aglycone, quercetin-3-*O*-rutinoside and luteolin-3-*O*-rutinoside. In both samples quercetin-3-*O*-rutinoside experience an increase in RDI olives with respect to control and caffeoyl-6’-secologanoside a decrease. In olives B1 the concentrations of comselogoside, luteolin and ligstroside also increased, whereas hydroxytyrosol glucoside, verbascoside, dihydro-oleuropein and oleuropein diglucoside decreased. With regard to B2 treatment, oleisode, oleuropein aglycone, verbascoside, oleuropein and 2-hydroxyoleuropein increased their concentration whereas oleoside diglucoside decreased.

Previous studies reported an increased in tyrosol when water deficit irrigation strategies were applied (Gómez-Rico et al., 2006), therefore, it is relevant to mention that hydroxytyrosol glucoside, oleuropein aglycone, verbascoside, dihydro-oleuropein, oleuropein diglucoside, oleuropein, lignoside and 2”-hydroxyoleuropein are formed from tyrosol.

Corcerning TO, an increase of concentration of luteolin-3-*O*-rutinoside, oleoside diglucoside and comselogoside were found on B1 at short moderate stress. However, long moderate stress (B2) increased dihydro-oleuropein and oleuropein concentrations but decreased oleoside.

As cited before, a previous study applying sustained deficit irrigation found an increase of polyphenols concentration as the stress increased (Sena-Moreno et al., 2018). Something similar was found in experiment B, where a high proportion of polyphenols increased their concentration when the stress was applied for a long time.

8.1.2.7. Pearson correlation between SI, and polyphenols, antioxidant activity, total phenol content and color.

For better understanding the relation between SI and some of the parameters studied, Pearson correlations were carried out. For instance, it was found a positive correlation in RO between SI and oleoside and dihydro-oleuropein, but a negative correlation with hydroxytyrosol glucoside, comselogoside, luteolin and ligstroside. After Spanish-style processing to TO, a positive correlation was found between SI and dihydro-oleuropein, caffeoyl-6’-secologanoside and oleuropein, but negatively correlated to comselogoside.

Regarding AA, FRAP assay was the one showing a positive correlation with SI in both RO and TO. It was observed the same behavior on experiment A, so it could be corroborated that FRAP assay is the best to study antioxidant potential of hydroSOStainable olives. A previous study with tomatoes from water deficit strategies (Bogale et al., 2016), found a positive correlation between FRAP assay and TPC so maybe the increase of phenols in experiment B contributed to the increase in FRAP assay, as polyphenols could be responsible components for the reducing potential (Fu et al., 2011; Kumar et al., 2015). As explained in the experiment A, FRAP assay could react with non-enzymatic components; for instance, Ilahy et al. (2011) found a positive correlation between FRAP and vitamin C. Therefore, it would be interesting to study the non-enzymatic antioxidants that could be increase by water stress as a hypothesis of this result.

Parameters *L** and *b** were positively correlated with SI in TO samples.

8.1.2.8. Antioxidant Activity, Total Phenol Content and its bioaccessibility after Gastrointestinal in vitro digestion

As it was previous explained, the test matrix (TO before in vitro digestion) of this experiment showed statistically significant differences among samples on DPPH$^{\cdot}$, FRAP and TPC. In DPPH$^{\cdot}$ and TPC, B1 showed the highest concentration while this treatment was the one with the lowest antioxidant potential on FRAP assay. After in vitro gastrointestinal digestion, no statistical differences were found among analyzed by any method. All of them presented lower concentrations than the test matrix so the % var was similar than the reported on the experiment A (~ - 89 %).

The percentage of bioaccessibility did not shown statistical differences among samples. B0 was 13.2 % bioaccessible, B1 was 12.2 % and B2 12.3 %.

The polyphenol profile of these treatments was analyzed (Sánchez-Rodríguez et al., 2019) and it was found an increase on elenoic acid glucoside, oleuropein, comselogoside and luteolin-3-*O*-rutinoside in some hydroSOStainable treatments but this increase was not reflected on the antioxidant potential after digestion nor in the bioaccessibility. Only 25 % of oleuropein and 20 % of comselogoside and elenoic acid derivatives are recovered during digestion (Fernández-Poyatos et al., 2019).

Flavonoids are usually present in food matrices in combination with sugars and that makes them not very soluble in organic or aqueous solvents (Tomás-Barberán, 2006), so the increase in some RDI treatments such as luteolin-3-*O*-rutinoside (Sánchez-Rodríguez et al., 2019) could not be recovered after the in vitro digestion simulation.

