UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE

ESCUELA POLITÉCNICA SUPERIOR DE ORIHUELA

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

EVALUACIÓN AGRONÓMICA, ANÁLISIS DE COMPUESTOS BIOACTIVOS Y PERFIL GENÉTICO DEL JINJOLERO (*Ziziphus jujuba* Mill.)

JUANA RECHE RUBIO

TESIS DOCTORAL

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CALIDAD DEL COMPENDIO DE CADA PUBLICACIÓN

Esta Tesis se presenta como compendio de las siguientes publicaciones:

Reche, J., Hernández, F., Almansa, M. S., Carbonell-Barrachina, Á. A., Legua, P., & Amorós, A. (2018). Physicochemical and nutritional composition, volatile profile and antioxidant activity differences in Spanish jujube fruits. *LWT-Food Science and Technology*, *98*, 1-8. *doi: 10.1016/j.lwt.2018.08.023*

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"PHYSICOCHEMICAL AND NUTRITIONAL COMPOSITION, VOLATILE PROFILE AND ANTIOXIDANT ACTIVITY DIFFERENCES IN SPANISH JUJUBE FRUITS"

Autores: Juana Reche, Francisca Hernández, Maria Soledad Almansa, Ángel Antonio Carbonell-Barrachina, Pilar Legua y Asunción Amorós.

Revista: LWT- Food Science and Technology

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2018				
Categoría JCR	Categoría Cuartil	Rango	Factor de impacto	Factor de impacto de los últimos 5 años
Food Science and Technology	Q1	23/135	3,714	4,00

"EFFECTS OF ORGANIC AND CONVENTIONAL FARMING ON THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF JUJUBE FRUIT"

Autores: Juana Reche, Francisca Hernández, María Soledad Almansa, Ángel Antonio Carbonell-Barrachina, Pilar Legua y Asunción Amorós.

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Autores: Juana Reche, Santiago García-Martínez, Pedro Carbonell, María Soledad Almansa, Francisca Hernández, Pilar Legua y Asunción Amorós.

Revista: Scientia Horticulturae

doi: 10.1016/j.scienta.2019.04.068

Editor: J.P. Bower, G. Colla, W.W. Guo, S. Kondo, P. Martínez-Gómez, B. Pennisi, D. Schwarz

ISSN: 0304-4238

Ámbito de la publicación: Horticultura

2018				
Categoría JCR	Categoría Cuartil	Rango	Factor de impacto	Factor de impacto de los últimos 5 años
Horticultura	Q1	5/36	1,961	2,315

"FATTY ACID PROFILE OF PEEL AND PULP OF SPANISH JUJUBES (*Ziziphus jujuba* Mill.) FRUIT"

Autores: Juana Reche, María Soledad Almansa, Francisca Hernández, Ángel Antonio Carbonell-Barrachina, Pilar Legua y Asunción Amorós.

Revista: Food Chemistry

doi: 10.1016/j.foodchem.2019.05.147

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ISSN: 0308-8146

Ámbito de la publicación: Food Science & Technology

2019				
Categoría JCR	Categoría Cuartil	Rango	Factor de impacto	Factor de impacto de los últimos 5 años
Food Sicence & Technology	Q1	10/124	5,399	5,488

"EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON THE PHYSIOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF SPANISH JUJUBE (*Ziziphus jujuba* Mill.) CV 'PHOENIX' DURING COLD STORAGE"

Autores: Juana Reche, María Emma García-Pastor, Daniel Valero, Francisca Hernández, María Soledad Almansa, Pilar Legua y Asunción Amorós.

Revista: Scientia Horticulturae

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UNIVERSITAS Miguel Hernández

Programa de Doctorado en Recursos y Tecnologías Agrarias, Ambientales y Alimentarias (ReTos-AAA)

Campus de Orihuela

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).

CERITIFICA:

Que la Tesis Doctoral titulada "***Evaluación agronómica, análisis de compuestos bioactivos y perfil genético del jinjolero (Ziziphus jujuba Mill.)"*** de la que es autora **Dña. Juana Reche Rubio**, ha sido realizada bajo la dirección de la **Dra. Dña. Pilar Legua Murcia** y la codirección de la **Dra. Dña. Mª Asunción Amorós Marco**, actuando como tutora de la misma la Dra. Francisca Hernández García. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Docotrado Re Tos-AAA por tanto, apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presnte ceritficado en Orihuela a once de marzo de dos mil veintiuno

Dra. Dña. Juana Fernández López

Coordinadora del Programa Doctorado Re Tos-AAA

Esta memoria ha sido presentada por Dña. Juana Reche Rubio, Ingeniero Agrónomo y Máster en Enología y Viticultura por la Universidad Miguel Hernández y Máster en Geomet: Geofísica y Meteorología por la Universidad de Granada, para obtener el título de doctor.

Fdo. Dña. Juana Reche Rubio

Esta Tesis Doctoral ha sido dirigida por la Dra. Pilar Legua Murcia, Profesora Titular de la Universidad Miguel Hernández de Elche del Departamento de Producción Vegetal y Microbiología y codirigida por la Dra. María Asunción Amorós Marco, Profesora Titular de la Universidad Miguel Hernández de Elche del Departamento de Biología Aplicada, quienes autorizan su presentación por compendio de publicaciones.

Fdo. Dña. Pilar Legua Murcia

Fdo. Dña. Mª Asunción Amorós Marco

Profesora Títular Universidad Miguel Hernández

Dpto. Producción Vegetal y Microbiología

Profesora Titular Universidad Miguel Hernández

Dpto. Biología Aplicada

Orihuela, marzo de 2021

'Dentro de un cucurucho de jínjoles, hay siempre algo más que no acabamos de encontrar. Y es tiempo acumulado, un ayer de dorados recuerdos. Con un puñado de jínjoles, de azufaifas, lo siento ahora recuperado y vivo y en esos nombres me siento afectivamente comprometido'

Salvador Jiménez en 'Murcia y la herida del tiempo', 1995

ÍNDICE

ABREVIACIONES Y SÍMBOLOS

ABREVIACIONES Y SÍMBOLOS

Abreviaciones

$ABTS^{\bullet+}$	2,2'-azino-bis (3-Ethyl-benzothiazoline-6-sulfonic acid)	$ABTS^{\bullet+}$	Ácido 2,2-azino-bis (3-etil-benzotiazolin-6-sulfónico)
AT	Titratable acidity	TA	Acidez titulable
BF_3	Boron trifluoride	BF_3	Trifluoruro de boro
BSA	Bovine serum albumin	ASB	Albúmina de suero bovino
CIELab	Commission Internationale de l'Eclairage	CIELab	Commission Internationale de l'Eclairage
Cv	Cultivar	Cv	Cultivar
DAD	Diode-array detector	DRD	Detector de red de diodos
$DPPH^{\bullet}$	2,2-diphenyl-1-picrylhydrazyl	$DPPH^{\bullet}$	2,2-difenil-1-picrilhidrazil
dw	Dry weigh	Ps	Peso seco
eq	Equivalent	eq	Equivalente
FAME	fatty acids methyl esters	EMAG	Ésteres metílicos de ácidos grasos
FRAP	Ferric reducing ability of plasma	FRAP	Capacidad reductora férrica del plasma
fw	Fresh weigh	Pf	Peso fresco
FU	Fertilizant units	UF	Unidades de fertilizante

g	Grams	g	Gramos
GC-MS	Gas chromatography-mass spectrometry	CG-MS	Cromatógrafo de gases-espectrometría de masas
GAE	Gallic acid equivalent	EAG	Equivalentes de ácido gálico
H-TAA	Hidrophilic- total antioxidant activity	AAT-H	Actividad antioxidante total de la fracción hidrofílica
HS-SPME	Headspace solid phase micro-extraction	HS-SPME	Micro-extracción en fase sólida
kg	Kilograms	kg	Kilogramos
L-TAA	Lipophilic- total antioxidant activity	AAT-L	Actividad antioxidante total de la fracción lipofílica
LSD	Least Significant Difference	DMS	Diferencia menos significativa
MI	Maturation index	IM	Índice de Madurez
mg	Miligrams	mg	Miligramos
mm	Millimeters	mm	Milímetros
MUFA	Mono-unsaturated fatty acids	AGMI	Ácidos grasos monoinsaturados
µg	Micrograms	µg	Microgramos
PCA	Principal components analysis	ACP	Análisis de componentes principales
PUFA	Poly-unsaturated fatty acids	AGPI	Ácidos grasos poliinsaturados

SFA	Saturated fatty acids	AGS	Ácidos grasos saturados
TPC	Total phenols content	CTF	Contenido total de fenoles
TFC	Total flavonoids content	CTFo	Contenido total de flavonoides
TSS	Total soluble solids	SST	Sólidos solubles totales

Símbolos

a*	Red-green	Rojo-verde
$AlCl_3$	Aluminium trichloride	Tricloruro de aluminio
b*	Yellow-blue	Amarillo-azul
Br	Bromine	Bromo
C*	Chroma	Croma
Ca	Calcium	Calcio
Cu	Copper	Cobre
C14:0	Myristic acid	Ácido mirístico
C14:1	Myristoleic acid	Ácido miristoleico
C16:0	Palmitic acid	Ácido palmítico
C16:1	Trans-palmitoleic acid	Ácido trans-palmitoleico
C16:1	Cis-palmitoleic acid	Ácido cis-palmitoleico
C17:0	Margaric acid	Ácido margárico
C18:0	Stearic acid	Ácido esteárico
C18:1n9c	Oleic acid	Ácido oleico

C18:1n7	11-octadecenoic acid	Ácido 11-octadecenoico
C18:1n9t	Elaidic acid	Ácido elaídico
C18:2	Linoleic acid	Ácido linoleico
C18:3	Linolenic acid	Ácido linolenico
Fe	Iron	Hierro
h*	Hue angle	Ángulo Hue
K	Potassium	Potasio
L*	Brightness	Luminosidad
Mg	Magnesium	Magnesio
Mn	Manganese	Manganeso
NaOH	Sodium hydroxide	Hidróxido de sodio
Rb	Rubidium	Rubidio
Zn	Zinc	Zinc

ESTRUCTURA DE LA TESIS

La presente Tesis Doctoral se ha escrito según la metodología basada en compendio de artículos de investigación, siguiendo la normativa exigida por la Universidad Miguel Hernández. Consta de las siguientes partes que se describen a continuación:

1. **Introducción.** Donde se resume la procedencia y distribución del jinjolero y se describe taxonómicamente, además de comentar sus usos y propiedades, por medio de una revisión bibliográfica.
2. **Objetivo**. En este apartado se explica cuál es el objetivo final de la presente investigación y los objetivos secundarios.
3. **Metodología**. En este apartado se explica la preparación de las muestras y su conservación, y las técnicas y métodos que se han seguido para poder realizar la investigación sobre el fruto del jinjolero. Asimismo, se describen los programas informáticos que se han utilizado para obtener los resultados estadísticos.
4. **Artículos.** Se han escrito 5 artículos, que son los que componen la Tesis doctoral. Todos ellos publicados en revistas de alto impacto (Q1).
    - **1ª publicación**: Se realizó una evaluación físico-química y nutricional del fruto del jínjol, así como de los compuestos volátiles y de la actividad antioxidante. Se ha estudiado por separado el contenido de fenoles y actividad antioxidante total en la piel y en la pulpa. Este artículo fue publicado por la revista *LWT- Food Science and Technology (Q1).*
    - **2ª publicación:** Consistió en un estudio comparativo del cultivo del jínjol mediante un manejo orgánico y otro convencional, analizando para ello las propiedades físicoquímicas y funcionales del fruto. Se publicó en la revista *LWT- Food Science and Technology (Q1).*
    - **3ª publicación:** Se realizó un estudio genético de las hojas de 5 cultivares españoles de jinjolero mediante marcadores ISSR, para conocer las relaciones filogenéticas entre los cultivares, además de una breve caracterización del fruto de cada cultivar en el estado óptimo de maduración. Se publicó en la revista *Scientia Horticulturae (Q1).*

- **4ª publicación:** Se realizó el perfil de los ácidos grasos contenidos en el fruto en la piel y la pulpa por separado. Se identificó y cuantificó cada ácido graso encontrado. Se publicó con la revista *Food Chemistry (Q1)*.
- **5ª publicación:** Se realizó un estudio de la capacidad de conservación del jínjol en atmósfera modificada durante 49 días, analizando la calidad de los jínjoles durante el periodo de conservación. Se publicó en la revista *Scientia Horticulturae (Q1)*.

5. **Resultados, discusión y conclusiones**. En este apartado se destacan los resultados obtenidos más relevantes en la investigación, así como la discusión con otros artículos de jínjoles de diferentes orígenes y las conclusiones específicas de cada artículo.
6. **Conclusiones generales e investigaciones futuras**. Se muestran cuáles fueron las conclusiones generales de la tesis. Después se muestran cuáles son las posibles vías de investigación del jinjolero en el futuro.
7. **Referencias.** Son los estudios de diferentes autores que se han consultado para la redacción de esta Tesis Doctoral sin tener en cuenta las referencias de los artículos.

RESUMEN/ ABSTRACT

RESUMEN/ABSTRACT

Resumen

Los jínjoles son frutos muy consumidos en China donde, desde la antigüedad, utilizan el fruto, la semilla y la hoja, tanto en alimentación como en medicina, por sus propiedades nutritivas y funcionales, disminuyendo así el riesgo de padecer algunas enfermedades.

Son frutos que varían sus características físico-químicas y funcionales dependiendo del cultivar. Dado que los cultivares españoles no han sido prácticamente estudiados, en esta Tesis Doctoral se evalúan las propiedades físico-químicas de los frutos, piel y pulpa, sus fenoles totales y actividades antioxidantes totales, así como el perfil de ácidos grasos de cinco cultivares españoles cultivados mediante un manejo orgánico y no orgánico. El cultivo del azufaifo en manejo ecológico hace que éstos sean de mejor calidad y más saludables, aunque más pequeños que los cultivados tradicionalmente.

También se ha hecho el perfil genético de cinco cultivares por ISSR. Este trabajo permitió diferenciar claramente tres grupos de jínjoles: por un lado, el cultivar 'Da' que fue el más diferente y alejado de todos, los cultivares 'Gab' y 'Gam' que fueron cercanos ente sí, y, entre estos dos grupos estarían los cultivares 'Me' y 'Pe'.

Finalmente, se ha hecho un estudio de conservación de estos frutos en atmósferas modificadas. Los jínjoles, una vez recolectados y almacenados en MAP, muestran mejores características y una mayor vida útil más del doble de tiempo, que los frutos almacenados sin ninguna película protectora.

Abstract

Jujubes are highly consumed fruits in China where, since ancient times, they use the fruit, the seed and the leaf, both in food and medicine, for their nutritional and functional properties. Thus reducing the risk of suffering from some diseases.

They are fruits that vary their physical-chemical and functional characteristics depending on the cultivar., Since Spanish cultivars have not been practically studied, this Doctoral Thesis evaluates the physico-chemical properties of the fruits, peel and pulp, their total phenols and antioxidant activities as well as the fatty acid profile of five Spanish cultivars grown under organic and non-organic management. The cultivation of jujube in ecological management makes them of better quality and healthier, although smaller than those traditionally cultivated.

The genetic profile of five cultivars by ISSR has also been done. This work allowed to clearly differentiate three groups of jujube: on the one hand, the cultivar 'Da' that was the most different and far from all, the cultivars 'Gab' and 'Gam' that were close to each other, and, between these two groups would be the cultivars 'Me' and 'Pe'.

Finally, a conservation study has been made of these fruits in modified atmospheres. The jujube, once collected and stored in MAP, show better characteristics and a longer shelf life more than twice as long as the fruits stored without any protective film.

1. INTRODUCCIÓN

1. INTRODUCCIÓN

El jinjolero es un frutal que presenta unas excelentes expectativas de cultivo debido: i) a que presenta unas altas propiedades nutricionales, ii) a que presenta unas excepcionales cualidades como alimento por sus propiedades medicinales iii) a que este cultivo es resistente a temperaturas extremas y tener pocos requerimientos hídricos por lo que es posible su cultivo en zonas semiáridas. Por ello, el conocimiento del material vegetal y la selección de nuevos individuos capaces de dar abundantes cosechas y frutos de calidad, unido al descubrimiento en occidente de sus numerosas propiedades nutricionales, farmacológicas, funcionales y de manufacturas, ha hecho que este frutal sea cada día más demandado por los consumidores. La investigación del jinjolero se ha centrado en variedades asiáticas, sin embargo, las variedades españolas no han sido prácticamente estudiadas al ser un cultivo menor en nuestro país.

Esta tesis doctoral se enmarca dentro de la línea de investigación que desde hace años vienen realizando varias investigadoras de las áreas de investigación de Fisiología Vegetal y Producción Vegetal de la Escuela Politécnica Superior de Orihuela de la Universidad Miguel Hernández sobre cultivos menores, y dentro de ellos el jinjolero. Dentro de las investigaciones de este grupo, esta tesis se enmarca específicamente en el proyecto de investigación titulado "Prospección, caracterización fisico-quimica, fisiológica, compuestos bioactivos y perfil genético de dos especies de cultivos "menores": jínjol (*Ziziphus jujuba* Mill.) y alcaparra (*Capparis spinosa* L.)", de la Consellería de Educación, Investigación, Cultura y Deporte de la Generalitat Valenciana, referencia AICO/2016/015.

El objetivo principal de la presente tesis doctoral ha sido estudiar los jínjoles de variedades españolas, realizar una caracterización físico-química y funcional de 14 cultivares diferentes de jínjol cultivados en el sureste español, en el fruto entero y por separado en piel y en pulpa, además del cultivo en ecológico y en convencional, hacer un estudio genético de diversas variedades españolas de jínjol para conocer su parentesco y, finalmente, hacer un estudio de su conservación postrecolección con técnica de atmósfera modificada. La finalidad ha sido conocer en profundidad estos cultivares y así poder seleccionar, para transferir al sector agroalimentario, aquellas variedades que mejores cualidades presenten para su producción.