The TPC of the residual fraction in olives from some irrigation treatments that previously had high concentrations of polyphenols in the test matrix could indicate that there are still antioxidant potential and polyphenols left that could be further bioavailable for human absorption. So the high proportion of antioxidants and polyphenols found on some hydroSOStainable TO are possibly available for absorption (Fernández-Poyatos et al., 2019) but further studies of bioavailability are need to corroborate this hypothesis

8.2. "Arbequina" olive oil

8.2.1. Experiment C

Results of Experiment C are showed on the sixth publication (irrigation, analytical parameters for olive oil grading, antioxidant activity, total phenol content, fatty acids, volatile compounds and descriptive sensory analysis).

8.2.1.1. Irrigation

In this experiment, three deficit irrigation treatments [C1 (optimal RDI), 197 mm of applied water; C2 (Confederation RDI), 160 mm; and C3 (Confederation SDI), 162 mm) and a control (full irrigated, 468 mm of applied water) were carried out. Although a high numeric difference could be found between treatments on SI, it was not significant due to irrigation problems (for a few weeks in July), due to the high variability of data from the same treatment no significant differences could be observed between treatments, although a trend ($p < 0.1$) was found. Control trees (C0) reached a lower stress level (53.4 MPa x day) than the deficit irrigation treatments. Confederation RDI (C2) showed the highest stress because of higher reduction of water in a short period of time (stage II). C1 received a higher volume of water than C3, but it had a higher stress (152 MPa x day) because of the concentration of the reduction of water during stage II while C3 (132 MPa x day) showed the lowest stress after control because the SDI applied the deprivation of water during the whole season. Similar behavior was found for Min Ψ_{stem} as it was affected by variability and no statistically significant differences were found ($p < 0.05$). As to yield and oil yield, they were not affected by the water restrictions applied.

8.2.1.2. Analytical parameters for Olive Oil Grading

With the purpose to study the commercial quality of olive oils under study, analytical parameters were studied following European Regulation EEC (2568/91) and EU (2016/2095). In this regulation, 3 categories were stablished for olive oil: (i) EVOO: extra virgin olive oil (highest chemical and sensory quality), (ii) VOO: virgin olive oil, and (iii) *lampante* olive oil, which need to be refined for human consumption. All samples, including control and deficit irrigation olive oil, were under the limit stablished by European Regulation (EEC, 2568/91; EU, 2016/2095) for acidity index, peroxide value and UV absorption characteristics (K_{232}, K_{270} and ΔK) to be categorized as EVOO. Therefore, it could be said that saving water did not affect olive oil grading. Previous studies agreed with this results (Gomez Del Campo and Garcia, 2013; García, et al., 2013; Caruto et al., 2014; García et al., 2017).

8.2.1.3.Antioxidant Activity (AA) and Total Phenol Content (TPC)

Antioxidant activity was measured by two assays, $ABTS^+$ and $DPPH^·$. No statistically significant differences were found among samples as analyzed by any assay, showing an average of 0.119 mmol Trolox eq L^{-1} for $ABTS^+$ and 0.250 mmol Trolox eq L^{-1} for $DPPH^·$. Oppositely, total phenol content showed significant differences being C0 and C2 the treatments with the highest concentration of TPC (259.8 and 267.3 mg GAE L^{-1} respectively) and C1 the lowest concentrated (126.8 mg GAE L^{-1}) while C3 showed an intermediate position (181.5 mg GAE L^{-1}).

Correlations between TPC and water stress variables were studied, and it was found a quadratic relationship between TPC and Min Ψ_{stem}. This showed that, TPC increased as Min Ψ_{stem} decreased until -4 MPa and, at less potentials, TPC start to decrease its concentration. Although Min Ψ_{stem} it is not the best way to define the stress suffered by olive tree, it is useful to describe oil features because of the reported extreme conditions.

Previous studies reported similar concentrations of antioxidants and TPC such as M. Servili et al. (2007), Gomez Del Campo and Garcia (2013) and Sarolic et al. (2014) in olive oil from different varieties, including “Arbequina”. Comparable results were found in the research by Gomez Del Campo and Garcia (2013) where TPC was higher with a 30 % water reduction during pit hardening stage while the other treatments, actually more intense, did not show an increase. The hypothesis proposed by (Horner, 1990) said that water stress in the tree can produce an increase in free phenylalanine (phenolic compounds precursor) and, therefore, phenols synthesis could be more sensitive when moderate water stress is applied.

8.2.1.4.Fatty Acids

Fatty acids are also relevant concerning olive oil composition. Twenty-two fatty acids were identified and quantified on oils of experiment C. Ten of them were saturated (SFAs) being palmitic and stearic acids the most concentrated. As to MUFAs, oleic acid was the predominant of the total of eight that were found. Regarding PUFA, linoleic acid is the one standing out by the total of five that were found. Irrigation made some changes on fatty acid composition. If SFA summary is considered, no statistically significant differences were found, although palmitic acid (the majority SFA) showed a significant decreased on deficit irrigated oils if compared to control, as well as lignoceric acid. In terms of MUFAs summary, all deficit irrigated treatments had higher content than control, for instance, oleic acid increased a 5.4 % in C1, 7.6 % in C2 and 7.1 % in C3 with respect to control (C0). Also, *cis*-9-heptadecenoic acid showed higher concentration in deficit irrigated oils than control. PUFAs did not shown statistically significant differences in any of the fatty acids neither the summary of them.

Some research has been done about the effect on fatty acids of oils coming from olive trees subjected to different deficit water strategies but not a clear trend was found. Studies of García et al. (2013) and Garcia et al. (2017) on "Arbequina" olive oil following different irrigation strategies showed similar concentrations than in the experiment C, although the performance of deficit irrigated samples was different. The first one found an increase of oleic acid, a decrease of linoleic acid, and MUFAs and SFAs were not affected while Garcia et al. (2017) found an increase of linoleic acid and MUFAs and a decreased of oleic acid and PUFAs as the water stress increased during the whole season. Other studies with different varieties also did not found a clear trend on fatty acid profile of oils as result of applying water restrictions with different conditions (Caruso et al., 2014; Gucci et al., 2019). Thus, it is not easy to reach a clear conclusion on the fatty acid profile of olive oils from deficit irrigation techniques, as many types of enzymes contribute to fatty acid synthesis from the beginning of pit hardening stage to the end of fruit maturation. Irrigation, including the timing and the stress level have a clear effect on the fruit composition (Garcia et al., 2017; Gucci et al., 2019).

Atherogenic and thrombogenic indexes (AI and TI respectively) were calculated for the oils of experiment C. Results showed one of the lowest AI and TI as compared with other oils (Ulbricht and Southgate, 1991). The lower the AI and TI, the healthier is the test matrix under study, as AI represent the possibility of atheroma formation (possibility of lipid adhesion to cells of the immune circulatory system) and TI values are associated with the formation of clots in the blood vessels (Ulbricht and Southgate, 1991).

Pearson correlation between fatty acids and SI was carried out, and a positive correlation was found on C17:1 *cis* and a negative correlation with linoleic acid and SFAs summary, so the higher the stress, the higher the concentration of C17:1 *cis* and the lower the concentration of SFAs and linoleic acid.

8.2.1.5. Volatile Compounds

A total of 30 volatile compounds that belonged to 5 chemical families were identified and quantified in oils of experiment C. The predominant family in oils from stress treatments were alcohols, concretely, C3 was the oil with the highest alcohol concentration as it presented high concentration of some compounds such as ethanol, 3-methylbutan-1-ol, 2-methylbutan-1-ol, pentan-1-ol, (*Z*)-pent-2-en-1-ol, (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol. Also, C1 and C2 presented higher concentration of alcohols than control, for instance, they (C1 and C2) experienced an increase of 3-methylbutan-1-ol, 2-methylbutan-1-ol, pentan-1-ol, (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol compounds. As aldehydes is concerned, some compounds experienced a decrease on deficit irrigation strategies. C1 was the oil with the smallest aldehyde concentration, with decreased o (E)-hex-2-enal, pentanal, hexanal and nonanal. Also 2-methylbutanal and heptanal decreased on C3 treatment. Ketone content showed different behavior in the deficit irrigated treatments, as it decreased in C1 (pentan-2-one decreased), but increased in C2 and C3 oils. However, pentan-3-one increased in all stressed olive oils. Total ester amount was increased on C1 and C2 oils as compared to control oil, as (*Z*)-hex-3-enyl acetate and hexyl acetate increased their concentration. Hydrocarbons of samples C1 and C3 were significantly lower than in control due to a decrease of 4,8-dimethylnona-1,7-diene and (*E*)-4,8-dimethylnona-1,3,7-triene compounds in C1 oils and (*E*)-β-ocimene in C3 treatment.

Main volatile compounds in olives are synthesized via Lipoxygenase (LPO) pathway from linoleic and linolenic acids during oil accumulation stage. Compounds formed by LPO pathway are hexanal, hexyl acetate, (*Z*)-hex-3-en-1-al, (*Z*)-hex-3-en-1-ol, (*E*)-hex-2-en-1-al, (*E*)-hex-2-en-1-ol, (*Z*)-hex-3-enyl acetate, and (*Z*)-hex-2-enyl acetate. Also alcohols, esters and ketones are formed by fatty acid metabolism (Kalua et al., 2007). As a result of volatile composition of experiment C (more concretely, the alcohol compounds increases noticed), it could be reported a probably increase on the activity of LPO pathway as a result of water stress, as previous reported by Garcia et al. (2017), Servili et al. (2007) and Stefanoudaki et al. (2009). Moreover, a previous study in “Arbequina” variety also reported the highest concentration of alcohols when deficit irrigation strategies were applied (Garcia et al., 2017). Also on “Koroneiki” variety an increase of 6C “green volatile” compounds, *trans*-3-hexen-1-ol, and hexyl acetate due to water stress was reported (Stefanoudaki et al., 2009). “Leccino” variety showed similar changes on aldehydes and alcohol (Servili et al., 2007) as well as “Frantoio” variety showed an increase on 2-hexen-1-ol as a result of water stress (Caruso et al., 2014).