Los objetivos específicos planteados y los resultados obtenidos durante la realización de esta tesis doctoral han dado lugar a las cinco publicaciones científicas que se exponen a continuación:

I. La primera publicación recoge los resultados obtenidos de evaluar las propiedades físicas y bioquímicas, de la piel y la pulpa de los jínjoles, así como las propiedades funcionales de 3 cultivares diferentes de jínjol. Este es el primer estudio que refleja la capacidad antioxidante de jínjoles en la piel y en la pulpa por separado. Este trabajo está publicado en la revista *LWT-Food Science and Technology*.

Reche, J., Hernández, F., Almansa, M. S., Carbonell-Barrachina, Á. A., Legua, P., & Amorós, A. (2018). Physicochemical and nutritional composition, volatile profile and antioxidant activity differences in Spanish jujube fruits. *LWT-Food Science and Technology*, 98, 1-8. doi: 10.1016/j.lwt.2018.08.023

II. La segunda publicación consistió en un estudio comparativo del cultivo del jínjol mediante un manejo orgánico y otro convencional, analizando para ello las propiedades físico químicas y funcionales de los jínjoles. Se publicó en la revista *LWT- Food Science and Technology*.

Reche, J., Hernández, F., Almansa, M. S., Carbonell-Barrachina, Á. A., Legua, P., & Amorós, A. (2019). Effects of organic and conventional farming on the physicochemical and functional properties of jujube fruit. *LWT- Food Science and Technology*, 99, 438-444. doi: 10.1016/j.lwt.2018.10.012

III. La tercera publicación consistió en la realización de un estudio genético de las hojas de 5 cultivares españoles de jinjolero mediante marcadores ISSR, para conocer las relaciones filogenéticas entre los cultivares, además de una breve caracterización del fruto de cada cultivar en el estado óptimo de maduración. Se publicó en la revista *Scientia Horticulturae*.

Reche, J., García-Martínez, S., Carbonell, P., Almansa, M. S., Hernández, F., Legua, P., & Amorós, A. (2019). Relationships between physico-chemical and functional parameters and genetic analysis with ISSR markers in Spanish jujubes (*Ziziphus jujuba* Mill.) cultivars. *Scientia Horticulturae*, 253, 390-398. doi: 10.1016/j.scienta.2019.04.068

IV. La cuarta publicación consistió en la realización del perfil de los ácidos grasos contenidos en los jínjoles en la piel y la pulpa por separado. Se identificó y cuantificó cada ácido graso encontrado. Se publicó con la revista *Food Chemistry*.

Reche, J., Almansa, M. S., Hernández, F., Carbonell-Barrachina, Á. A., Legua, P., & Amorós, A. (2019). Fatty acid profile of peel and pulp of Spanish jujube (*Ziziphus jujuba* Mill.) fruit. *Food Chemistry*, 295, 247-253. doi.org/10.1016/j.foodchem.2019.05.147

V. La quinta publicación consistió en la realización de un estudio de la capacidad de conservación del jínjol en atmósfera modificada durante 47 días, analizando sus propiedades funcionales durante el periodo de conservación Se publicó en la revista *Scientia Horticulturae*.

Reche, J., García-Pastor, M. E., Valero, D., Hernández, F., Almansa, M. S., Legua, P., & Amorós, A. (2019). Effect of modified atmosphere packaging on the physiological and functional characteristics of Spanish jujubes (*Ziziphus jujuba* Mill.) cv 'Phoenix' during cold storage. *Scientia Horticulturae*, 258, 108743. doi.org10.1016/j.scienta.2019.108743

1.1.Origen y distribución

Los jínjoles pertenecen al género *Ziziphus* tourn. Ex. L. *Ziziphus*, dentro de la familia de las Rhamnaceae.

El nombre ziziphus se relaciona con el antiguo persa “zizfum o zizafun”, el griego “zizifon” o la palabra árabe “zizoufo” que también se utiliza para *Ziziphus lotus.*

Existen numerosos tipos de jínjoles asilvestrados y que se destinan a diferentes usos como *Z. nummularia* (Burm.) Wight & Arn. y *Z. spina-christi* (L.), para leña en zonas áridas y semiáridas (Adams et al., 1978) y otras para miel, forraje y en protección ambiental (Von Maydell, 1986).

Por otro lado, destacan dos grandes jínjoles domesticados, y que han sido cultivados a lo largo del continente asiático a diferentes escalas; *Ziziphus mauritiana* Lam. de India, denominado azufaifa o ber y *Ziziphus jujuba Mill.*, de China, denominado jinjolero o azufaifo o azufaifo chino, que es el más conocido, y su fruto es el más común y objeto de esta tesis. Éstos han despertado en los últimos años cierto interés en su cultivo e investigación.

El origen del jínjol se encuentra en el continente asiático, en la zona templada, principalmente en China (Vavilov, 1951). El área donde se domesticó *Ziziphus jujuba* posiblemente sea en la llanura del rio Huailhe, en el área del Rio Amarillo. Prueba de ello son las grandes zonas cultivadas en la provincia de Shandong, aunque Shaanxi y Shanxi son consideradas las originarias del fruto (Figura 1) (Chen et al., 2017). Hace más de 2000 años, en la Edad del Bronce, se hablaba del jínjol en poemas y canciones (Qu, 1983). Se tiene constancia de que la primera descripción del azufaifo fue en la Poesía Clásica (1046-771 a.C.). Según un libro de canciones del año 1.000 a.C., en un poema muy famoso, el azufaifo se cultivaba asociado al arroz desde hace más de 4.000 años (Qu, 1983).

Figura 1: Principales áreas de cultivo en China. Shaanxi y Shanxi se consideran las zonas originarias del jínjol (Chen et al., 2017).

El jínjol cuenta con una larga historia en China y se tiene registro de los usos del árbol, de sus frutos, hojas, semillas y raíces, en un antiguo libro sobre hierbas medicinales, "Shennnong Bencao Jing" (300 a.C.-200 d.C.), que consideró el jínjol como una de las hierbas medicinales superiores que prolonga la vida mediante la mejora del sueño, su poder nutritivo, regulando el sistema sanguíneo y el sistema digestivo (Chen et al., 2013). Otro libro posterior de medicina herbal, "Huangdi Neijing" (475-221 a.C.), lo describió como una de las cinco frutas más valiosas de China.

La expansión del cultivo se relaciona con el transporte de antiguas rutas comerciales y los imperios mongoles. Kazvini escribió sobre la existencia de excelentes frutos de jínjoles de los califas abasíes en la provincia de Jurjan, cerca de Persia, que florecían dos veces al año cuando los árboles tenían entre 2-3 años (Le Strange, 1905). Según De Candolle (1883) en el siglo VII este árbol fue tomado por Plinio y llevado a Roma desde Oriente Medio, para extenderlo en la costa este de Europa y el norte de

África (Brizicky, 1964). Así, el cultivo se extendió al Mediterráneo y según Vavilov (1951) también al Oriente, Corea y Japón.

Ziziphus jujuba se introdujo en más de 30 países, ya que es un cultivo muy adaptado a zonas subtropicales y templadas con clima cálido y seco en la etapa de crecimiento y fresco en su latencia, tolerando bajas temperaturas. Cada día aumenta su popularidad, por su fácil manejo, por sus múltiples usos y por el poder nutritivo y funcional del fruto (Lin et al., 2003). Actualmente, sólo en Beijing, Hebei, Sahndong, Shanxi y Henan, se cultivan más de 40 cultivares (Gao et al., 1999).

La ciudad de Geshi, en Ningyang, produce más de 30 millones de kg. Ha sido nombrada como "Base industrial del Azufaifo" por el ministerio de Silvicultura. Tienen un certificado nacional de indicación geográfica. El Centro de Desarrollo de Alimentos de China lo clasificó, en 2008, como un producto nacional de grado A de alimentos verdes.

Imagen 1: Imágenes de "El Jardín del Azufaifo de la Buena suerte" en Geshi (China).

Allí existe "El Jardín del Azufaifo de la Buena Suerte", como se puede ver en la Imagen 1. Cuenta con más de 10 km de caminos y árboles de azufaifo de diferentes

cultivares. Es un lugar donde confluye la agroecología con un parque geológico, fomentando el ecoturismo del jínjol y la mejora de la economía local agrícola (Imagen 2).

Imagen 2: Recolección local de jínjoles en Xianjang.

1.2.Taxonomía

Existen oficialmente 86 especies dentro del género (Evreinoff, 1964; Johnston, 1972), pero hay otros autores que indican que puede haber hasta 170 especies (Liu & Cheng, 1995). Existe cierta confusión con los nombres pues son reducidos por sinonimia y los autores utilizan los mismos nombres para nombrar a diferentes especies que pueden ser originarias de diferentes áreas geográficas. La hibridación es otra cuestión a tener en cuenta para comprender la compleja taxonomía de las especies de *Ziziphus*.

A continuación, se describe la clasificación sistemática del jinjolero (Trópicos, 2015; The Plant List, 2015):

- Clase: Equisetopsida C. Agardh.
- Subclase: Magnolidae Novák ex. Takht.
- Superorden: Rosanae Takht.
- Orden: Rosales Bercht. & J. Presl.
- Familia: Rhamnaceae Juss.

- Género: *Ziziphus* Mill.
- Especie: *Ziziphus jujuba* Mill.
- Sinónimos: *Z. sativa* Gaertn., *Z. vulgaris* Lam., *Z. flexuosa* Wall., *Z. nitida* Roxb., *Z. sinensis* Lam., *Z. zizyphus* (L.) Karst., *Z. mairei* Dode, *Z. officinarum* Med., *Z. chinensis* D.C., *Z. chinensis* Watt.
- Nombre común: jinjolero, azufaifo, azofaifo, azufaifa, azofeifa.

La mayoría de los cultivares provienen de poblaciones heterogéneas, que han sido propagados de forma espontánea o por esqueje y han estado protegidos por la población local. Se contabilizan hasta 400 cultivares distintos del azufaifo chino (Hayes, 1945) y 700 cultivares según Qu & Wang (1993). Estos se pueden dividir en los silvestres que son amargos y se utilizan como portainjertos, para forrajes en alimentación animal, medicamentos, y los que se cultivan para su consumo por el sabor del fruto (Cinimata, 1996). Esta diversidad y propagación se debe a la rusticidad, a la capacidad de retoñar y a la gran adaptabilidad que tiene el árbol (Azam-Ali et al., 2006).

Estas variaciones se deben a la polinización cruzada de los jínjoles, que se refleja en las características vegetativas, en la hoja, flores y fruto, además de la diversidad en la calidad y en las propiedades fitoquímicas del fruto (Shobha et al., 2001).

Pueden ser árboles (Imagen 3) o arbustos caducifolios con ramas muy ramificadas, rígidas y flexibles, y ramillas colgantes de color verde que hacen un zig-zag y son espinosas. Las hojas forman espigas y son alternas, tienen forma de oblonga a oval con bordes un poco dentados. Las flores axilares son pocas y las de la cima son más grandes. Se disponen en grupos y son de color amarillo-verdoso de unos 3-4 mm. Tienen un disco lobulado y con 2 estilos. Florecen de julio a agosto. La fruta es una drupa globosa y comestible, con un tamaño que oscila entre el de una aceituna al de una manzana. Son carnosos, de pulpa harinosa y dulces, pero ligeramente ácidos, conforme avanza la maduración la carne se vuelve más acorchada. El color de la piel del fruto va cambiando a rojizo conforme aumenta su grado de maduración desde mitad del mes de agosto a octubre en el hemisferio norte.

Imagen 3: Árbol de jinjolero en cultivo ecológico.

Se multiplican por semillas y esquejes, y también se injerta el cultivar.

Son resistentes a bajas temperaturas en invierno y a altas en verano y aptos para su producción en zonas desérticas (Mizrahi et al., 2002). Puede florecer en junio o en septiembre.

Tabla 1: Diferentes nombres por los que se conoce el jínjol en cada país.

CONTINENTE	PAÍS	NOMBRE
ASIA	Afghanistan	Berra
	Bangladesh	Bozoi, Kool, Kul
	Camboya	Putrea
	China	Hong tsao, Lang tsao, Tsao Tse
	India	Bogori, Kul, Ber, Bor, Bordi, Boyed, Ber Beri, Badari, Baer, Bogari, Bore, Egsi, Elasi, Ilanji, Ilisi, Jelachi, Karkhamdhu, Yolachi, Bhor, Baher, Bardi,Badaram, Badari, Koilam, Lantu, Elanda, Perintutati, Unals, Ajapriya, Balastha, Dridhabija, Dviparmi, Ghonta, Gudaphala, Kantaki, Karkaramadhu, Koli, Kuvali, Madhuraphala, Madadebara, Nakhi, Nripabadari, Nripeshta, Prithukoli, Phalashayasshira, Rajabadari, Rajakoli, Rajavallabha, Sukshmaphala, Skshmapatrika, Srigalakoli, Svachha, Sukorapriya, Suphala, Tanubija, Ubhayakantaka, Jangri, Adidarum, Attiram, Ilandai, Iradi, Koli, Kandai, kullari, Kulvali, Padari, Sivagam, Badari, Vettiram, Veyam, Elanda, Badaramu, BAdari, Gangaregu, Gangarenu, Karkhanduvu, Regu, Renu, Barholi, Badokoli, Bodori, Koli
	Indonesia	Widara, Dara, Bidaru
	Irán	Kanar, Kunar, Nabik
	Iraq	Aunnaberhindi, Nabid, Sidr
	Japón	Sanebuto-Natsume
	Laos	Than
	Malasia	Bidaru, Epal siam, jujube
	Miamar	Zi, Zee-pen, Zizidaw, Ziben
	Nepal	Baer
	Pakistán	Ber, Berwarter, Kunar
	Filipinas	Manznites, Manzanas

	Sri Lanka	Ilanda, Mahadebara, Masada
	Tailandia	Phutsa, Putsa, Man tan
	Vietnam	Tao, Tao-nhuc
ÁFRICA	Etiopía	Abateri, Gaba-argie, Gewa-ortigi
	Kenia	Mkunazi, Kwkurrah, Ekalati, Olongo, Tolumuro
	Malawi	Masawo, Msondoka
	Somalia	Gob, Bheb, Jujuba
	Sudán	Sidr nabk, Nabbag elfil
	Tanzania	Mkunazi
	Uganda	Esilang
	Zaire	Kankole
	Zambia	Masau, Musawce, Akasongole
	Zimbawe	Masua, Yanja, Musawu
	Este de África y Sahel	Dono, Ntomono,Surgo ntomono, Tomboro, Tomonou, Batenluongu, Bu sakonhionabu, Inakpayuani, Nan janlwane, Magaria, Magunuga, Mugulga, Mugulanga, Muegunga, Mugunuga, Mug-niga, Djabe, Djabi, Tabi, N´giobi, Ngit, Ajzen, Dem, Dim, Sedem
EUROPA	Inglaterra	Jujube, Indian jujube, Indian plum, Indian cherry, Indian date, Chinese jujube, Chinese date, Chinese fig, Cottony jujube
	Francia	Jujubier, Date chinoise
	Grecia	Tzintzola
	Italia	Guiggiolo
	Portugal	Jujubeira, Maceira
	España	Azufaifo, Azufaifa, Yuyuba, jínjol

FAO, 1988; Von Maydell, 1986; Pareek, 2001; Sundararaj y Balasubra-Manyam, 1959.

Existe una gran variabilidad de nombres por los que se conoce al jinjolero según el lugar donde es cultivado (Tabla 1). Los árboles *Ziziphus* tienen un gran número de usos, agroforestal, etnobotánicos (Arndt y Kayser, 2001), aunque el principal es el consumo del fruto. Actualmente se está despertando cierto interés en los usos del jínjol,

existiendo una gran cantidad de artículos que estudian su composición y beneficios en China.

1.3. Importancia económica del cultivo del jínjol

Hoy día, el cultivo del jínjol en Europa no es destacable en la agricultura. En España es considerado marginal y suele crecer de forma espontánea en linderos de parcelas, márgenes de los ríos, o en huertos familiares para autoconsumo. También es utilizado en algunas regiones como planta ornamental en jardinería (Melgarejo y Salazar, 2003). En el sureste peninsular se conocen algunas fincas comerciales de este fruto, pero son tan mínimas que no existe censo alguno por su escasa importancia económica. Se encuentra dentro del grupo llamado "frutales menores" o "frutales subutilizados".

Imagen 4: Puestos de jínjol en el mercado chino.

En cambio, en China, se cultiva con una gran superficie, de 1,5 millones de hectáreas (Yi et al., 2012) y más de 700 variedades diferentes que dan una producción de 400.000 toneladas al año. Se viene utilizando desde hace más de 4.000 años por sus

propiedades medicinales, además de ser una gran fuente alimentaria. La producción ha ido en aumento a lo largo de los años, siendo la producción nacional de 5,62 millones de toneladas (peso seco) en 2017. Esto se debe a un considerable aumento de 3,9 millones de toneladas desde el año 2005, siendo el crecimiento medio de 10,36% durante los siguientes 12 años (每日期货, 2019).

La industria china del jínjol se caracteriza por el autoconsumo y la venta al por menor (Imagen 4), siendo el volumen de exportación relativamente pequeño. Según datos de la aduana de China, las exportaciones en la época de producción estaban por debajo de 10.000 toneladas aproximadamente durante los años de 2009 a 2015, cifra que se vió superada en 2016 con 11.027 toneladas (农民日报, 2019).

Imagen 5: Sistema de secado del fruto de Shanxi Jishan Banzao.

En la última década la producción ha crecido por el aumento de la demanda en el mercado alimentario (Imagen 5) y farmacéutico, ya que tiene innumerables aplicaciones (Yan y Gao, 2002).

1.4. Composición del fruto

El jínjol tiene un alto contenido en antioxidantes, vitamina C, fenoles y polifenoles, como flavonoides, flavonoles, etc. (Gao et al., 2013), siendo un alimento saludable (Imagen 6).

La composición del fruto puede variar según el cultivar, el estado de madurez, el manejo agronómico, la orografía y si es fresco o está procesado. La pulpa del fruto es una fuente nutritiva, rica en fibra, azúcares, ácidos orgánicos, proteínas y vitaminas, además de minerales como potasio, magnesio, fósforo, calcio y zinc.

Imagen 6: Jínjol verde.

1.4.1. Ácidos orgánicos y azúcares

La glucosa y fructosa son los azúcares mayoritarios en el jínjol, junto con la sacarosa, y ramnosa, que son los principales azúcares en azufaifo y también el sorbitol pero en menor contenido. Se han identificado diferentes ácidos orgánicos como el cítrico y succínico, además de ácido málico.

1.4.2. Vitaminas y minerales

El elevado contenido en vitamina C del jínjol ha despertado un gran interés nutraceútico, convirtiéndose en una importante fuente de vitaminas, ya que también destaca por su alto contenido, aunque en menor medida, en vitamina A, vitamina B1 (tiamina) y B2 (riboflavina), niacina y vitamina B6 y vitamina P (bioflavonoide) que mejora la acción de la vitamina C (Baratov et al., 1975). El contenido en vitamina C puede variar entre 192 y 359 mg 100 g^{-1}, vitamina B1 0,04 y 0,08 mg 100 g^{-1} y vitamina B2 0,05 y 0,09 mg/100 g aproximadamente (Li et al., 2007).

El jínjol es una importante fuente de minerales como el magnesio, fósforo, potasio y calcio, que son los que mayor contenido muestran, además de hierro, sodio, zinc y cobre, pero en menores cantidades (Li et al., 2007) (Tabla 2). El mineral más elevado significativamente fue el potasio (7797 mg kg^{-1} ps), que está por encima del contenido en potasio de la granada (2500 mg kg^{-1} ps) (Galindo et al., 2015).

Tabla 2: Composición nutricional del jínjol fresco (USDA Base de datos del Departamento de Agricultura de Estados Unidos, 2011).

Tipo	Nutrientes	Unidades	Contenido (100 g)
Principios inmediatos	agua	(g)	77,86
	energía	(kcal)	79
	proteína	(g)	1,20
	lípidos totales	(g)	0,20
	carbohidratos	(g)	20,23
Minerales	Ca	(mg)	21
	Fe	(mg)	0,48
	Mg	(mg)	10
	P	(mg)	23
	K	(mg)	250
	Na	(mg)	3
	Zn	(mg)	0,05
Vitaminas	vitamina C	C	69
	Vitamina B1	(mg)	0,02
	Vitamina B2	(mg)	0,04
	niacina	(mg)	0,9
	Vitamina B6	(mg)	0,081
	Vitamina A (RAE)	(µg_RAE)	2
	Vitamina A (IU)	(IU)	40

1.4.3. Ácidos grasos

El azufaifo es una fuente de ácidos grasos que son esenciales y saludables para el organismo. Pueden variar según su estado de madurez. Este fruto contiene un gran número de ácidos insaturados (monoinsaturados y poliinsaturados) tanto en la piel como en la pulpa, siendo los más abundantes el ácido oleico y el palmítico (Guil-Guerrero et

al., 2004), además del linoleico y palmitoleico (San y Yildirim, 2010). El fruto es rico en lípidos, especialmente el linoleico (omega-6), que el organismo no es capaz de producir. Se identificaron 33 ácidos grasos en la pulpa–del jínjol, mayoritariamente ácidos monolínicos, y los ácidos palmíticos, que son, en parte, responsables del aroma del fruto (Gusakova et al., 1999).

1.4.4. Compuestos volátiles

El jínjol cuenta con un bajo número de compuestos, entre los que destacan elbenzaldehido, ácido hexanoico, α-felandreno, nonanal, and *p*-cimeno. Y en menor proporción hexanal, β-mirceno, heptanal, limoneno, trans-2-hexenal, octanal, 1-octen-3-ona, 2-heptenal, 6-metil-5-hepten-2-ona, 2-octenal, 6-metil- 1-heptanol y hexadecanoato de metilo (Reche et al., 2018). Estos son indicativos de un bajo olor e intensidad de aroma. Aún así, se han identificado 78 compuestos volátiles donde los ácidos alifáticos y los carbonilos muestran valores de 62,97% y 29,56% del total de los volátiles (Wong et al., 1996). Los compuestos volátiles más abundantes fueron el hexanal con un 45% del área total de compuestos identificados, trans-2-hexenal con un 25% y benzaldehído con un 10%, que son descriptores del sabor a fruta verde y dulce, como la manzana. En cambio, tienen concentraciones más bajas de compuestos volátiles de descriptores sensoriales grasos y Aceitosos (Galindo et al., 2015).

1.4.5. Capacidad antioxidante y compuestos fenólicos

La oxidación es un proceso biológico que se produce en el organismo y libera radicales libres que alteran y dañan las células y sus funciones. Los antioxidantes, en bajas concentraciones, pueden retrasar ese proceso y reducir los daños (Halliwell, 1995). Los antioxidantes de origen natural son más seguros y mejores que los sintéticos (Anagnostopoulou et al., 2006). Una dieta rica en frutas y verduras crea un efecto protector frente a la oxidación (Lampe, 1999; Scalbert y Williamson, 2000).

En los últimos años, se han estudiado las propiedades del jínjol, destacando su alta capacidad antioxidante por la presencia de compuestos fenólicos (Li et el., 2005; Zhang et al., 2010; Sun et al., 2011).

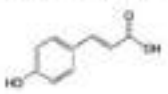
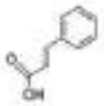
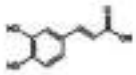
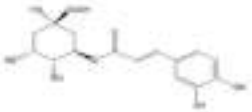
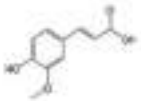
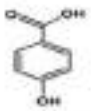
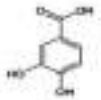
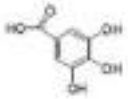
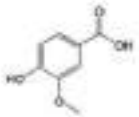
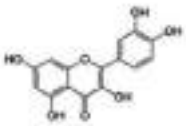
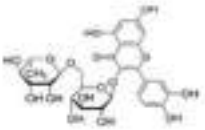
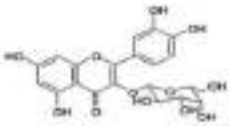
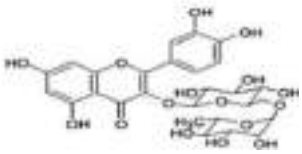
El jínjol tiene una gran cantidad de fenoles totales (275,6-541,8 mg EAG 100 g^{-1} pf que suelen ser medidos mediante el ensayo de Folin-Ciocalteu. Este contenido es mucho mayor que en otras frutas comunes que nos podemos encontrar en el mercado, como el caqui (112,1 mg EAG 100 g^{-1} pf), la uva (80,3 mg EAG 100 g^{-1} pf), la manzana (74,0 mg EAG 100 g^{-1} pf) o la cereza (114,6 mg EAG 100 g^{-1} pf) (Carlsen et al., 2010). Según algunos estudios, el contenido fenólico total puede ser 5-6 veces mayor en la piel que en la pulpa (Xue et al., 2009). La genética, altitud y precipitaciones, son factores que afectan significativamente al contenido fenólico en el azufaifo que, cultivado a elevada altitud y con sequía severa o estrés hídrico, puede producir una mayor cantidad de compuestos fenólicos, mostrando una mayor actividad antioxidante en comparación con los frutos cultivados en otras fincas bajo diferentes condiciones (Sun et al., 2011).

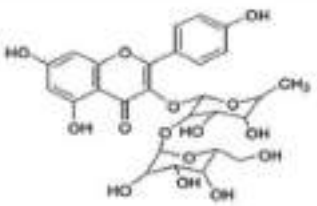
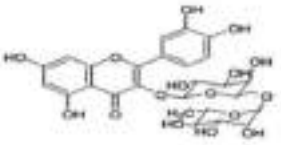
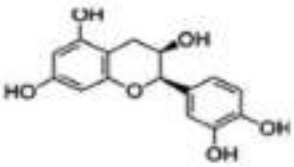
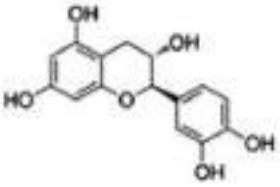
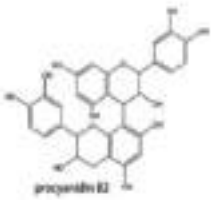
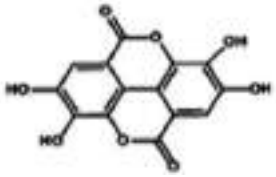
El contenido en ácidos fenólicos en el jínjol se encuentra, principalmente, en forma insolube unida en la piel y semilla, mientras que en la pulpa se encuentra en forma glucosídica, representando un 44,7% en la pulpa, 11,6% en la semilla y un 22,3 % en la piel del fruto (Wang et al., 2011).

Se han encontrado diferentes tipos de flavonoides como flavolones y flavanonas en el fruto de *Ziziphus* (Gao et al., 2012) y que pueden variar según el cultivar y el estado de madurez (Imagen 7) (Guo et al., 2011; Choi et al., 2012). Los flavonoides que se han identificado en jínjoles y su estructura (Choi et al., 2011; Choi et al., 2012; Guo et al., 2011), se pueden ver en la Tabla 3.

Imagen 7: Jínjoles clasificados en 6 estados de madurez diferentes.

Tabla 3: Compuestos fenólicos del fruto de *Ziziphus jujuba*

main class	sub-class	name	chemical structure
phenolic acids	hydroxycinnamic acids	*p*-coumaric acid	
		cinnamic acid	
		caffeic acid	
		chlorogenic acid	
		ferulic acid	
	benzoic acids	*p*-hydroxybenzoic acid	
		protocatechuic acid	
		gallic acid	
		vanillic acid	
flavonoids	flavonols	quercetin	
		rutin	
		quercetin-3-galactoside	
		quercetin-3-rutinoside	

main class	sub-class	name	chemical structure
		kaempferol-glucosyl-rhamnoside	
		quercetin-3-robinobioside	
	Flavan-3-ols	epicatechin	
		catechin	
		procyanidin B2	
ellagic acid			

(Zhang et al., 2010)

1.5.Propiedades funcionales

El jínjol es una fuente alimentaria con una gran cantidad de propiedades funcionales, que contiene el fruto, la hoja, la semilla e incluso la raíz y la corteza del árbol (Figura 2). Se ha utilizado en la medicina tradicional china desde hace 4.000 años, en la que los frutales cobran un importante papel, destacando el uso del jínjol en

diferentes formas procesadas. Se ha utilizado como tratamientos frente a enfermedades como la anorexia, problemas relacionados con el bazo, en estados de fatiga, etc (Guo et al., 2010). También se ha utilizado como anticonceptivo, analgésico, antidiabético, insomino y ansiedad (Erenmemisoglu et al., 1995) y estimulante inmunológico (Benammar et al., 2010).

Figura 2: Propiedades funcionales de *Ziziphus jujube* (Gao et al., 2013).

Tiene propiedades beneficiosas para el cerebro, calmando la mente y mejorando el sueño, cuya actividad se relaciona actualmente con efectos neurobeneficiosos, debido a la neuroprotección frente a la acción neurotrófica, como el insomnio y la depresión (Chen et al., 2013). Los nucleótidos cíclicos y derivados influyen en la regulación de procesos fisiológicos en el organismo, mostrando efectos neuroprotectores (Schmidt et al., 2000 y Urushitani et al., 2000) como el adenosín monofosfato cíclico (AMPc) que muestra en el fruto una elevada cantidad mucho mayor que la de cualquier otra fruta (Cyong y Hunabusa, 1980 y Liu y Wang, 1991). Esto puede servir para tratar enfermedades depresivas o de neurodegeneración, por ello numerosas investigaciones se centran en la extracción de AMPc (Chi y Zhang, 2009). Estas propiedades beneficiosas

para el cerebro también mejoran el aprendizaje (Zhang et al., 2014). Se ha comprobado que el extracto hídrico de jínjol, puede proteger a las neuronas del estrés oxidativo de algunas enfermedades como el Parkinson y el Alzheimer (Lin y Beal, 2006).

Se consume en forma de té para el insomnio por su efecto sedante e hipnótico (Dey, 2013), demostrado por las saponinas y flavonoides (Jiang et al., 2007).

La oxidación es un proceso vital en el organismo para producir energía, pero libera radicales que dañan las células y sus funciones, pudiendo desembocar en enfermedades cardiovasculares, hepáticas e incluso cancerígenas. En numerosos estudios se analiza la actividad antioxidante de frutas y verduras. El jínjol se presenta como un antioxidante natural que puede mejorar el daño oxidativo de estos radicales libres, como hemos dicho en el apartado anterior (Anagnostopoulou et al., 2006), y por lo tanto disminuye el riesgo de padecer ciertas enfermedades. Los componentes bioactivos del azufaifo, como los flavonoides, tienen un efecto neuroprotector contra la oxidación y su prevención (Zhu et al., 2007). Destacan diferentes tipos de flavonoides como kaempferol 3-O- rutinósido, quercetina 3-O-rutinósido que han demostrado tener actividad en la reducción de la disfunción de la memoria (Huang et al., 2007). La espinosina, presente en la semilla, ha mostrado una importante actividad sedante (Cheng et al., 2000).

El estado de madurez del fruto también influye en el organismo, dependiendo si está más inmaduro o maduro, su composición varía modificando sus propiedades. El jínjol fresco inmaduro tiene una alta actividad antioxidante que se refleja en propiedades de protección frente a la oxidación de las células del organismo. En cambio, en el estado maduro tiene un mejor efecto de inhibición neuronal (Chen et al., 2015). Estas diferencias son muy interesantes para usos específicos en diferentes estados de madurez.

1.6. Usos

El azufaifo es una planta con múltiples usos, que se viene utilizando en países asiáticos desde hace miles de años, aunque es muy desconocida en occidente. Por el gran potencial como alimento y como medicina, el jínjol se puede consumir de diferentes formas pues es un suplemento para la salud: fresco o deshidratado, en

extracto hídrico, o en decocción que puede consumirse a diario, sólo o combinado con otros alimentos, en sopa o en polvo para postres en forma de harina, sirope, colorante e incluso para la producción de vino y vinagre. Son muy numerosas las formas de consumo, casi tantos como beneficios aporta, utilizando la flor, el fruto, la cáscara, la semilla, la corteza, la madera y la raíz. La infusión o decocción es la forma más popular y consumida. En la Imagen 8 se pueden ver diferentes tipos de infusiones comerciales de jínjol.

Imagen 8: Diferentes formatos de venta de infusiones de jínjol, que en la caja anuncia sus beneficios para la salud.

El uso histórico del jínjol dentro de la cultura china es como hierba medicinal, que alivia la tensión mental y calma la mente. Se recomienda su uso como una sola hierba o en combinación con otras hierbas para tratar el insominio y el olvido. Además de su uso tradicional medicinal chino para tumores y enfermedades cardiovasculares relacionados con el estrés oxidativo (Zhang et tal., 2010).

Desde época antestral, en China, el jínjol se ha utilizado tanto fresco como secado. Dentro de la medicina herbal china, lo más utilizado es una mezcla de hierbas llamada Fu Fang, que en combinación con otras hierbas tiene efectos beneficiosos para

el organismo. A día de hoy, esta combinación no ha cambiado, y hay miles de preparados herbales más que son muy conocidos y que aparecen escritos por el erudito de la medicina china Zhang Zhongjing (219 a. C - 150 a.C.), de la dinastía Han de China, en el libro recetario Jingui Yaolue. Las más comunes son Gamai Dazao Tang, que contiene regaliz, trigo y jínjol, y era tradicionalmente usada para la ansiedad. Esta mezcla fue una de las mejores 10 fórmulas herbales chinas para tratar el insomnio (Chen et al., 2011). Otra mezcla muy conocida es Chaihu Guizhi Tang que se viene usando para trastornos depresivos (Pang et al., 2008). Estas mezclas herbales tienen como ingrediente principal y esencial el azufaifo, con el objetivo de armonizar el bazo, el estómago, para aumentar las defensas y para calmar la mente (Imagen 9).

Imagen 9: Ganmai Dazao Tang. A. Preparado casero. B. Sobres para infusionar. C. Extracto de la receta ancestral china. Según la caja es "rica en fosfato de hidrógeno, en lisina, aumenta el calcio y aumenta la sabiduría".

El fruto deshidratado se ha utilizado como alimento, por el poder nutricional, como aditivo alimentario y saborizante desde hace miles de años (Li et al., 2007). La forma popular más utilizada en el continente asiático, China, Corea y Japón es en forma seca o semiseca (Li et al., 2009 y Sun et al., 2009). Una vez recolectado, secado al sol (Imagen 10) se comercializa así o en polvo (Kim y Joo, 2005). Este tipo de consumo en seco está aumentando en Asia por el aporte nutricional y funcional. Por ello es importante la investigación del polvo y sus características bioquímicas para la industria (Zhang et al., 2005; Lee et al., 2008; Fang et al., 2009).

Otro consumo habitual del fruto es procesado como pasta o puré, en compota o concentrado como jarabe (Imagen 11), así mejora y mantiene la salud y ayuda en las digestiones (Huang et al., 2008 y Guo et al., 2010).

Generalmente, para el procesado del fruto en bebidas o pasteles, no se suele utilizar la piel del azufaifo, pero es más rica en compuestos fenólicos que la pulpa (Xue et al., 2009), por lo que se podría utilizar como subproducto por ser una fuente natural de antioxidantes naturales. Se utiliza en forma de harina, pasta, siropes, colorantes, gominolas, saborizantes o en crema en la industria hostelera (Imágenes 12 y 13), en forma bebible como zumos, agua, aceite, vino y refrescos (Imagen 14) y en la industria farmaceútica (Imagen 15).

Imagen 10: Proceso del fruto secado al sol en China.

Imagen 11: El jínjol procesado en diferentes formas en hostelería. A. Sirope. B. Pasta. C. Bizcocho de jinjoles. D. Bizcocho hecho con harina de jínjol. E-F. Forma común de servir el jínjol de postre en China.

Imagen 12: A-B Azufaifos secos o deshidratados. C-D-F El fruto en almíbar. Según el envase, "Método comestible: 1. En invierno puede ser una bebida caliente, en verano puede ser una bebida fría. 2. Aplicar la mermelada de azufaifo sobre pan. 3. Agrega yogurt para hacer un

delicioso yogurt de azufaifo. 4. Añadir al vino blanco y hacer vino de azufaifo". E-F. Polvo de azufaifo seco.

Imagen 13: Gominolas y chucherías saludables a base de azufaifo. B. El fruto en polvo. C. Sopa de jínjol. D. Fruto fresco envasado al vacío. E. Bote de jínjol laminado y seco. F. Azufaifo confitado.