Moreover, Pearson correlation between volatiles identified and SI was carried out and a negative correlation was found for aldehydes sum, but a positive with esters. It was also found a positive correlation with some compounds such as 2-methylbutanal, 2-methylbutan-1-ol, (*Z*)-hex-3-en-1-ol, (*Z*)-hex-3-enyl acetate, hexyl acetate and (*Z*)–hexyl-2-enyl acetate. These compounds are related to apple, fruity, sweet, fresh, green and grass sensory notes.

To summarize, hydroSOStainable olive oils presented higher concentration of volatiles than control, so it could make them more attractive to consumers due to their aromatic notes in addition to the water saving that it entails. More concretely, C2 and C3 were the treatments with a highest aromatic concentration.

8.2.1.6.Descriptive Sensory Analysis

The official panel determined the oils as EVOO with an average of 4.0 on fruity attribute. “Food quality and safety” research group panel conducted descriptive sensory analysis and all hydroSOStainable olive oils presented lower concentration of green-herbs notes and sourness as compared to control, but higher intensities of almond and walnut notes and sweetness. With regard to C1 most attributes under study presented smaller intensities than control (fruity-olive, fruity-green, floral, green-grass and bitter). As C2 is concerned, fruity-olive, fruity-green and green-herbs attributes increased their concentration and C3 oils increased intensities of fruity-olive and woody but decreased green-herbs notes. No defect (negative attributes) was detected on the oils and, finally, mouthfeel descriptors showed an increase of astringency and viscosity on C2 and C3 oils.

As it was previously reported, changes in polyphenol, volatile and fatty acid composition are correlated with the sensory analysis of olive oil (Servili et al., 1995; Motilva et al., 2000; Kalua et al., 2007; García-Mesa et al., 2008; Dabbou et al., 2010; Campestre et al., 2017). For instance, the increased found on astringency on C2 and C3 samples could be directly correlated with the increased of polyphenols. For “Koroneiki” and “Leccino” varieties, olive oils from deficit irrigation strategies were more pungent and bitter as a result of their higher polyphenolic content (Stefanoudaki et al., 2009; Servili et al., 2007) as compared to control oil. For “Arbequina” olive oils coming from water stressed trees, Garcia et al. (2017), did not found an effect on sensory analysis, but

Gomez Del Campo and Garcia (2013) found that stress intensity and the phenological stage could affect the bitterness. In experiment C, bitterness and astringency decreased their intensities on C1 treatment, the one that also decreased polyphenol, aldehydes and ketones concentrations.

Pearson correlation between sensory attributes and SI showed a positive correlation for almond, walnut, sweet and astringent and a negative one for green-herbs and sour.

In a general point of view, it could be said that deficit irrigation strategies studied on experiment C affected several sensory attributes and C2 was the one with the highest intensities, as it suffered the highest stress.

8.2.2. Experiment D

Results of Experiment C are showed on the seventh publication (irrigation, analytical parameters for olive oil grading, antioxidant activity, total phenol content, fatty acids, volatile compounds and descriptive sensory analysis).

8.1.2.1. Nutritional tree status, yield and Irrigation

Water saving techniques did not affect tree nutrition status as no deficiency was found in any treatment, although significant differences were found among them. Barium, sulfur, sodium and calcium showed statistically significant different concentrations in each treatment. Calcium reduction on water stress treatments could be explained as a closure of stomata aperture due to stress, as calcium is moved by xylem.

No statistic differences were found for yield. Neither canopy productivity and fruit fresh weight showed significant differences among treatments. Previous studies had demonstrated that a light water stress (ψ_{stem} of -3.5 MPa) did not affected yield (Ahumada-Orellana et al., 2017).

Regarding the stress integral, when stress was applied during pit hardening stage (DOY 185 to 239). Control, D0, presented the lowest stress, while D3 was the most stressed treatment. D1 and D2 showed an intermediate SI between D0 and D3 treatments.

8.1.2.2. Analytical parameters for Olive Oil Grading

Results of acidity, peroxide value and UV absorption characteristics (K_{232}, K_{270}, and ΔK) showed that oils for all the irrigation treatments under study could be classified as

EVOO following European Regulation EEC (2568/91) and its last amend EU (2016/2095). As no statistical differences were found among irrigation treatments, it could be said that saving water techniques analyzed in experiment D did not affect final commercial quality of olive oil. As same occurred on experiment C, it could be stated that hydroSOStainable olive oil reached the highest quality standards as EVOO.