Imagen 14: Bebidas y productos líquidos. A. Vino de azufaifo. B. Bebida gaseosa o en zumo de jínjol. C. Zumo elaborado con el jínjol en 3 estados de madurez diferente. D. Bebida del fruto seco. E. Aceite. F. Agua de jínjol para deportistas.

Imagen 15: Productos farmacológicos. A-B. Pastillas y sobres solubles en agua que según la caja aporta grandes benficios al organismo. "Buena absorción, buena digestión, y buena bebida. Cuerpo débil, que suele permanecer despierto toda la noche, personas que son propensas a la fatiga y el mareo. Después del período fisiológico femenino, los enfermos se nutren y los jóvenes crecen. Radioterapia contra el cáncer, suplemento nutricional después de la quimioterapia, cirugía quirúrgica, ajuste nutricional." C. Extracto de jínjol en cápsulas

El aceite de las semillas también es utilizado por su actividad antimicrobiana y para el cremimiento del cabello (Yoon et al., 2010).

La miel del azufaifo (Imagen 16) es muy consumida y apreciada en China por su sabor y aroma, además de no sufrir la cristalización y es muy rica en potasio (Zhou et al., 2013). Se produce principalmente en zonas de Shaanxi, Shanxi y Henan.

Imagen 16: Miel obtenida de las flores del jinjolero. En el bote se puede leer en chino "zaohua Fengmi" que significa "miel de azufaifo".

Además de su consumo destacan otros usos, como la utilización de la madera del árbol para la construcción de barcos, carros, instrumentos musicales y artesanía (Imagen 17), por ser una madera longeva, fuerte pero también flexible. También se usa como forraje animal para ganado caprino, vacuno y camellos (Outlaw et al., 2002).

Imagen 17: La madera del jinjolero (A) es muy apreciada para muebles (B), accesorios (C) e instrumentos musicales (D) .

2. OBJETIVOS

2. OBJETIVOS

El objetivo principal de la presente Tesis Doctoral ha sido la caracterización físico-química y genética de diferentes cultivares de jínjol cultivados en el sureste español, realizada en el fruto entero y por separado en piel y en pulpa, además de estudiar el fruto en cultivo en ecológico y en convencional, y un estudio postrecolección de los jínjoles.

Para alcanzar este objetivo principal se siguieron los siguientes objetivos secundarios:

I. Caracterización morfológica del fruto.
II. Caracterización química, funcional y aromas del fruto.
III. Caracterización funcional de la piel y de la pulpa del fruto.
IV. Caracterización química, funcional y aromas del fruto en cultivo ecológico y en convencional.
V. Identificación de los ácidos grasos en la piel y en la pulpa del jínjol.
VI. Estudio de las relaciones genéticas de diversas variedades con un estudio de marcadores ISSR.
VII. Determinación de la longevidad del fruto en conservación bajo atmósfera modificada.

La finalidad de esta tesis es conocer un cultivo que se considera marginal pero que tiene un gran potencial de cultivo en regiones semi-áridas y seleccionar los mejores cultivares para su uso como consumo en fresco y en la industria alimentaria y farmaceútica.

3. RESUMEN DE LA METODOLOGÍA

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El material vegetal investigado procedió de 4 tipos de variedades diferentes de jinjolero. La variedad el de tamaño 'Grande' fue cultivado en ecológico y en convencional. En la siguiente tabla (Tabla 4) se clasifican los jínjoles estudiados:

Tabla 4: Clasificación de los jínjoles estudiados en la presente tesis.

Tipo	Nombre	Origen	Agricultura
Grande	'GAL-E', 'Gam'	San Isidro (Albatera)	Ecológica
Grande	'GAL-T', 'Gab', 'Grande de Albatera'	San Isidro (Albatera)	Convencional
Mediano	'MSI', 'Me', 'Isidro'	San Isidro (Albatera)	Convencional
Pequeño	'PSI', 'Pe', 'Rate'	San Isidro (Albatera)	Convencional
Dátil	'DAT', 'Da', 'Phoenix'	San Isidro (Albatera)	Convencional

El cultivar **'GAL-E' o 'Gam'** (Imagen 18), llamado así por ser de tamaño grande, fue cultivado en ecológico y procedió de San Isidro de Albatera (latitud 38º10´22.29´´N, longitud 0º51´36.138´´E, a 19 metros sobre el nivel del mar) conun marco de plantación de 4x4. El suelo era de textura limosa, con una baja conductividad eléctrica y un alto contenido en materia orgánica. El agua de riego tenía una conductividad eléctrica de 0.8-1.1 dS m^{-1}. El bio-abonado consistió en 80 unidades de nitrógeno, Bombandier (producto certificado para agricultura ecológica por Schiscert para

Imagen 18: Porte del árbol GAL-E.

el mercado europeo), 90 unidades de potasio del producto Horisul® (autorizado por el CE normativa Nº 834/2007 y CE Nº 889/2008) y 30 unidades de bactoneco, una formulación bacteriana beneficiosa: *Pseudomonas* spp y *Bacillus* spp (usado normalmente en agricultura ecológica y certificado por Sohiscert). No se utilizaron pesticidas ni herbicidas. El agua consumida fue de 4.500 m^3 $ha^{-1.}$

El cultivar '**GAL-T'** (Imagen 19), o 'Grande de Albatera' es la misma variedad que la anterior, cultivada en otra finca comercial de San Isidro de Albatera pero en agricultura convencional. Se recolectaron los frutos de árboles de 21 años podados en vaso con un marco de plantación de 4x4. La fertilización fue de 46 unidades de nitrógeno (UF), 25 unidades de fósforo (UF), 82 unidades de potasio (UF) y 36 unidades de óxido de calcio (UF). El agua de riego aplicada fue de 5.500 m^3 $ha^{-1.}$

En el estudio comparativo del jínjol en agricultura ecológica y convencional, se recolectaron a mano 100 frutos de 4 árboles (25 por árbol), en su punto óptimo de madurez fisiológica e inmediatamente fueron transportados bajo condiciones adecuadas al laboratorio para su posterior análisis (Imagen 20). Una vez allí, se seleccionaron 30 frutos para su análisis físico mientras que los otros 70 se destinaron para los parámetros químicos.

Imagen 19: Frutos de el cultivar GAL-T.

Los cultivares '**MSI',** 'Me' e **'Isidro'** son de tamaño mediano; los cultivares, '**PSI**', **'Pe'** y **'Rate'** son de tamaño pequeño; los cultivares '**DAT**', **'Phoenix'** y '**Da'** (Imagen 21), son similares a la forma de un dátil. Todos ellos pertenecen a diferentes fincas ubicadas en San Isidro, cultivadas en agricultura convencional y en un marco de

plantación de 4x4. Los frutos fueron recolectados de árboles de 20 años de edad y podados en vaso y se recogieron entre las dos últimas semanas de agosto excepto el cultivar 'DAT' 'Da' y 'Phoenix' que fueron recolectados a mediados de octubre. Se recolectaron un total de 100 frutos de 5 árboles diferentes, es decir, 20 frutos por árbol, en su estado óptimo de madurez, con 15º Brix. Para los análisis de ácidos grasos se recolectaron 100 frutos de 4 árboles, lo que supone 25 frutos por árbol.

El estudio de conservación se realizó con 27 lotes de 15 jínjoles cultivar 'Phoenix', de los que 3 se analizaron el mismo día de su recogida y, por otro lado, se almacenaron 12 lotes en las cámaras de refrigeración sin película, denominados control. Los otros 12 lotes se dispusieron en bandejas termoselladas para el tratamiento con MAP. El film estaba compuesto de poliéster (12 µm)-polipropileno (60 µm) (Amcor Flexibles, Barcelona, España) con permeabilidad a O_2 = 75 ml O_2 m^{-2} $día^{-1}$ atm^{-1}, CO_2 = 350 ml CO_2 m^2 $día^{-1}$ atm^{-1} y vapor de agua = 75 mL H_2O mL m^{-2} atm^{-1}. Se almacenaron a 5 °C y 90% HR. Se tomaron tres muestras a los 7, 21, 35 y 49 días después de la recolección.

Para el análisis genético, se recolectaron 40 hojas de 4 árboles diferentes durante la fase vegetativa, lo que supone 10 hojas por árbol. Después de recolectarlas se llevaron al laboratorio y se congelaron a -80º C hasta su análisis.

Imagen 20: Recolección de los jínjoles.

Todos los frutos se transportaron en condiciones óptimas de ventilación, y una vez en el laboratorio, se escogieron 30 frutos para la caracterización física.

El **peso** (g) se midió con una balanza digital (modelo BL-600; Sarotirus, Madrid, España). Se midió el **diámetro equatorial y la altura del fruto** (mm) usando un calibre digital (modelo CD-15 DC; Mitutoyo (Uk) Ltd, Telford, UK). **El color** fue medido en dos puntos diferentes de la superficie del fruto, de acuerdo con la comisión internacional CIELab y expresada con los parámetros L*, a*, b*, chroma y el ángulo Hue, con un espectofotómetro Minolta C-300 Chroma Meter (Minolta Corp., Osaka, Japón) y con el procesador de datos Minolta DP-301. **Los sólidos solubles totales (SST)** se obtuvieron del zumo en fresco mediante un refractómetro (modelo ATAGO N20; Minato-Ku, Tokio, Japón). La **acidez titulable (AT)** se determinó por titulación automática (modelo pH-Matic 23, Crison Instruments, S.A., Barcelona, España) con 0,1 mol L^{-1} de NaOH hasta pH 8,1, en la dilución de 1 mL de zumo de jínjol en 25 ml de agua destilada. Los resultados se expresaron en ácido málico equivalente a 100 g^{-1} pf. **El índice de madurez (IM)** se calculó por la relación SST y AT.

Imagen 21: Fruto del cultivar 'DAT'.

A continuación, se separó la pulpa del hueso para realizar la caracterización química y, en otros casos, también se separó la pulpa de la piel para analizar los componentes bioquímicos por separado y conocer las propiedades de la pulpa y de la piel. El hueso también fue caracterizado fisicamente. Se congelaron a -80ºC hasta su análisis en fresco. Para el análisis de sus propiedades en seco, se procedió a liofilizar la muestra y posteriormente triturarla en un molinillo y conservarla en las condiciones óptimas (Imagen 22).

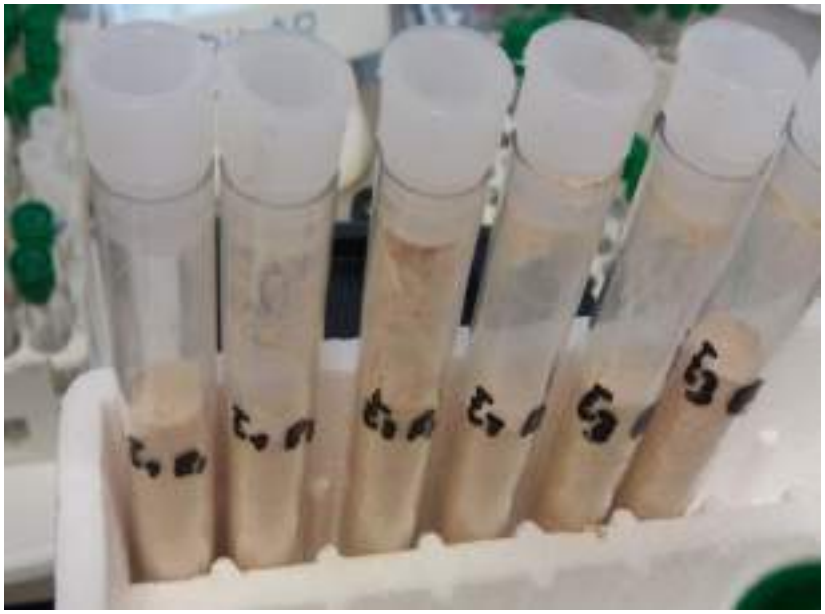

Imagen 22: Muestras de jínjol liofilizadas.

Se estudiaron los contenidos en **clorofilas *a* y *b*,** extraídas de la pulpa, usando acetona al 85% en un relación de 1:2 (p:v) y fueron cuantificadas según AOAC (1990). Cada muestra se trituró en un mortero de mano con arena de mar (Imagen 23) y después fue centrifugada a 12.000 rpm durante 20 minutos a 4 ºC. En un espectofotómetro Helios Gamma (modelo UVG 1002E; Helios, Cambridge, UK) se midió la absorbancia a 664 nm y 647 nm. Los resultados se expresaron en mg $100g^{-1}$ de peso fresco (pf).

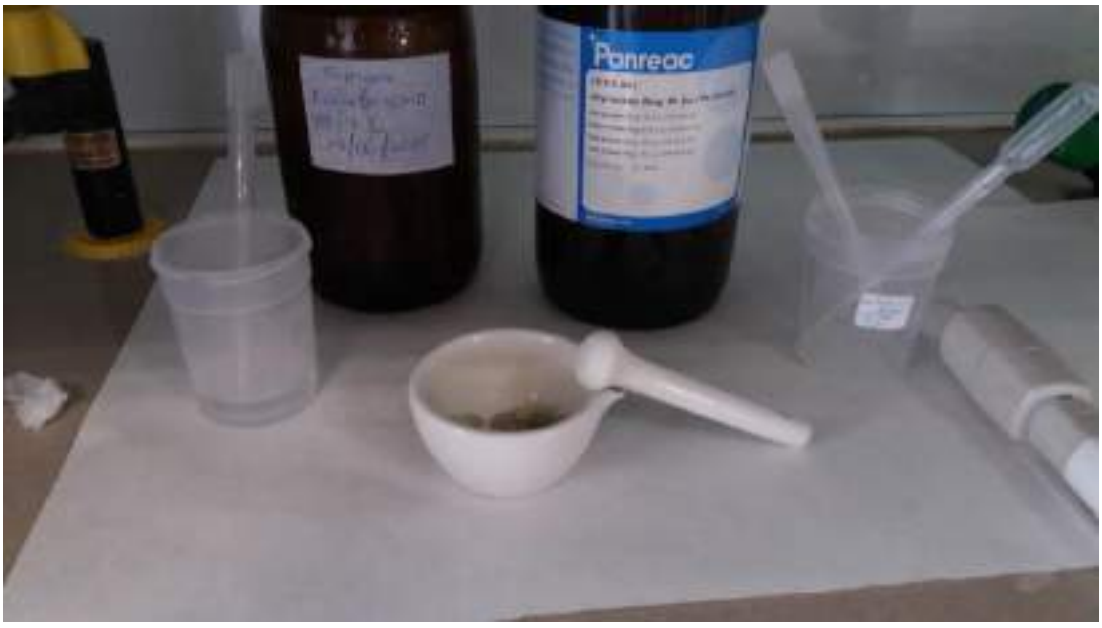

Imagen 23: Extracción de clorofilas mediante mortero de cerámica.

Los **carotenoides** se extrajeron con etil acetato y se midió la absorbancia a 450 nm. Los resultados se expresaron en mg equivalente β-caroteno 100 g^{-1} pf, teniendo en cuenta $\varepsilon^{1\%}_{cm}$ =2560.

Para cuantificar los **azúcares y ácidos orgánicos**, se utilizaron 6 gramos del fruto y se homogeneizaron con 6 mL de tampón Tris-acetato 50 mM a pH 6.0, 10 mM CaCl y 6 mL de etil acetato, se centrifugaron y se separaron las fases. 1 ml de la fase hidrofílica se pasó por un filtro de 0.45 mm y a continuación se identificaron y cuantificaron los azúcares y ácidos orgánicos mediante cromatografía líquida de alta eficacia (HPLC) (Hewlett-Packard HPLC series 1100; Hewlett-Packard, Wilmington, DE, USA) en tampón fosfato al 0.1% con un flujo de 0.5 mL min^{-1}. Se utilizó una columna Supelco (Columna Supelcogel TM C-610 30x7.8 mm) y una precolumna Supelguard (5 cm x 4.6 mm; Supelco, Inc., Bellefonte, PA, USA). La absorbancia fue medida a 210 nm con un detector de diodo-array (DAD). Se usaron estándares obtenidos de Sigma (Poole, Dorset, UK) de azúcares (glucosa, fructosa, sacarosa y sorbitol) y de ácidos orgánicos (L-ascórbico, oxálico, cítrico, málico, succínico, fumárico, tartárico, quínico y shikímico). Se cuantificaron los azúcares y ácidos orgánicos por su tiempo de retención, expresando los resultados en g 100 g^{-1} pf.

Mediante el reactivo Bio-Rad se obtuvo el contenido en **proteínas** del jínjol, por el método de Bradford (1976) (Imagen 24) y con la curva de calibración de suero de albúmina bovino (BSA) se expresaron los resultados en mg 100 g^{-1} pf.

La **materia seca** se expresó en g 100 g^{-1} (ps) y fue determinada mediante secado a 55 ºC hasta peso constante.

El contenido en **minerales** de los jínjoles se obtuvo mediante un espectofotómetro de absorción-emisión atómico (Solaar 969, Uncam Ltd, Cambridge, UK), expresando los resultados en g kg^{-1} (ps) de los macroelementos (Ca, Mg, K y Na) y en mg kg^{-1} (ps) de los microelementos (Fe, Cu, Mn y Zn). En la Imagen 25 se puede observar un detalle del proceso de este método.

Imagen 24: Viales para cuantificar las proteínas.

Los **compuestos volátiles** del jínjol se extrajeron con una microextracción de la fase sólida (HS-SPME), mediante cromatografía de gases (GC-MS) Shimazdu GC-17 (Shimadzu Corporation, Kyoto, Japón) y para la identificación y semicuantificación de los volátiles se utilizó un espectrofotómetro de masas GC-MS QP-5050A con una columna TRACSIL Meta X5 (Tenolroma S. Co. Ltd., Barcelona, España). Los resultados se expresaron en porcentaje del área total representada por cada componente. La identificación se realizó con:

- Índice de retención de los componentes.
- Tiempo de retención GC-MS de los componentes estándar.
- Espectro de masas y base de datos Wiley 229.

Imagen 25: Detalle del proceso de extracción de componentes volátiles y de minerales.

El contenido total en fenoles, flavonoides, flavonoles y actividad antioxidante total (AAT) se realizó en el fruto entero y en la piel y pulpa por separado.

En la fase hidrófila se cuantificaron los **fenoles totales** según Singleton et al., (1999). Se tomaron 25 µl de la fase hidrosoluble y 2,5 mL del reactivo Folin-Ciocalteu, se incubaron durante 120 segundos a temperatura ambiente y después se añadieron 2 mLde carbonato de sodio (75 g L^{-1}) y se agitó. Esta mezcla se incubó durante 5 minutos a 50 ºC y a continuación se midió la absorbancia a una longitud de onda de 760 nm. La recta patrón se hizo con ácido gálico, por lo que los resultados se expresaron en mg EAG 100 g^{-1} pf.

Los **flavonoides y flavonoles** se extrajeron con metanol al 80% y los resultados se expresaron en mg equivalentes de rutina 100 g^{-1} pf. Para los **flavonoides totales** se utilizó $NaNO_2$ al 5%, $AlCl_3$ al 10% y NaOH 1M, y se midió la absorbancia a una longitud de onda de 512 nm en un espectrofotómetro Gamma de Helios (modelo UVG 1002E; Helios, Cambridge, UK). Para expresar los resultados se hizo la recta patrón de rutina que resultó y=4.479 x + 0.06773 con una correlación R^2= 0,9988. Los **flavonoles totales** se midieron a 440 nm usando 2 mg mL^{-1} de $AlCl_3$ y 50 mg mL^{-1} de acetato de sodio. Para expresar los resultados se hizo la recta patrón de rutina que resultó y=3.408 x + 0.0297 con una correlación R^2=0,9901.

La actividad antioxidante total (AAT) se midió en la fase hidrófila (H-AAT) y en la lipófila (L-AAT) (Imagen 26) con el tampón Tris-acetato pH 6.0 y etil acetato para separar las dos fases y así cuantificar la actividad, mediante 10 mM $ABTS^{\bullet+}$, 1 mM de peróxido de hidrógeno y 10 mM de peroxidasa en un volumen total de 1 ml de 50 mM de tampón glicina HCl con un pH 4.5. A continuación, se midió la absorbancia a una longitud de onda de 730 nm, hasta que fuera estable, en un espectrofotómetro Helios UNICAM (Cambridge, UK). Los resultados se expresaron en mg equivalentes de Trolox 100 g^{-1} pf.

Imagen 26: Proceso de separación de las fases hidrófila y lipófila y su posterior preparación para la AAT.

La capacidad de la AAT fue determinada por 3 métodos diferentes $ABTS^{+}$, $DPPH^{\bullet}$ y FRAP.

$ABTS^{\bullet+}$: antes de la generación del radical libre se añaden compuestos antioxidantes y se mide la inhibición de la formación de radicales libres.

$DPPH^{\bullet}$: emplea el radical libre 2,2-difenil-1-pricrihidrazilo por su habilidad para donar un electrón.

FRAP: mide la capacidad para reducir el complejo férrico a ferroso en un pH bajo (Imagen 27).

Se hicieron curvas patrón en el rango de 0.5-5.0 mmol Trolox L^{-1}, para los tres métodos que mostró una buena linearidad (R^2=0,998). Los resultados se expresaron en mmol Trolox kg^{-1} pf.

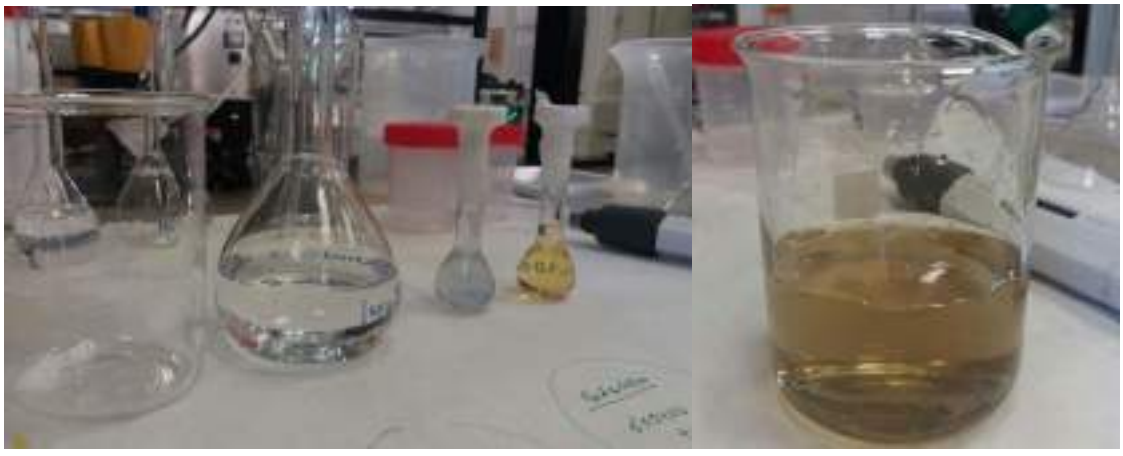

Imagen 27: Reactivos para determinar la actividad antioxidante por el método FRAP.

La extracción de los **ácidos grasos,** de la piel y de la pulpa, se realizó pesando 0.5 g de pulpa o piel liofilizada en un tubo de ensayo, y se agregaron 60 µl de C17:0 en solución de n-hexano, a 20 mg mL^{-1} de solución en HPLC n-hexano como patrón interno. Se añadieron 100 µl de cloruro de metileno y 1 mL de NaOH 0,5 N en metanol. Los tubos se calentaron al baño María a 90 ºC durante 10 minutos, se añadió 1 mL de BF_3 en metanol y se dejaron enfriar a temperatura ambiente (25 ºC) media hora. Después se añadió 1 mL de agua destilada y 600 µl de hexano, y se agitó fuertemente durante 1 minuto para separar los ácidos grasos mediante centrifugado (Imagen 28) y almacenarlos a -20 ºC hasta su cuantificación e identificación en GC-MS con una columna SupraWax-280, 100% polietilglicol (Teknokroma S. Co. Ltd., 165 Barcelona, España; 30 mx 0.25 µm de espesor de película). Para la separación de los ácidos grasos se siguió el siguiente perfil de temperaturas en el cromatógrafo:

- Temperatura inicial de 80 ºC durante 2 minutos.
- Incremento de 8.0 ºC min^{-1} hasta 160 ºC.
- Incremento de 4 °C min^{-1} de 160 a 220 °C durante 13 minutos.
- Incremento de 10 °C min^{-1} de 220 a 260 ° C durante 6 min.

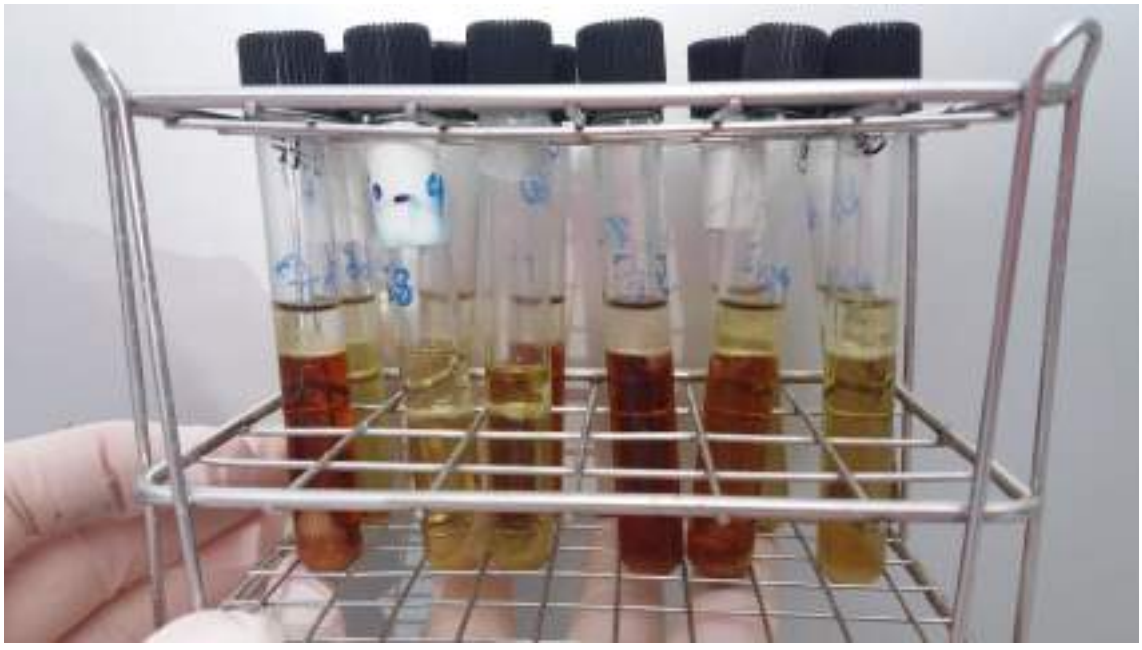

Imagen 28: Ácidos grasos de los jínjoles extraídos para su cuantificación e identificación en GC-MS.

Las temperaturas del inyector y detector fueron de 230 ºC y 260 ºC respectivamente. Se usó helio como gas portador a un caudal de 1,1 mL min^{-1} y se inyectaron 0,5 µl de muestra.

La identificación de los picos se realizó mediante los tiempos de retención de estándares de Sigma-Aldrich y comparando espectros de masas de la biliboteca Wiley 09 MS (Wiley, Nueva York, USA) y NIST14 (Gaithersburg, MD, USA) y la base de datos espectrales. El resultado se expresó en porcentaje total.

Con las hojas jóvenes recogidas del jinjolero (Imagen 29), se realizó **el estudio genético** siguiendo el método CTAB con algunas modificaciones (Doyle y Doyle, 1990). Se extrajo el ADN y se disolvió en agua destilada a una concentración ajustada de 15 mg μl^{-1} con un espectrofotómetro Nanodrop (ThermoFisher Scientific, Waltham, EE.UU.) para proceder a su uso con 6 marcadores del UBC primer set # 9 de la University of British Columbia Biotechnology Laboratory (Vancouver, Canadá), y 12 marcadores del trabajo de Al-Safadi et al. (2014).

Imagen 29: Árbol de jinjolero.

La PCR se realizó a 53 °C con los primers reproducibles y de bandas consistentes. Las reacciones se realizaron en un volumen de 25 µl con 30 ng de plantilla de ADN, 0.5 U TaqDNA polimerasa, dNTP 10 mM, cebador 10 µM en una reacción que contenía tampón Tris-HCl 10 mM (pH 8,3), KCl 50 mM y $MgCl_2$ 2,5 mM. El perfil de temperatura en Eppendorf Mastercycler Gradient (Hamburgo, Alemania) fue:

- Desnaturalización a 94 ºC durante 2 minutos.
- 35 ciclos consistentes en:
    - Desnaturalización a 94 ºC durante 30 segundos:

— Un paso de recorrido a 53 ºC durante 30 segundos.

— Un paso de extensión a 72 ºC durante 60 segundos.

— Pase final a 72 ºC durante 5 minutos.

Las condiciones de electroforesis consistieron en que los productos amplificados se cargaron en gel de agarosa al 1,5% y se separaron con el tampón 1 × tampón TAE a 100 V. Los geles se tiñeron con bromuro de etidio y se visualizaron bajo luz ultravioleta y sistema de análisis de imagen (Vilber Lourmat, Collégien, Francia).

Para analizar los datos los patrones de las bandas se calificaron como presentes o ausentes y sólo se consideraron los fragmentos que eran claros y repetibles para un nuevo análisis genético. El tamaño de la banda fue determinado por el peso molecular del marcador GeneRuler100 bp más ADN Ladder (ThermoFisher Scientific, Waltham, USA). Se calcularon 3 índices MR (Multiplex Ratio), PIC y RP (Poder de Resolución). Las relaciones filogenéticas entre accesiones fueron estimadas de datos de caracterización molecular, se procesaron en el paquete NTSYSpc 2.0 y el dendograma se construyó utilizando el método no ponderado de grupo de pares con promedio aritmético (UPGMA) del análisis de agrupamiento basado en las matrices de coeficientes de similitud genética. La estabilidad estadística de las ramas del cluster fue estimado por el análisis de bootstrap con 1000 réplicas, usando el programaWinboot.

El estudio de conservación de los jínjoles se realizó mediante MAP (Imagen 30). Se cuantificó la **composición del gas** en cada una de las bandejas termoselladas. Las **concentraciones de CO_2 y O_2** se cuantificaron por duplicado, extrayendo con una jeringuilla 1 mL de la atmósfera del espacio superior. Posteriormente, cada muestra se inyectó en un cromatógrafo de gases GC 14B (Shimadzu, Tokio, Japón) equipado con un detector de conductividad térmica (TCD), con las características explicadas en Díaz-Mula et al. (2011a). Los resultados se expresaron como kPa CO_2 y kPa O_2 dentro de las bandejas (n = 6).

Imagen 30: Jínjoles en bandejas termoselladas con atmósfera modificada (MAP).

La **tasa de respiración** y **producción de etileno** de los jínjoles se midió poniendo los frutos en botes de cristal de 750 mL sellados herméticamente con una tapa de goma durante 30 minutos para medir la producción de etileno (Imagen 31). Para la cuantificación de **etileno y CO_2**, se extrajo 1 mL de la atmósfera por cada medición que se inyectó en el cromatógrafo. Los resultados de etileno fueron la media ± SE de las determinaciones para seis réplicas y se expresaron como nL g^{-1} h^{-1}. Los resultados de CO_2 se expresaron como mg kg^{-1} h^{-1} y fueron la media ± SE.

Imagen 31: Botes herméticos para extraer los gases de la respiración de los frutos.

El análisis estadístico fue realizado mediante el programa SPSS 18.0 y 2.0 para Windows (SPSS Science, Chicago, IL, USA). Se realizó una estadística descriptiva básica, y un análisis de la varianza (ANOVA) para comparar medias. Se hizo el Test de Rango Múltiple de Fisher de las diferencias mínimas significativas (LSD) a un nivel de confianza del 95.0%. El análisis de componentes principales y el análisis discriminante se realizó para el contenido en ácidos grasos de los diferentes cultivares, donde cada valor fue la media de 6 repeticiones, 3 por año y 2 por experimento (n=6).

En la estadística del análisis genético se estudió la correlación entre los parámetros en R usando el paquete ´reshape2´v.1.4.3. (Wickham, 2007). El análisis de los componentes principales se realizó para determinar la combinación de atributos de la diversidad fenotípica de la población en R usando el paquete ´FactoMineR´v.1.41.

4. PUBLICACIONES

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4.1. Publicación 1

PUBLICACIÓN 1
Physicochemical and nutritional composition, volatile profile and antioxidant activity differences in Spanish jujube fruits
Juana Reche, Francisca Hernández, María Soledad Almansa, Ángel A. Carbonell-Barrachina, Pilar Legua y Asunción Amorós. ***LWT- Food Science and Technology 2018, 98, 1-8*** ***doi: 10.1016/j.lwt.2018.08.023***

PUBLICACIÓN 1: TRANSCRIPCIÓN LITERAL

Physicochemical and nutritional composition, volatile profile y antioxidant activity differences in Spanish jujube fruits.

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Abstract

In Spain, jujube can be considered a minor crop; thus, its cultivars have not yet been investigated in depth. The objective of this study was to evaluate the composition, nutritional profile, and volatile composition of three cultivars of Spanish jujube fruits. The total antioxidant activity and phenolic compounds in peel and pulp of the fruits were studied. The obtained results proved that 'Isidro' cultivar presented the largest fruits, had the highest ascorbic acid content, with a good color intensity, total soluble solid content, and glucose and fructose. In addition, fruits of the 'Isidro' cultivar presented together with the 'Phoenix' ones, the highest protein content and juiciness. The cultivar 'Phoenix' behaved differently than the other two cultivars studied; its fruits had significantly lower contents of total phenols, flavonoids and flavonols and these compounds were mainly accumulated in the pulp. In the other two cultivars, bioactive compounds reached higher values and were mainly accumulated in their peels. 'Isidro' was the cultivar that presented maximum amounts of phenols and total flavonoids. Thus, both cultivars 'Isidro' and 'Phoenix' could be promising cultivars to be used in future breeding programs, to obtain fruit with high content of bioactive compounds as well as interesting organoleptic properties.

Keywords:

Ascorbic acid

Benzaldehyde

Flavonoids

Flavonols

Phenols

1. Introduction

Jujube (*Ziziphus jujuba* Mill.) tree has its origin in China, where it is popular for its high nutritional value and medicinal uses. Different parts of the jujube plant are used as pharmacological agents (Jiang, Huang, Chen & Lin, 2007). It seems that there are significant differences among the contents of bioactive compounds in peel and pulp of the jujube fruit (Xue, Feng, Cao, Cao & Jiang, 2009; Zhang, Jiang, Yue, Ye & Ren, 2010); however, the studies of Xue et al. (2009) and Zhang et al. (2010) were conducted using Chinese jujube cultivars. This same trend has been previously reported in other fruits, such as nectarines, peaches, plums (Tomás-Barberán et al., 2001), orange, banana, and tangerine (Faller & Fialho, 2010).

In Western countries, there is great interest in antioxidant rich diets, ingredients and/or raw materials. There is a current trend towards replacement of chemical additives with "natural" antioxidants (Cheng, Liu, Zhang, Chen& Wang, 2018). Jujubes can be presented as an alternative additive in complex food, due to their high contents in antioxidants, phenolic compounds, potassium, iron, and vitamin C. Despite being a marginal crop in Spain and its low consumption, its demand is increasing (Hernández, Noguera-Artiaga, Burló, Wojdyło, Carbonell-Barrachina & Legua, 2016; Wojdyło, Carbonell-Barrachina, Legua & Hernández, 2016).

However, there is scarce information on the phytochemical composition of Spanish jujubes and the effect of the cultivar factor on key nutritional (Hernández et al., 2016; Wojdyło et al., 2016). Therefore, the objective of this work was to study, for the first time, three cultivars of Spanish jujube and to establish their composition, the nutritional value and volatile profile, as well as the antioxidant capacity of the peel and

the pulp of these fruits. This will be the first study describing the differences in the antioxidant capacity of the peel and pulp from Spanish jujube cultivars.