As Rancimat test is concerned, results showed that stability time of oils proportionally increased with the water stress, as D3 oil was the one with highest stability (14.4 h), D1 and D2 presented intermediate stability (12.5 h and 12.9 h, respectively) and finally, control, D0 was the one with the lowest stability (9.39 h). It was previous reported that olive oils with high proportion of antioxidants and polyphenols are more stable in time (Martinez-Nieto et al., 2010).

8.1.2.3. Antioxidant Activity (AA) and Total Phenol Content (TPC)

$ABTS^{+}$ and $DPPH^{\cdot}$ assays are commonly used to study AA in olive oil samples (Baiano et al., 2009; Giuffrè et al., 2017). In both assays, hydroSOStainable olive oils showed higher activity than control, although for $ABTS^{+}$, D1 and D3 presented higher values than D2 and in $DPPH^{\cdot}$ no statistical differences were found among the three deficit irrigated treatments. With regard to TPC, also oils from saving water strategies had more concentration than control; D3 was the oil with highest concentration while D1 and D2 showed similar TPC concentration.

Servili et al. (2007) found evidence about the effect of water on polyphenols: tree water status is inversely correlated with polyphenols content in olive oil. Similar results on “Arbequina” olive oil were reported by Sena-Moreno et al. (2018) where polyphenol profile and AA increase in oils coming from deficit irrigation during all the season. It was also demonstrated by Gucci et al. (2019) that timing of deficit irrigation influence the polyphenol synthesis. This study reached the conclusion that when deficit irrigation was carried out during pit hardening stage the polyphenol synthesis was increased. Experiment C showed a quadratic correlation between Ψ_{stem} and TPC where TPC increased with Ψ_{stem} but it started to decreased when Ψ_{stem} reached -4 MPa. As in experiment D the maximum Ψ_{stem} reached is -4 MPa, this decreased was not showed.

For further understanding of SI effect on AA and TPC, Pearson correlation were carried out among these parameters. $ABTS^{+}$ and TPC were positively correlated with SI, which means that higher stress produced higher concentration in these assays, although, TPC presented a higher significance than $ABTS^+$.

Therefore, hydroSOStainable olive oils of experiment D showed an improvement on its AA and TPC composition that could be linked to the polyphenolic health claims for olive oil (5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil contribute to the protection of blood lipids from oxidative stress) (EU, 2012), although it would be necessary to study the polyphenolic profile of olive oils to determine hydroxytyrosol concentration to fully support this statement.

8.1.2.4. Fatty Acids

Fatty acid profile of olive oils of experiment D was identified and quantified. Oleic acid was the compound with the highest concentration in samples and hydroSOStainable oils presented higher concentration than control. Continuing with MUFAs, 17:1 *cis*9 compound showed the highest concentration in D2 treatment. With regard to the MUFA sum, D1 and D2 olive oils had higher concentrations than control (D0) and D3. Continuing with SFAs sum, it is important to mention that hydroSOStainable oils showed a lower concentration than control, which is due to a decrease on palmitic and lignoceric acids. Finally, PUFAs summary showed statistically equivalent content on D0 and D2 and a slight decrease on D1 and D3 oils. Linoleic acid experienced a decrease in all hydroSOStainable oils, and 20:4 n6- *cis*-5 *cis*-8 *cis*-11 *cis*-14 decrease on D2 and D3 olive oils. Similar fatty acid concentrations were reported on “Arbequina” variety by Aparicio and Harwood (2013) and Garcia et al. (2017).

To further study the possible quality improvements of the hydroSOStainable olive oil, atherogenic and thromogenic indexes have been studied. D1 sample showed the lowest AI and TI, but also D2 and D3 showed slight decreased if they are compared to control. AI and TI of olive oil are among the lowest values on foods. These decreases indicated lower possibility of lipid adhesion to cells of the immune circulatory system (atheroma formation) and lower possibility of formation of clots in the blood vessels (Ulbricht and Southgate, 1991). Similar values than in the current experiment were previous reported for olive oil (Ulbricht and Southgate, 1991) but no information about AI and TI are found in studies with water deficit strategies techniques.

Some contradictory information is found about fatty acids on olive oil from water deficit strategies. For instance, it was reported an increase in the oleic/linoleic ratio in

“Arbequina” variety coming from different water deficit strategies by Garcia et al. (2017) and Gomez Del Campo and Garcia (2013) but the opposite effect was found on “Koroneiki” variety by Stefanoudaki et al. (2009). This is a clear indicator of the effect of variety on fatty acid composition of olive oils from water deficit strategies. But not only the variety, also the location, as in experiment C (located in Sevilla), it was found an increase on MUFAs, but no effect was reported on SFAs, PUFAs, AI and TI.