2. Materials and methods

2.1. Experimental conditions and plant material

The experiment was carried out in a commercial farm with 21-years-old jujube trees (latitude 38º10'22, 29''N x longitude 0º51'36, 138''W, 19 m above sea level) in Albatera (Alicante) from Spain. Trees were trained as a vase and were spaced 4 m × 4 m. The jujube fruits under study belonged to three cultivars: (i) 'Phoenix', (ii) 'Isidro', and (iii) 'Rate' (**Figure 1**). One hundred fruits from four trees (25 fruits per tree) of each cultivar were hand-harvested at commercial maturity (above 15 ºBrix). Fruits were immediately transported, under ventilated conditions, to the laboratory. Thirty fruits were taken for the physical analyses, while the other seventy fruits were used for the analyses of the biochemical parameters.

2.2. Physical parameters

The following physical parameters were measured in 30 fruits: equatorial diameter and fruit length (mm) using a digital caliper (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK); fruit weight (g) was measured using a digital balance (model BL-600; Sartorius, Madrid, Spain). Instrumental color was on the surface of the jujubes at two opposite points of the equatorial zone. Color was assessed according to the *Commission Internationale de l'Eclairage* (CIE*Lab*) and expressed as *L**, *a**, *b**, chroma, and Hue angle, with a spectrophotometer Minolta C-300 Chroma Meter (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor. Then, the pulp was separated from

the stone of each fruit, and in each stone the equatorial diameter, length and weight (g) were measured.

2.3. Chemical and biochemical parameters

All parameters included in this section were measured in triplicate.

Chlorophylls *a* and *b* were extracted from each sample using 85% acetone in a ratio 1:2 (w:v) (AOAC, 1990). The sample was crushed with sea sand in a mortar and, then, centrifuged at 12,000 rpm for 20 minutes at 4°C. Absorbance of the supernatant was read at 664 and 647 nm, using a Helios Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). Results were expressed as mg 100 g^{-1} fresh weight (fw). Total carotenoids were extracted according to Valero et al. (2011), with acetone and diethyl ether; 10% NaCl was used to promote the phases separation. The lipophilic phase was used to estimate the total carotenoids content, by reading the absorbance at 450 nm, and results were expressed as mg of β-carotene equivalent 100 g^{-1} fw, taking into account the $\varepsilon^{1\%}_{cm} = 2560$.

The total soluble solids (TSS) were measured in triplicate using an ATAGO N20 refractometer (Minato-Ku, Tokyo, Japan).

The sugars and organic acids were extracted according to Almansa, Hernández, Legua, Nicolás-Almansa & Amorós (2016). Five grams of jujube fruit were homogenized with 6 ml of 50 mM Tris-acetate buffer pH 6.0, 10 mM CaCl, and 6 mL of ethyl acetate, centrifuged, and, then, the aqueous and organic phases were separated. The aqueous phases was utilized for the identification and quantification of sugar and organic acid profiles, according to Almansa et al. (2016). One mL of the hydrophilic

extract was filtered through a 0.45 mm Millipore filter and, then, used for high-performance liquid chromatography (HPLC) (Hewlett–Packard HPLC series 1100; Hewlett-Packard, Wilmington, DE, USA). The elution buffer consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL min^{-1}. Organic acid was isolated using a Supelco column (Supelcogel TM C-610H column 30 cm x 7.8 mm) and a precolum Supelguard (5 cm x 4.6 mm; Supelco, Inc., Bellefonte, PA, USA), and absorbance was measured at 210 nm using a diode-array detector (DAD). These same HPLC conditions (elution buffer, flow rate, and column) were used for the analysis of sugars. Standards of organic acids (L-ascorbic, oxalic, citric, tartaric, malic, quinic, shikimic, succinic, and fumaric acids) and sugars (glucose, fructose, sucrose, and sorbitol) were obtained from Sigma (Poole, Dorset, UK). Calibration curves were used for the quantification of organic acids, showing good linearity (r^2 = 0.999). Results for both organic acids and sugars were expressed as g 100 g^{-1} fw.

The mineral contents were analyzed using an atomic absorption-emission spectrometer (Solaar 969, Unicam Ltd, Cambridge, UK), according to Hernández et al. (2016). Macro-elements (Ca, Mg, K, and Na) were expressed as g kg^{-1} dry weight (dw) and micro-elements (Fe, Cu, Mn, and Zn) were expressed as mg kg^{-1} dw.

The protein content was analyzed by the Bradford (1976) method using the Bio-Rad reactive. A standard curve of pure bovine serum albumin (BSA) was used for quantification according to Almansa et al. (2016). Results were expressed as mg 100 g^{-1} fw.

The dry matter was determined by drying at 55 ºC until constant weight and was expressed as g 100 g^{-1} fw.

The volatile composition of the jujubes was studied by using headspace solid phase micro-extraction (HS-SPME), according to the methodology previously described by Hernández et al. (2016). A gas chromatograph (GC-MS) Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A was used in the identification and semi-quantification of the volatile compounds of jujube. A TRACSIL Meta X5 column (Telnolroma S. Co. Ltd., Barcelona, Spain) was used. The identification of each volatile compound was reached using three methods: 1) retention index of the problem compound, 2) GC-MS retention time of authentic standard, and 3) mass spectra of authentic chemical and database Wiley 229. Results were expressed as percentage of the total area represented by each compound.

Total phenols, flavonoids and flavonols and total antioxidant activity (TAA) were quantified in both pulp and peel of jujube, separated with a vegetable peeler.

Total phenolic compounds were quantified in the hydrophilic phase according to Singleton, Orthofer & Lamuela-Raventos (1999), and using the Folin–Ciocalteu reagent. Briefly, 25 µL of hydrophilic extracts were mixed with 2.5 mL of Folin–Ciocalteau. The mixture was incubated for 2 min at room temperature and 2 mL of sodium carbonate (75 g L^{-1}) were added and vortexed. Finally, the mixture was incubated at 50ºC for 5 min and the absorbance was measured at 760 nm. A calibration curve was performed with gallic acid and results were expressed as mg GAE 100 g^{-1} fw.

Flavonoids and flavonols were extracted using 80% methanol from the peel and pulp of jujubes following the method of Zhuang (1992). The analysis of total flavonoids (mg rutin equivalents 100 g^{-1} fw) was performed by spectrophotometry after using 5% $NaNO_2$, 10% $AlCl_3$ and 1 M NaOH; absorbance was measured at 512 nm on a Helios

Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). Quantification of total flavonols (mg rutin equivalents 100 g^{-1} fw) was done according to Kumaran, Kutty, Chatterji, Subrayan & Mishra (2007), using $AlCl_3$ (2 mg mL^{-1}) and sodium acetate (50 mg/mL), and absorbance was measured at 440 nm.

For the antioxidant activity determination, a methanol extract was prepared as described by Wojdyło, Oszmiański & Bielicki (2013). The free radical scavenging capacities were determined by three methods, $ABTS^{\bullet+}$ method (Re et al., 1999), $DPPH^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams, Cuvelier & Berset, 1995), and FRAP (ferric reducing antioxidant power) method (Benzie & Strain, 1996). Calibration curves, in the range 0.5–5.0 mmol Trolox L^{-1}, were prepared for all three methods and showed good linearity (R^2= 0.998). All antioxidant capacity analyses were run in triplicate, and results were expressed as mmol Trolox kg^{-1} fw.

The activity of H-TAA (hydrophilic-TAA) and L-TAA (lipophilic-TAA) of jujube fruit (peel or pulp) were determined in the aqueous and organic extracts used for the sugar and organic acid analyses. The reaction mixture contained 10 mM $ABTS^{\bullet+}$, 1 mM hydrogen peroxide, and 10 mM peroxidase in a total volume of 1 mL of 50 mM glycine-HCl buffer (pH 4.5) for H-TAA, or ethyl acetate for L-TAA. The reaction was monitored at 730 nm until a stable absorbance was obtained using a UNICAM Helios spectrophotometer (Cambridge, UK). After that, a suitable amount of jujube fruit extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with Trolox as standard antioxidant for both H-TAA and L-TAA. (Arnao, Cano & Acosta, 2001). The results were expressed as mg Trolox equivalent 100 g^{-1} fw.

2.4. Statistical analysis

Statistical analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance test (ANOVA) for mean comparisons. The method used to discriminate among the means (multiple range test) was Fisher's LSD (Least Significant Difference) procedure at a 95.0% confidence level.

3. Results and discussion

3.1. Dimensions of jujube fruit

Data showed that there was a significant difference in size among cultivars (**Table 1**). The 'Isidro' cultivar had a higher equatorial diameter than the other two cultivars; however, the one with the longest length was 'Phoenix', followed by 'Isidro' and 'Rate'. These differences in the size led to differences in the weight, with the cultivar 'Isidro' (thickest fruit) having the highest value and 'Phoenix' (longest fruit) the lowest one.

The morphology of the stones followed the same trend than that described for the whole fruits (**Table 1**), with the highest equatorial diameter and weight being found in the 'Isidro' samples, and the longest length in the 'Phoenix' one; the 'Rate' fruits and stones had intermediate values of all morphologic parameters. The fruit weight and dimensions, including the edible portion and the non-edible stones, might be influenced by several factors, such a cultivar genotype, also depends on crop load (Gao et al., 2012 b).

The pulp yield of the three cultivars was very similar and ranged between 94.90 % ('Rate') and 96.04 % ('Phoenix'), without significant statistical differences. These final weights, dimensions and pulp yields were within the normality ranges reported previously in other Spanish cultivars 'Jínjoles Grandes' and 'Jínjoles Medianos' (Almansa et al., 2016) and 'Grande de Albatera', 'MSI', 'PSI' and 'Dátil' (Hernández et al., 2016), but also in Korean (Choi, Ahn, Kozukue, Levin & Friedman, 2011), Chinese (Gao et al., 2011; 2012b; Wang et al., 2012) and Ukrainian (Grygorieva, Abrahamová, Karnatowská, Bleha & Brindza, 2014) jujube cultivars.

3.2. Color of jujube fruit

Color is one of the main quality parameters of agricultural products as it determines consumers' acceptance (Pathare, Opara & Al-Said, 2013). The fruits modify their coloration due to the synthesis and degradation of pigments throughout their development and maturation (Almansa et al., 2016; Pék, Helyes & Lugasi, 2010). All jujubes were harvested at their commercial maturity; however, slight changes in the reflection color were observed. The 'Rate' fruits presented a more reddish and darker color, due to their highest values of the green-red coordinate and the lowest of lightness. The other two cultivars, 'Isidro' and 'Phoenix' followed the same trend regarding external color, and had the highest values of lightness, blue-yellow coordinate, chroma, and Hue angle (**Table 2**).

The color parameter values reported here were very similar to those previously reported by other researchers for Spanish cultivars 'Jínjoles Grandes' and 'Jínjoles Medianos' (Almansa et al., 2016), 'Grande de Albatera' (Collado-González et al., 2014; Galindo et al., 2015) and even Chinese jujube cultivars (Wang et al., 2012).

Fruits from the cultivar 'Isidro' had the highest contents of chlorophylls, with the other two cultivars being grouped and with significantly smaller contents; this trend was valid for chlorophyll *a*, chlorophyll *b*, and total chlorophylls (**Figure 2**). All three cultivars presented equivalent contents of total carotenoids (~0.20 mg eq β-carotene 100 g^{-1} fw).

3.3. Biochemical properties of jujube fruit

The 'Rate' cultivar had the lowest value of TSS, with the other two cultivars ('Isidro' and 'Phoenix') having significantly higher and statistically equivalent contents (**Table 3**). The reported values of TSS agreed with those found in Spanish 'Grande de Albatera' (Galindo et al., 2015) and Chinese cultivars (Gao et al., 2011; 2012b; Wu, Gao, Guo, Yu & Wang, 2012).

The trend showed in TSS was corroborated by the sugars profiles, with fruits from the cultivars 'Isidro' and 'Phoenix' having significantly higher contents of glucose, fructose and consequently total sugars, than fruits of the cultivar 'Rate' (**Table 3**). These sugar contents were higher than those found in Spanish cultivars 'Jínjoles Grandes' and 'Jínjoles Medianos' (Almansa et al., 2016) and 'Grande de Albatera', 'MSI', 'PSI' and 'Dátil' (Hernández et al., 2016), and also Chinese cultivars (Gao et al., 2012 a, b; Wu et al., 2012). The predominant sugars were sucrose and fructose, being glucose the less abundant sugar. This trend agreed with the one reported by Hernández et al. (2016). However, Wu et al. (2012) obtained a maximum content of fructose followed by glucose and almost no sucrose was found; while, Gao et al. (2012b) reported a predominance of glucose, with low contents of sucrose and fructose. These differences may be due to the fact that during the development of the jujubes, sucrose degradation

occurs with a parallel increase in the synthesis of glucose and fructose, leading to slightly higher contents of fructose than glucose (Almansa et al., 2016). Therefore, small differences in the jujubes harvest time can lead to relatively high differences in the ratios of these three sugars.

The predominant organic acid was succinic (**Table 3**). Regarding the effect of the factor cultivar, the 'Isidro' fruits had the highest concentration of all identified acids and consequently of the total content; while, 'Phoenix' fruits had the lowest contents. This study showed higher ascorbic acid content, ranging from 0.33 ('Phoenix') to 0.65 g 100 g^{-1} ('Isidro'), as compared to other common fruits which are well-known for their high ascorbic acid content, such as strawberries (0.046 g 100 g^{-1}), oranges (0.031 g 100 g^{-1}) (Roberts & Gordon, 2003), and kiwi fruits (0.029-0.080 g 100 g^{-1}) (Nishiyama et al, 2004). These three jujube cultivars showed higher ascorbic acid values than Spanish, 'Grande de Albatera', 'MSI', 'PSI' and 'Dátil' (Hernández et al., 2016) and Chinese (Gao et al., 2012 b) cultivars. Ascorbic acid is a powerful water soluble antioxidant, which plays an important role in the suppression of free radicals (Zhang et al., 2010). Therefore, it appears that these Spanish jujube cultivars are a good source of vitamin C in the diet.

The total sugars/total acids ratio is very interesting because it gives an idea of the potential fruit taste. The cultivars 'Isidro' and 'Rate' had practically the same value, around 5 (**Table 3**), which indicates that although they were sweet, they presented a moderate sour taste; on the other hand, the sweet taste clearly predominated in the cultivar 'Phoenix', with a ratio of 13.7. These values were similar those reported for Chinese and Spanish jujube cultivars with a similar maturity stage (Wu et al., 2012; Hernández et al., 2016), with the exception of the cultivar 'Phoenix', which had the

highest value reported in the literature up to now and shows the high interest of this cultivar for fresh consumption.

The content of proteins in jujubes ranged from 0.37 ('Rate') to 0.61 mg g^{-1} fw ('Phoenix'), with statistically significant differences between these two cultivars (**Table 4**). These protein contents were within the normal range for Spanish cultivars 'Jínjoles Grandes' and 'Jínjoles Medianos' (Almansa et al., 2016), but were lower than those reported in Chinese (Li, Fan, Ding & Ding, 2007) and Korean (Choi et al., 2012) cultivars.

The 'Rate' fruits had the highest dry matter content (**Table 4**), and can be considered as the less juicy ones. The range of this parameter found in the literature fluctuated between 15.3-25.7 g 100 g^{-1} fw in Spanish cultivars 'Grande de Albatera', 'MSI', 'PSI' and 'Dátil' (Hernández et al., 2016), and Chinese (Li et al., 2007) and Korean jujube fruits (Choi et al., 2012).

Jujubes were a good source of K and Ca (Hernández et al., 2016). Potassium was the predominant mineral with contents of ~ 5 g kg^{-1} dw (**Table 4**). This content was low compared to other Spanish cultivars, but higher than that reported in Chinese ones. The mean contents of Mg and Na were 1.3 and 1.0 g kg^{-1} dw, while those of Zn, Cu, Mn, and Fe were 4.7, 1.6, 3.1, and 11.9 mg kg^{-1} dw, respectively. These contents proved that the three studied jujube cultivars had interesting contents of both macro- and micro-elements.

No significant differences were found in any mineral element among the three cultivars studied, except for the Ca content, which was higher in the ‘Isidro’ cultivar. Several studies showed that Ca is an effective pressure lowering agent (Osborne et al.,

1996); thus, a high Ca content can be beneficial for health. It is important to mention that the Fe content (7.5-17.3 g kg^{-1} dw) was typical of other Spanish cultivars but lower than those of other countries, such as China (Li et al., 2007). This fact can be due to the immobilization of Fe in Mediterranean soils with high pH and low content of organic matter (Hernández et al., 2016).

Eighteen volatile compounds were found in the volatile profile of jujube fruits of the three studied cultivars (**Table 5**). The low number of volatile compounds (18) seemed to indicate that the odor and aroma (perception of volatile compounds outside or inside the mouth, respectively) of these fruits is not one of their most relevant sensory attributes (Galindo et al., 2015). The predominant compound was benzaldehyde, followed by hexanoic acid, α-phellandrene, nonanal, and *p*-cymene. The quantitative analysis showed that 'Rate' jujubes had higher total concentration of volatile compounds that the other two cultivars. This trend was basically linked to the benzaldehyde content, which was about four time higher in the 'Rate' fruits than in the cultivar 'Isidro' and ten times than in 'Phoenix'. But, in general, the volatile profiles of fruits of the cultivars 'Isidro' and 'Rate' were similar between them and different from that of 'Phoenix' fruits. In this way, the most abundant compounds in 'Phoenix' were *trans*-2-hexenal, nonanal, 2-octenal, and benzaldehyde only occupied the fourth position in the abundance order.

3.4. Phenol compounds of jujube fruit

The cultivar factor significantly affected the total phenols content (TPC) in peel and pulp of jujubes (**Table 6**). The content of total phenols was 1.18 and 1.45 times higher in peel than in pulp in cultivars 'Isidro' and 'Rate', respectively; while, in the cultivar 'Phoenix' the content was 1.35 times higher in pulp than in peel. Xue et al.