Pearson correlation between SI and fatty acids were studied and 17:1 *cis* showed a positive correlation but a negative correlation was found for 18:2 *cis* and the AGSs sum. Same behavior was found on experiment C.

As a result, hydroSOStainable olive oils from experiment D offered higher content of MUFAs, lower SFAs and better values of AI and TI than control, so the fatty acid profile was improved.

8.1.2.5. Volatile Compounds

Identification and quantification of volatile compounds in samples of experiment D were those carried out from the extraction on PDMS/DVB fiber as it was the one recovering more compounds. DVB/CAR/PDMS fiber was also studied but lower quantity of compounds were identified, so PDMS/DVB was the most suitable fiber to capture olive oil volatiles.

Starting with the volatile compound families, alcohols were the predominant chemical family. Alcohols showed a decrease of concentration as water stress increased. D0 and D1 treatments showed the highest concentrations of ketones, D2 the largest amount of aldehydes and esters. Regarding the total volatile compounds, a decrease of concentrations was found as water restrictions increased. However, D2 showed a profile with the highest concentration of aldehydes and esters but the second lowest content of alcohols. Previous studies reported an increase in aldehydes when water stress were applied (Servili et al., 2007; Stefanoudaki et al., 2009).

(*E*)-hex-2-en-1-ol was the most abundant compound in D0 oil and it decreased as water stress increased although it was also the most abundant compound on D1 sample. (*Z*)-hex-3-en-1-ol and hexan-1-ol also decreased as irrigation water decreased reaching the last one values close to zero on D2 and D3. Nevertheless, (*E*)-hex-2-enal increased its concentration on hydroSOStainable olive oils having D2 the largest quantity. (*E*)-hex-2-enal and hexan-1-ol compounds were previously reported in “Arbequina” variety showing a negative correlation with water stress (Garcia et al., 2017) but the opposite occurred with (*E*)-hex-2-enal in the current experiment. This difference could be explained as previous studies applied water restrictions during the whole season and in experiment D, RDI was applied during pit hardening stage. There are some other minor compounds such as ethyl acetate, 3-methylbutanal and 2-methylbutanal that appeared on hydroSOStainable olive oils and they were not detected on D0. Other compounds, for instance, 6-methylhepta-1,5-diene, 4,8-dymethylnona-1,7-diene and hexyl acetate showed same concentration on D1 than D0, but decreased on D2 and D3 treatments. (*Z*)-hex-3-enyl acetate presented same concentration on D2 than control. Regarding hexyl-acetate, also Stefanoudaki et al. (2009) and Servili et al. (2007) found a decrease with water stress on “Koroneiki” and “Leccino” varieties respectively.

Regarding experiment C, a different behavior was found and location proved to be a relevant factor, again, for volatile compounds as it was found an increase on alcohols and a decrease on aldehydes, just the opposite than in experiment D.

As explained on experiment C, LPO pathway is the main responsible for volatile synthesis (Kalua et al., 2007). As alcohols decreased their concentration while aldehydes increased, it can be assumed that water stress caused a change on LPO pathway decreasing the activity of alcohol dehydrogenase, but further research is needed to corroborate this hypothesis. Also Stefanoudaki et al. (2009) found a correlation between LPO pathway and water stress on “Koroneiki” variety.

Regarding Pearson correlation of volatiles with SI, 3-methylbutanal and (*Z*)-hex-3-en-1-ol were positively correlated but (*E*)-hex-2-en-1-ol, hexan-1-ol and the alcohols sum were negatively correlated. These results could be linked to the sensory descriptors linked to these volatiles; for instance, an increase in fruity, apple, fresh and grass notes, but a decrease on apple, fruity, grass, banana and tomato so a final balance between loss and profit could be found.

8.1.2.6. Descriptive Sensory Analysis

Olive oils of experiment D were sent to a certified panel to be classified and all samples were categorized as EVOO; D0 and D2 presented a 4.0 average of fruity and D1 and D3 4.5 of fruity.

To obtain further details about the sensory characteristics of the oils, a descriptive sensory analysis with 28 descriptors was carried out. D1 and D3 samples presented higher intensity than control of fruity (general and under-ripe olive), floral, green (artichoke, herbs and grass) astringent and viscosity attributes. A balance between astringency and pungency was found on these oils. D2 samples were given higher scores of apple and woody than control. Previous studies reported that olive oils from stress olive trees produced unbalanced notes between bitter and pungency (Stefanoudaki et al., 2009). These authors also reported high woody characteristics and low fruity intensity but the contrary effect was found on “Arbequina” olive oil from RDI technique during pit hardening stage.

HydroSOStainable olive oils of experiment D presented an increase of intensities of some attributes, so it made them more attractive for consumers. RDI during pit hardening stage made more balanced and intense olive oils than control.