(2009) reported higher values in peel than pulp, with the only difference that the pulp contents were lower than the current ones. Zhang et al. (2010) reported a similar trend ($TPC_{peel}>TPC_{pulp}$) in two cultivars, but in another cultivar contents were similar ($TPC_{peel}\cong TPC_{pulp}$). This trend is not specific of jujubes and, for instance, Faller & Fialho (2010) found more soluble phenols in peel than in pulp in oranges, bananas, and tangerines, although in apples, papayas and mangos, they found equivalent contents. Therefore, it seems that phenolic compounds accumulate in epidermal plant tissues, because they have a potential role in the protection against ultraviolet rays, also because they act as attractants in the dispersion of fruits, and as defensive chemicals against pathogens and predators (Xue et al., 2009). Polymeric proanthocyanidins were the predominant compounds among the flavan-3-ols phenolics in Spanish jujubes cultivars (Wojdyło et al., 2016). The flavan-3-ols (monomer, dimer, and polymeric proanthocyanidins) represented 89-94% of the total phenolic contents (Collado-González et al., 2013; Wojdyło et al., 2016).

This study showed higher TPC values in jujubes as compared to other fruits, such as persimmon (112 mg GAE 100 g^{-1} fw), pomegranates (147 mg GAE 100 g^{-1} fw), and apples (73.9 mg GAE 100 g^{-1} fw) (Fu et al., 2011). It is known that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested in the form of a diet rich in fruits and vegetables (Tanaka, Kuei & Nagashima, 1998). Therefore, our results have demonstrated the jujubes, and especially their peel, can be considered a good source of phenolic compounds and, therefore, be good for health. It is recommended to consume jujubes with peel, but after proper washing to ensure maximum intake of phenolic compounds.

The total flavonoids content (TFC) was 5 times higher in peel than in pulp in 'Isidro' jujubes (**Table 6**). However, TFC was almost double in the pulp than in peel in 'Rate' and 'Phoenix' fruits. The trend found in these last two cultivars ($TFC_{peel}<TFC_{pulp}$) is new; Zhang et al. (2010) found more flavonoids in peel than in jujube pulp, between 1.27 to 4.7 times higher. The flavonoids found in *Ziziphus jujuba*, such as spinosin and its derivatives, are its main pharmacologically active compounds, and have been proven to be responsible for its sedative properties, protection against NMDA-induced neuronal cell damage, among other effects (Bai, Wang, Liu & Li, 2010).

Total flavonols content (TFoC) were low and not detectable in several cases, especially in fruits of cultivars 'Rate' and 'Phoenix' (**Table 6**). Wojdyło et al. (2016) found that flavonols (6-11 %) were the second major group of the total phenolic compounds in Spanish jujubes cultivars 'Grande de Albatera', 'MSI', 'PSI' and 'Dátil' (only 0-2.5 % in the current study); these same authors reported that quercetin and its derivatives, especially the quercetin-3-*O*-rutinoside, were the predominant flavonols.

3.5. Antioxidant activity of jujube fruit

The total antioxidant activity was quantified in jujubes peel and pulp by three methods, FRAP, $ABTS^{\bullet+}$, and $DPPH^{\bullet}$. In general, the TAA was higher in peel than in pulp, although the differences were not statistically significant in the 'Phoenix' fruits (**Table 7**). These differences between cultivars were also found by other authors in jujubes (Gao et al., 2012b; Choi et al., 2012). Factors such as geographical source, genotype, but mainly maturity stage at harvest may account for the observed divergence. Besides, the differences between peel and pulp values were higher in the FRAP method, implying that this method is better suited to this specific fruit. Xue et al.

(2009) and Zhang et al. (2010) also observed significantly higher FRAP values in peel than in pulp, but their differences were smaller (1.4-3.9 higher in peel than in pulp) as compared to results of the current experiment (12.2-40.2 higher in peel than in pulp).

The $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ methods showed lower values in peel activity but higher in that of pulp, as compared to results of the FRAP method in 'Isidro' and 'Rate' cultivars, and again no differences were found in the 'Phoenix' cultivar (**Table 7**). The variation of antioxidant capacity between peel and pulp extracts may be due to differences in antioxidant compounds and their effectiveness (Robards, Prenzler, Tucker, Swatsitang & Glover, 1999). The jujube peel of fruits of the cultivar 'Rate' showed the highest values of FRAP and L-TAA.

In the three studied cultivars, the peel had higher L-TAA than the pulp (**Table 7**), and showed significant differences among cultivars. The H-TAA activity was very high in both peel and pulp in the three cultivars, being in most of the cases significantly higher in the pulp than in the peel.

It is interesting to note that the cultivar 'Phoenix' presented significantly lower TPC contents, both in peel and pulp, than in the other two cultivars, leading to lower H-TAA and L-TAA, in peel and pulp, and, lower FRAP, $ABTS^{\bullet+}$, and $DPPH^{\bullet}$ activities.

A significant positive correlation was obtained among TPC and H-TAA in both peel and pulp (R^2=0.8595), while between TPC and L-TAA this correlation was only significant for the pulp (R^2=0.873). These positive correlations seemed to indicate that phenols are soluble in water, and that different compounds should be responsible for the antioxidant activity of the lipophilic fraction.

4. Conclusion

Physico-chemical and bioactive compounds in three cultivars of Spanish jujubes were compared. Due to the high antioxidant properties and the presence of important bioactive compounds in these fruits, it is considered that their cultivation has a great interest. The high resistance of this crop to arid conditions existing in the Mediterranean region of Spain, suggested it can be a useful crop in the Mediterranean countries and the cultivars studied can be promising for cultivar selection. The only advantage of the cultivar 'Rate' was that had the highest amount on volatile compounds. The cultivar 'Isidro' had the biggest and heaviest fruits, and together with 'Phoenix' fruits had the better color characteristics, as well as the highest contents of sugars, organic acids, proteins, and moisture. In this study, the content of H-TAA, L-TAA, FRAP, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, total phenols, total flavonoids, and total flavonols was studied for the first time in the peel and pulp of these three jujube cultivars. It can be concluded that the cultivar ‘Isidro’ can be considered as the most interesting one, and have a huge potential for its fresh consumption, besides the antioxidant activity, total phenols and total flavonoids were higher generally in peel than in pulp; thus, it is recommended to eat this fruit without peeling. Thus, both cultivars 'Isidro' and 'Phoenix' could be promising cultivars to be used in future breeding programs, to obtain fruit with high content of bioactive compounds as well as attractive organoleptic properties.

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TABLES

Table 1. Morphology of jujube fruits as affected by cultivar.

Cultivar	Fruit			Stone		
	Weight (g)	**Equatorial diameter (mm)**	**Length (mm)**	**Weight (g)**	**Equatorial diameter (mm)**	**Length (mm)**
'**Isidro**'	12.7±0.5 b[†]	29.08±0.38 b	29.55±0.36 b	0.62±0.02 c	9.21±0.13 c	17.80±0.27 a
'**Rate**'	10.4±2.5 ab	22.98±0.22 a	28.43±0.20 a	0.53±0.01 b	8.12±0.08 b	19.55±0.27 b
'**Phoenix**'	9.1±0.3 a	23.90±0.49 a	39.59±0.51 c	0.36±0.02 a	6.81±0.10 a	26.97±0.36 c

[†] *Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) ($n=30$).*

Table 2. External color of jujube fruits as affected by cultivar.

Cultivar	*L**	*a**	*b**	*C*	H^o
'**Isidro**'	71.82±0.37 b†	-0.36±1.01 a	34.48±1.02 b	35.30±0.33 b	89.40±2.76 b
'**Rate**'	63.54±0.63 a	3.49±0.41 b	30.94±0.51 a	31.23±0.47 a	83.27±0.84 a
'**Phoenix**'	72.10±0.32 b	-0.20±0.21 a	34.86 ± 0.19 b	34.94 ± 0.19 b	90.29 ± 0.42 b

†*Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) (n=60). L*, lightness; a*, green/red coordinate; b*, blue/yellow coordinate; C, chroma; H°, hue angle.*

Table 3. Total soluble solids, TSS (ºBrix), sugars (g 100 g^{-1} fw) and organic acids (g 100 g^{-1} fw) profiles of jujube fruits as affected by cultivar.

Cultivar	TSS	Sucrose	Glucose	Fructose	Total sugars	Citric acid	Malic acid	Ascorbic acid	Succinic acid	Total acids	Sugars/acids
	(ºBrix)	(g 100 g^{-1} fw)									
'**Isidro**'	22.40±0.81 b†	8.84 ± 0.31 a	6.06 ± 0.25 b	8.40 ±0.33 b	23.30 ± 0.98 b	0.95 ± 0.02 c	0.13 ±0.02 a	0.65 ±0.01 c	2.87 ± 0.08 c	4.60 ± 0.13 c	5.06
'**Rate**'	17.73±0.18 a	7.38 ± 0.33 a	4.81 ± 0.14 a	6.76 ± 0.28 a	18.95 ± 0.74 a	0.83 ± 0.05 b	0.17 ± 0.01 a	0.52 ±0.02 b	1.94 ± 0.08 b	3.46 ± 0.16 b	5.48
'**Phoenix**'	24.07±0.34 b	9.51 ± 0.14 a	6.02 ± 0.10 b	8.84 ± 0.09 b	24.37 ± 0.35 b	0.36 ± 0.04 a	0.26 ± 0.01 b	0.33 ± 0.06 a	0.82 ± 0.02 a	1.77 ± 1.00 a	13.77

†*Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) (n=3).*

Table 4. Protein (mg g^{-1} fresh weight, fw), dry matter (g $100g^{-1}$ fw), and minerals content (g or mg kg^{-1} dry weight, dw) in jujube fruits as affected by cultivar.

Cultivar	Protein (mg g^{-1} fw)	Dry matter (g 100 g^{-1} fw)	Macro-elements (g kg^{-1} dw)				Micro-elements (mg kg^{-1} dw)			
			Potassium	Sodium	Magnesium	Calcium	Zinc	Copper	Manganese	Iron
'**Isidro**'	0.56 ± 0.032 b	22.17 ± 0.48 a	5.0 ± 0.5 a	0.02 ± 1.04 a	1.4 ± 0.08 a	4.0 ± 0.34 b	5.68 ± 1.13 a	1.95 ± 0.20 a	4.26 ± 0.17 a	7.53 ± 1.15 a
'**Rate**'	0.37 ± 0.014 a	25.50 ± 0.27 b	5.2 ± 0.1 a	2.00 ± 3.42 a	1.5 ± 0.29 a	2.6 ± 0.23 a	3.69 ± 0.69 a	0.79 ± 0.39 a	2.40 ± 0.29 a	10.95 ± 5.16 a
'**Phoenix**'	0.61 ± 0.017 b	22.15 ± 0.53 a	5.0 ± 0.4 a	1.10 ± 3.38 a	0.9 ± 0.15 a	2.0 ± 0.48 a	4.81 ± 1.95 a	1.95 ± 0.38 a	2.55 ± 0.38 a	17.32 ± 5.32 a

†Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) (n=3).

Table 5. Retention time (min), retention indexes (Exp.=experimental, and Lit.=literature) used for identification of the volatile compounds (µg kg^{-1}) found in jujube fruits as affected by cultivar.

Compound	Retention time (min)	Retention Indexes		ANOVA	Concentration (µg kg^{-1})		
		Exp.	Lit.		'Isidro'	'Rate'	'Phoenix'
Hexanal	7.24	1071	1075	*†	13.4 a‡	15.2 a	57.4 b
α-Phellandrene	9.42	1148	1158	**	126 b	134 b	8.0 a
β-Myrcene	9.55	1152	1156	NS	14.4	16.1	0.7
Heptanal	10.29	1177	1176	NS	7.1	6.7	14.4
Limonene	10.48	1183	1189	NS	40.4	39.4	16.6
β-Phellandrene	10.76	1192	1196	*	59.6 b	72.7 b	7.1 a
trans-2-Hexenal	11.30	1210	1211	**	53.8 a	64.8 a	484 b
p-Cymene	12.80	1260	1268	***	97.6 b	179 c	5.6 a
Octanal	13.50	1284	1288	NS	14.5	10.5	15.3
1-Octen-3-one	13.84	1295	1300	NS	5.7	7.2	4.2
2-Heptenal	14.49	1317	1320	**	13.5 a	18.5 a	81.6 b
6-Methyl-5-hepten-2-one	14.92	1331	1337	NS	19.5	19.2	10.6
Nonanal	16.58	1387	1389	*	119 b	45.1 a	129 b
2-Octenal	17.63	1422	1416	**	53.5 a	78.1 a	112 b
Benzaldehyde	20.39	1515	1508	***	257 a	1024 b	106 a
6-Methyl-1-heptanol	20.84	1530	1520	**	21.3 a	10.2 a	105 b
Hexanoic acid	30.05	1846	1843	*	210 b	215 b	64.4 a
Methyl hexadecanoate	39.54	2210	2217	NS	36.5	19.9	27.6
Total				**	1162 a	1976 b	1250 a

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

‡Values (mean of 3 replications) in each row followed by the same letter, within the same factor, were not significantly different ($p < 0.05$).

Table 6. Total phenols, flavonoids, flavonols contents of jujube fruits as affected by cultivar.

Cultivar		Total phenols (mg GAE 100 g^{-1} fw)	Total flavonoids (mg eq. rutin 100 g^{-1} fw)	Total flavonols (mg eq. rutin 100 g^{-1} fw)
'**Isidro**'	**Peel**	457 ± 20 e[†]	101 ± 3 d	6.18 ± 0.56 a
	Pulp	386 ± 17 d	19.82 ± 0.10 a	9.55 ± 0.32 b
'**Rate**'	**Peel**	453 ± 15 e	31.82 ± 1.77 b	ND
	Pulp	313 ± 21 c	70.60 ± 0.20 c	ND
'**Phoenix**'	**Peel**	178 ± 24 a	16.23 ± 0.35 a	6.18 ± 0.26 a
	Pulp	241 ± 21 b	29.23 ± 0.60 b	ND

[†]*Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) (n=3).*

Table 7. Antioxidant activity of jujube fruits as affected by cultivar.

Cultivar		FRAP	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	H-TAA	L-TAA
		(mmoles Trolox kg^{-1} fw)			(mg Trolox 100 g^{-1} fw)	
'Isidro'	**Peel**	165 ± 27 b[†]	43.63 ± 2.75 c	67.84 ± 1.22 c	562 ±13 cd	363 ± 4 c
	Pulp	13.42 ± 1.61 a	37.37 ± 2.13 b	61.70 ± 0.56 b	606 ± 23 d	60.15 ± 1.68 b
'Rate'	**Peel**	372 ± 55 c	46.54 ± 0.22 c	70.10± 1.80 c	513 ± 10 c	427 ± 4 d
	Pulp	9.24 ± 0.07 a	32.99 ± 0.62 b	61.45 ± 2.58 b	595 ± 17 d	52.87 ± 0.81 b
'Phoenix'	**Peel**	18.93 ± 0.87 a	11.45 ± 0.59 a	11.05 ± 0.35 a	246 ± 28 a	367 ± 27 c
	Pulp	14.82 ± 0.95 a	10.08± 1.22 a	9.10 ± 0.31 a	374 ± 11 b	18.34 ± 1.33 a

[†]*Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) (n=3).*

FIGURES

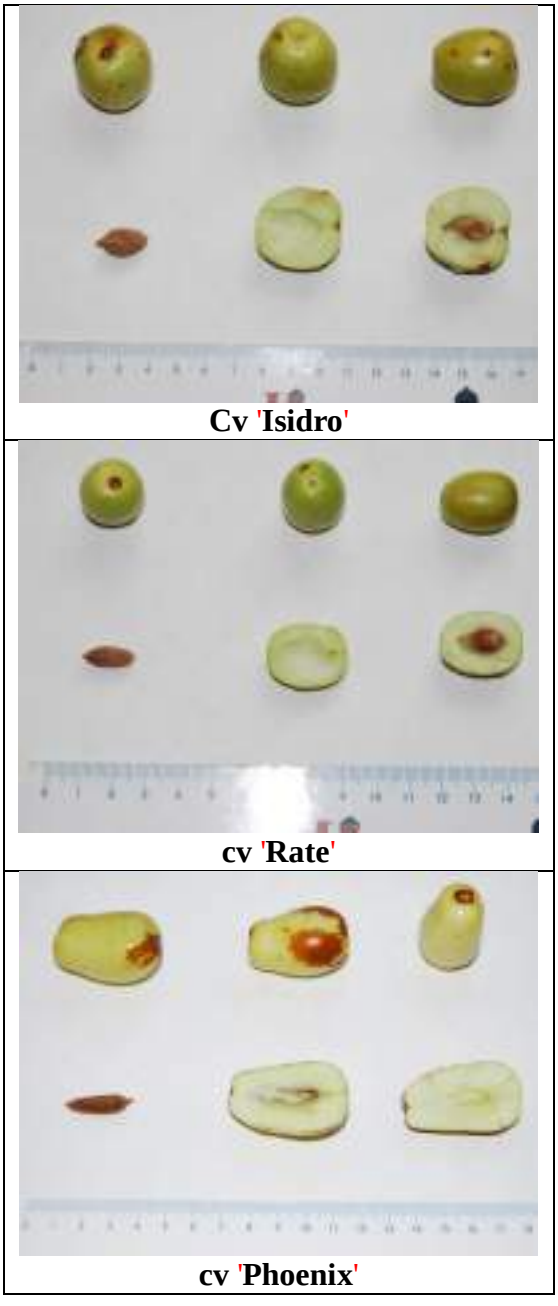

Fig. 1: Photographs of jujube fruits and stones of 'Isidro', 'Rate' and 'Phoenix' cultivars.