As to Pearson correlation with SI, fruity-olive, green-artichoke, floral, green-herbs and green-grass showed a positive correlation with SI. Although similar irrigation treatments were carried out on experiment C, a negative correlation was found for green-herbs attribute, maybe due to location.

Sensory descriptors were also correlated with volatile compounds by Pearson test and some interesting interaction was found to further understand how changes in volatile composition affected the sensory perception of the oils. The attribute fruity-olive was positively correlated with ethyl acetate, pentan-2-one, (*E*)-hex-2-en-1-ol, hexan-1-ol and hexyl acetate. Fruity-green also was positively correlated with ethyl acetate, hexan-1-ol and hexyl acetate. Floral showed a positive correlation with ethyl acetate, but negative with (*E*)-hex-2-en-1-ol and hexyl acetate. For green-artichoke only a positive correlation was found with hexyl acetate but three negatives (pentan-2-one, (*E*)-hex-2-en-1-ol and hexan-1-ol). Green-grass was positively correlated to (*E*)-hex-2-en-1-ol. Apple attribute was correlated negatively with ethyl acetate and positively to (*E*)-hex-2-enal. Finally, woody was positively correlated to 3-methylbutan-1-ol and (*E*)-hex-2-enal.

9. CONCLUSIONS AND CONCLUSIONES

9.1. CONCLUSIONS

9.1.1. General conclusions

Morphological quality of hydroSOStainable olives showed some changes as compared with control. In Experiment A, RDI strategies produced rounder, harder, lighter and greener olives while in Experiment B the size was slightly reduced but the pulp proportion was maintained. Regarding mineral composition, antioxidant activity, TPC and organic acids and sugars of Experiment A, the hydroSOStainable olives showed the same values than control, nutritional and functional quality was maintained and RDI strategies did not affected olives quality. Several volatile compounds were affected by the RDI treatments, as well as the intensity of some sensory descriptors. As Experiment B, when RDI was highest, antioxidant activity, TPC and MUFA content were increased. It was also found a positive correlation between de SI and the FRAP assay to determine antioxidant activity in several olive samples in both experiments.

With respect to hydroSOStainable olives oil, both experiments showed an increase of MUFAs and decreased SFAs, improved, balanced volatile profiles and sensory attributes when the water restriction was applied during pit hardening in a moderate stress.

The Spanish-style processing produced a decreased in the concentration of all polyphenols due to the osmosis effect during fermentation and brining. HydroSOStainable olives polyphenol profile was improved when trees were submitted to a moderate stress in both experiments. HydroSOStainable TO are healthier for consumers due to an increase of some polyphenols such as oleuropein.

Affective sensory analysis was carried out with TO of Experiment A in three locations. Consumers preferred TO with hydroSOStainable logo and were willed to pay a higher price for them. The logo created an effect on consumers as they marked these TO with higher green-olive flavor and overall liking.

Antioxidant activity and phenolic content after *in vitro* gastrointestinal digestion simulation showed different behavior in Experiment A and B. In the first (A), small differences were found for TPC, ABTS^{+} and DPPH$^{\cdot}$ assays between irrigation treatments but in the latter, no differences were found. As a whole, a total amount of 1 g GAE kg^{-1} was extracted after digestion, so ~12 % of bioaccessible polyphenols were found on control and hydroSOStainable TO. Eating 10 hydroSOStainable TO per day involve the daily intake of 40 mg of bioaccessible polyphenols for protective effect against chronic diseases, which involves the 7 % of the daily recommendations.

Therefore, it could be concluded that, if the water reduction is applied during pit hardening stage (Experiment A), fruit size and yield are maintained with no significant differences in composition, and when the water deficit is applied during rehydration stage (Experiment B), olive size is reduced but improved the functional quality of olives.

9.1.2. Future work

First of all, it would be interesting to continue studying the effect of the irrigation treatments studied in this thesis in more olive varieties and locations. It would be good to study different varieties at the same location and repeat the study in other locations to be able to study the effect of water stress in the varieties and also how the location affects.

Furthermore, in this thesis hydroSOStainable olive oil was studied only for one year, so it is necessary to continue this investigation, and also to study the polyphenol profile and to do some studies with consumers to know the acceptability of this product in the market.

Following the study of phenols bioaccessibility, it would be interesting to study the phenols bioavailability.

To further study the correlation between LPO pathway (volatile compounds synthesis) and water stress.

To study the effect of water stress in the non-enzymatic antioxidants synthesis as a result of the positive correlation found between FRAP assay and water SI.

9.2. CONCLUSIONES

9.2.1. Conclusiones generales

La calidad morfológica de las aceitunas hidroSOStenibles se vio afectada por las estrategias de riego deficitarias estudiadas. En el Experimento A, las aceitunas hidroSOStenibles tuvieron una forma más redondeada, una textura más dura y fueron más luminosas y verdes que el control. En cambio, en el Experimento B, las aceitunas hidroSOStenibles presentaron un tamaño inferior que el control, pero se mantuvo la proporción de pulpa. En relación a la composición mineral, actividad antioxidante, fenoles totales, ácidos orgánicos y azúcares del Experimento A, no se encontraron diferencias estadísticas significativas entre las aceitunas hidroSOStenibles y el control. Se mantuvo la calidad funcional y nutricional. Algunos compuestos volátiles se vieron afectados por los tratamientos de riego, al igual que la intensidad de algunos atributos sensoriales. En relación al Experimento B, a mayor estrés hídrico, mayor actividad antioxidante, contenido total de fenoles y de ácidos grasos monoinsaturados. También se encontró una correlación positiva entre la integral de estrés y el método FRAP para determinar actividad antioxidante en ambos experimentos y varias de las muestras estudiadas.

Con respecto a los aceites de oliva hidroSOStenibles, en ambos experimentos se encontró un aumento de los ácidos grasos monoinsaturados y un descenso de los saturados, un aumento del contenido total de polifenoles y un perfil de volátiles e intensidades de atributos sensoriales equilibrados cuando las restricciones de agua de riego se realizados durante el endurecimiento del hueso a un nivel moderado.

El proceso de fermentación para transformar las aceitunas crudas en aderezadas por el estilo español produjo una reducción en la concentración de todos los polifenoles debido al efecto de ósmosis. Las aceitunas hidroSOStenibles mejoraron el perfil polifenólico cuando el estrés aplicado fue moderado en ambos experimentos. Las aceitunas hidroSOStenibles producen más beneficios a la salud de los consumidores debido al incremento de algunos polifenoles como la oleuropeína.

El análisis sensorial afectivo fue llevado a cabo en las aceitunas de mesa del Experimento A en tres localizaciones. Los consumidores prefirieron las aceitunas de mesa marcadas con el logo hydroSOStenible e indicaron que estaban dispuestos a pagar más por ellas que por las marcadas como convencionales. Los consumidores indicaron que estas aceitunas (marcadas con el logo hydroSOStenible), les gustaban más de forma general y también les gustaba más el sabor verde-aceituna.

La actividad antioxidante y el contenido total de polifenoles tras la simulación de digestión *in vitro* de aceitunas de mesa mostraron un comportamiento diferente en los experimentos A y B. Se encontraron pequeñas diferencias en los ensayos TPC, $ABTS^{+}$, y $DPPH^{\cdot}$ en el experimento A entre los tratamientos de riego, mientras que en el experimento B no se encontraron diferencias significativas. En resumen, después de la digestión, se extrajeron una cantidad total de 1 g GAE kg^{-1}, lo que supone, aproximadamente, de una bioaccesibilidad del 12 % de los polifenoles de las aceitunas de mesa hidroSOStenibles. Teniendo en cuenta una ingesta diaria recomendada de 40 mg de polifenoles bioaccesibles por día para favorecer el efecto protector contra enfermedades crónicas, podríamos decir que comiendo 10 aceitunas hidroSOStenibles se cumple con el 7 % de esta recomendación.

Por lo tanto, se puede concluir que, cuando la reducción de agua de riego se produce durante la fase de endurecimiento del hueso (Experimento A), el tamaño de las aceitunas y el rendimiento de producción no se ve afectado y tampoco la composición nutricional y funcional de las aceitunas, mientras que, si el déficit hídrico se produce durante la fase de rehidratación (Experimento B), la productividad se puede ver afectada, pero se mejora la calidad funcional de las aceitunas.

9.2.2. Futuras investigaciones

En primer lugar, sería interesante continuar estudiando el efecto de los tratamientos de riego en más variedades de aceituna y en diferentes localizaciones. Más concretamente, hacer un ensayo con diferentes variedades en la misma localización, y repetirlo en diferentes localizaciones para así poder estudiar el efecto en la variedad y también cómo afecta la localización.

Además, se debería seguir estudiando los ensayos con aceite de oliva hidroSOStenibles, ya que en esta tesis sólo se han realizado estudios un año. También se podría estudiar el perfil polifenólico de estos aceites y hacer estudios con consumidores para saber la aceptación de este producto en el mercado.

Continuado con el estudio de la bioaccesibilidad de los fenoles, sería interesante estudiar la biodisponibilidad.

Estudiar en profundidad la correlación entre la ruta de la lipooxigenasa (síntesis de compuestos volátiles) y el estrés hídrico.

Estudiar el efecto del estrés hídrico en la síntesis de antioxidantes no enzimáticos como resultado de la correlación positiva que se ha encontrado entre el método FRAP y la integral de estrés hídrico.

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