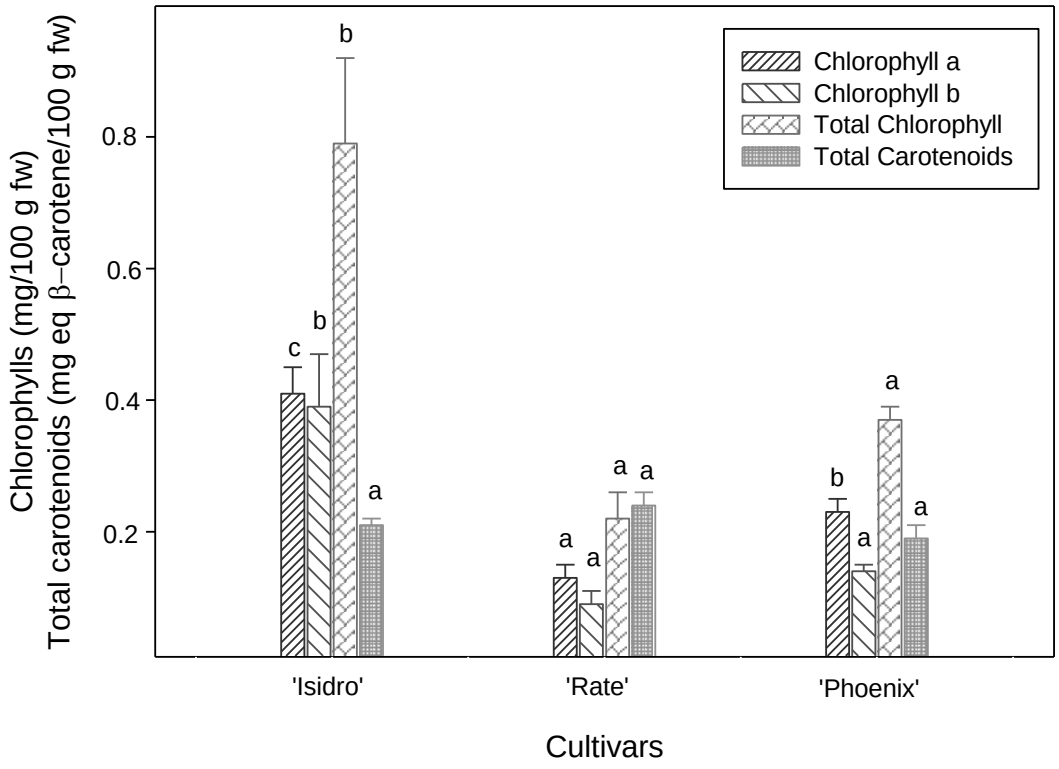

Fig. 2: Chlorophylls and carotenoids of jujube fruits 'Isidro', 'Rate' and 'Phoenix' cultivars. Different letters on top of bars indicate significant differences according to Fisher's LSD procedure at 95% confidence level (n=3).

4.2.Publicación 2

PUBLICACIÓN 2
Effects of organic and conventional farming on the physicochemical and functional properties of jujube fruit
Juana Reche, Francisca Hernández, María Soledad Almansa, Ángel A. Carbonell-Barrachina, Pilar Legua y Asunción Amorós. ***LWT- Food Science and Technology 2019, 99, 438-444*** ***doi:*** **10.1016/j.lwt.2018.10.012**

PUBLICACIÓN 2: TRANSCRIPCIÓN LITERAL

Effects of organic and conventional farming on the physicochemical and functional properties of jujube fruit

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ABSTRACT

Organic food is associated with improved nutritional properties, and this consumer expectation has led to increasing demand for organic fruits and vegetables. The objective of this study was to evaluate the changes in physical, chemical, and nutraceutical parameters of the jujube fruits 'Grande de Albatera' cultivar, grown under organic or conventional production systems. Results showed that the organic jujubes were smaller, with a slightly more intense yellow and red color, with higher contents of chlorophylls, carotenoids, sugars, organic acids, and total volatile compounds, but with lower protein and flavonoids, contents than conventional jujubes. Therefore, it can be concluded that the market quality of organic jujubes was similar to that of the conventional ones because fruits were smaller but with a more intense coloration. However, the flavor quality was better as they had more sugars, acids, and volatile compounds, making the flavor of fruits more attractive for consumers. Finally, there were no significant differences in the antioxidant attributes, as organic and conventional jujubes had similar total contents of phenols and antioxidant activity.

Keywords:

Minor crops

Organic foods

Phenolic compounds

Volatile compounds

Ziziphus jujuba

1. Introduction

Organic food is associated by the general public with improved nutritional properties, as well as to non-contaminating sustainable agricultural practices (Zanoli & Naspetti, 2002). This consumer perception has led to an increasing demand for organic fruits and vegetables (Raigón, Rodríguez-Burruezo & Prohens, 2010). Many consumers believe that organic foods are healthier than conventionally produced foods and that they are produced in a more environmental friendly way (Zanoli & Naspetti, 2002). This consumer expectation challenges scientists with the aim of proving this hypothesis (Woese, Lange, Boess & Bögl, 1997), tests must be done to compare the composition and quality of conventional and organic foods. According to Lester & Saftner (2011), the quality of fruits and vegetables can be categorized into market, sensory, and nutritional attributes; the last ones being mainly linked to high antioxidant activities (Gao, Wu & Wang, 2013).

Jujube fruits are widely consumed in Asian countries as a food and food additive due to its high nutritional value (Almansa, Hernández, Legua, Nicolás-Almansa & Amorós, 2016).

Jujube is an important plant in traditional Chinese medicine and is recommended for the treatment of some diseases, since have multiple beneficial health activities, such as anticancer, anti-inflammatory, hepatoprotective, gastrointestinal protective, antioxidant, antinsomnia, immunostimulating and neuroprotective effects (Guo et al,. 2015).

There are many jujube cultivars, each one with different physicochemical, physiological and functional characteristics. Most studies have been done on Asian

cultivars; however, the Spanish cultivars are still very poorly studied (Almansa et al., 2016; Hernández et al., 2016). Besides, it is known that the peel of these fruits may contain a great deal of compounds with high antioxidant activity and this content is higher in the peel as compared to the pulp (Wojdyło, Carbonell-Barrachina, Legua & Hernández, 2016).

For all of this, the aim of this work was to study the effects of organic *versus* conventional farming on the composition and quality of Spanish jujubes 'Grande de Albatera' cultivar. The quality of jujubes has been studied following the similar three attributes categories established by Lester & Saftner (2011): (i) *market quality* (weight and size of fruits and stones, instrumental color, and contents of chlorophylls and carotenoids), (ii) *flavor and nutritional quality* (total soluble solids, sugars, organic acids, moisture, volatile compounds, proteins and mineral composition), and (iii) *nutraceutical compounds* (total antioxidant activity, and total contents of phenols, flavonoids, and flavonols). The nutraceutical parameters were evaluated separately in the peel and pulp to be able to recommend or not their consumption with or without peel.

2. Material and methods

2.1. Experimental conditions and plant material

The experiment was carried out in 2015 and 2016 at one farm located in San Isidro (38° 10′ 22.29″ N, 0° 51′ 36.138″ W, 19 masl; province of Alicante, Spain). All data are the average of the two years (similar results and trends were obtained), and the results were expressed as the mean values of both years. In this farm, two plots

separated 500 m were taken, one plot of organic system and another plot of conventional system. Both plots consisted of 12-year-old jujube trees ('Grande de Albatera' cultivar) planted at 4 × 4 m. The soil of the farm had a sandy loam texture, very low electrical conductivity, high lime content, and low organic matter content. The irrigation water had an electrical conductivity of 0.8-1.1 dS m^{-1}. The water consumption was 4500 m^3 ha^{-1}. The nutritional conditions were the following in each plot:

- The organic plot relied only on organically certified fertilizers and pesticides and used no soil fumigation. This plot was biofertilized with 55 N fertilizer units (FU), based on Bombardier (certified product for organic farming by Sohiscert for European markets), 90 K FU based on Hortisul® (authorized according to CE regulations Nº 834/2007 and CE Nº 889/2008, to be used in organic farming), and 30 units of bactoneco®, a formulation based on specific strains of beneficial bacteria: *Pseudomonas* spp and *Bacillus* spp (suitable for organic farming and certified by Sohiscert).
- In the conventional plot inorganic fertilizers and synthetic pesticides were used. This plot was fertilized with 46 N FU, 25 P FU, 82 K FU, and 36 CaO FU.

For each farm, 200 fruits (50 fruits *per* tree) by year were hand-harvested, from the 4 central trees of the plot to avoid border trees, at physiological maturity, and immediately transported under ventilated conditions to the laboratory; avoiding border trees is essential to ensure in the case of the organic trees to avoid any pollution coming from adjacent plots. Thirty fruits from each farm were taken for the analyses of the physical parameters, while the other one hundred seventy fruits were used for the analyses of the chemical and sensory parameters, including the volatile composition each year.

The determination of the majority parameters (chlorophylls, carotenoids, total soluble solids, moisture, volatiles, proteins and minerals) was made in three samples that were quantified in duplicate (n = 6 each year). For each sample, three different whole fruits were used. The rest of parameters (antioxidant activity, total phenols, flavonoids and flavonols) were determined in similar samples but separating the peel and the pulp.

2.2. Market quality

Once in the laboratory, equatorial diameter and length (mm) of fruits and stones were measured with a digital caliper with 0.01 mm accuracy; fruits and stones weight (g) was measured using a digital balance, with an accuracy of 0.01 g; Color was assessed according to the *Commission Internationale de l'Eclairage* (CIE*Lab*) and expressed as *L**, *a**, *b** coordinates, chroma, and Hue angle, with a Minolta C-300 Chroma Meter (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor. Color measurements were made on the whole fruit on two opposite faces at the equatorial zone, according to Almansa et al. (2016). These parameters were measured in 30 fruits. The moisture content was determined by drying at 55 ºC in a Binder oven until reaching constant weight and expressed as a percentage, and stored -80ºC until further analysis.

Chlorophylls *a* and *b* were extracted from whole fruit (mixing peel and pulp) using 85% acetone in a ratio 1:2 (w:v) (AOAC, 1990). The sample was crushed with sea sand and, then, centrifuged. Absorbance of the supernatant was read at 664 and 647 nm, using a Helios Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). Results were expressed as mg 100 g^{-1} fresh weight (fw). Total carotenoids were

extracted with acetone and diethyl ether and quantified from whole fruit (peel and pulp) according to methodology previously described by Valero et al. (2011), with acetone and diethyl ether. The lipophilic phase was used to estimate the total carotenoids content, by reading the absorbance at 450 nm. Results were expressed as mg of β-carotene equivalent per 100 g^{-1} fw, taking into account the $\varepsilon^{1\%}_{cm}$ = 2,560.

2.3. Flavor and nutritional quality

The total soluble solids (TSS) were measured with a refractometer. The sugar and organic acid profile were quantified according to Hernández et al. (2016). Briefly, ~5 g of jujube fruit were homogenized with 6 mL of 50 mM Tris-acetate buffer pH 6.0, 10 mM $CaCl_2$, and 6 mL of ethyl acetate. The aqueous phase was used for the identification and quantification of sugar and organic acid profiles, according to Almansa et al. (2016). 1 mL of the hydrophilic extract was used for high-performance liquid chromatography (HPLC) (Hewlett–Packard HPLC series 1100; Hewlett-Packard, Wilmington, DE, USA). The elution buffer consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL min^{-1}. Organic acid was isolated using a Supelco column (Supelcogel TM C-610H column 30 cm x 7.8 mm) and a precolum Supelguard (5 cm x 4.6 mm; Supelco, Inc., Bellefonte, PA, USA). Sugars and acids were quantified using refractive index and diode-array detectors, respectively. Standards of organic acids (L-ascorbic, oxalic, citric, tartaric, malic, quinic, shikimic, succinic, and fumaric acids) and sugars (glucose, fructose, sucrose, and sorbitol) were obtained from Sigma (Poole, Dorset, UK). Calibration curves were used for the quantification of organic acids, showing good linearity (R^2 = 0.999). Results for both organic acids and sugars were expressed as g 100 g^{-1} fw.

The volatile composition of the jujube fruits was studied by using headspace solid phase micro-extraction (HS-SPME), according to the methodology previously described by Hernández et al. (2016). A gas chromatograph (GC-MS) Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan) coupled with a Shimadzu mass spectrometer detector GC-MS QP-5050A was used in the identification and semi-quantification of the volatile compounds of jujube. A TRACSIL Meta X5 column (Telnolroma S. Co. Ltd., Barcelona, Spain) was used. Results were expressed as µg kg^{-1} fw.

The protein content was analyzed by Bradford (1976) method using the Bio-Rad reactive. A standard curve of pure bovine serum albumin (BSA) was used for quantification according to Almansa et al. (2016). Results were expressed as mg g^{-1} fw.

The mineral contents were analyzed using an atomic absorption-emission spectrometer (Solaar 969, Unicam Ltd, Cambridge, UK), according to Hernández et al. (2016). Macro-elements (Ca, Mg, K, and Na) were expressed as g kg^{-1} dry weight (dw) and micro-elements (Fe, Cu, Mn, and Zn) were expressed as mg kg^{-1} dw.

2.4. Nutraceutical compounds

Total antioxidant activity (TAA), phenols, flavonoids and flavonols were quantified in both pulp and peel of jujube fruit.

The extracts of jujube fruit (peel or pulp) for the analysis of H-TAA (hydrophilic-TAA) and L-TAA (lipophilic-TAA) were obtained with 50 mM tris-acetate buffer pH 6.0, and ethyl acetate to separate the aqueous and organic phases and used to quantify H-TAA and L-TAA, respectively. The reaction mixture contained 10 mM $ABTS^{\bullet+}$, 1 mM hydrogen peroxide, and 10 mM peroxidase in a total volume of 1 mL of 50 mM

glycine-HCl buffer (pH 4.5) for H-TAA, or ethyl acetate for L-TAA. The reaction was monitored at 730 nm until a stable absorbance was obtained using a UNICAM Helios spectrophotometer (Cambridge, UK). A calibration curve was performed with Trolox as antioxidant standard for both H-TAA and L-TAA (Arnao, Cano & Acosta, 2001). The results were expressed as mmol Trolox kg^{-1} fw.

For the antioxidant activity determination by the $ABTS^{+}$, $DPPH^{\bullet}$ and FRAP methods, a methanolic extract was prepared described previously by Wojdyło et al. (2016). The free radical scavenging capacities were determined using the $ABTS^{\bullet+}$ method described by Re et al. (1999), $DPPH^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams, Cuvelier & Berst (1995) and FRAP (ferric reducing antioxidant power) method described by Benzie & Strain (1996). Calibration curves, in the range 0.5–5.0 mmol Trolox L^{-1} were used for the quantification of antioxidant activity by the three methods showing good linearity (R^2= 0.998). The results were expressed as mmol Trolox kg^{-1} fw.

Total phenolic compounds were quantified in peel and pulp in the hydrophilic phase according to the method described by Singleton, Orthofer & Lamuela-Raventos (1999), using the Folin–Ciocalteu reagent, and measuring absorbance at 760 nm. A calibration curve was performed with gallic acid and results were expressed as mg GAE 100 g^{-1} fw.

Flavonoids and flavonols were extracted from peel and pulp of jujube following the method of Zhuang (1992) with 80% methanol. The analysis of total flavonoids was performed by spectrophotometry following the method of Zhuang (1992) with 5% $NaNO_2$, 10% $AlCl_3$ and 1 M NaOH and the absorbance was measured at 512 nm. Results of total flavonoids were expressed in mg rutin equivalents 100 g^{-1} fw. For this, a

rutin calibration line was performed whose equation was y = 4.479x + 0.06773 with a correlation of 99.88%. Quantification of total flavonols was performed by spectrophotometry following the method of Kumaran, Kutty, Chatterji, Subrayan & Mishra (2007) with $AlCl_3$ (2 mg mL^{-1}) and sodium acetate (50 mg mL^{-1}) and the absorbance was measured at 440 nm. The results of total flavonols were expressed in mg rutin equivalents 100 g^{-1} fw. For this, a rutin calibration line was performed whose equation was y = 3.408x + 0.0297 with a correlation of 99.01%.

2.5. Statistical analysis

Statistical analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance test (ANOVA) for mean comparisons. The method used to discriminate among the means (Multiple Range Test) was Fisher's LSD (Least Significant Difference) procedure at a 95.0% confidence level.

3. Results and discussion

3.1. Market quality

The experimental fruit weights were similar to those previously reported in Spanish (Hernández et al., 2016), Korean (Choi, Ahn, Kozukue, Levin & Friedman, 2011), and Chinese jujube cultivars (Wang et al., 2012). Conventionally grown jujubes were significantly heavier than those cultivated under organic conditions (Table 1). This higher weight was due to larger size of the traditional jujubes and higher values of

equatorial diameter and length. However, the stone of both types of jujubes were similar in weight and equatorial diameter. These experimental findings regarding fruit size and weight were predictable, because conventional farming provides easily available nutrients for plant uptake. The conventional system applied synthetic fertilizer, whereas the organic system only used certified organic products, which nutrient release was slow but sustainable. These differences between the farming types were in agreement with those previously reported in other species such as grapefruit (Lester, Manthey & Buslig, 2007) and strawberry (Conti et al., 2014). In these two cases the fruit size, the fruit number per plant and the yield decreased under organic conditions, although the weight and longitudinal diameter of ripe passion fruit were statistically equivalent in both cropping systems (De Oliveira et al., 2017).

Significant differences in the dry matter content of jujubes were observed, with organic fruits having higher content (Table 1). This same trend was previously reported in tomatoes (Caris-Veyrat et al., 2004). Dry matter is often higher in organically grown plants than in conventionally grown ones, for leafy and root vegetables and tubers, although in vegetables and fruit the trend is not so clear (Woese et al., 1997).

The values of the CIE*L*a*b** color coordinates were very similar to those described in other Spanish (Collado-González et al., 2014) and Chinese jujube cultivars (Wang et al., 2012). The fruits of both farming types were harvested on the same date; however, slight changes in the color values were observed (Table 2). The organic jujubes presented a slightly, but significant, more reddish color (higher *a** value) and more yellowish (higher *b** value) color. These differences in the basic color coordinates (*a** and *b**) led to a more color intensity (higher chroma values), although differences were below 2 units, which are not perceptible by the human eye (no significant

differences in H^o parameter). These data agreed with those found by Lester et al., (2007), who found that organically grown grapefruits presented higher chroma index than the conventional ones; this trend was reported in both external color of the fruit and the fruit juices. Caris-Veyrat et al. (2004) they also found that more lycopene and carotene in organic tomatoes than in conventional tomatoes. However, López, Fenoll, Hellín & Flores, (2013) did not find differences in the color of organic and conventional peppers, but using the Hunter Lab System.

The organic jujubes presented significantly higher content of pigments, including both chlorophylls and carotenoids (Figure 1), as compared to the conventional fruits, which agreed with a higher color intensity (higher chroma value; Table 2). Several authors (Caris-Veyrat et al., 2004; Conti et al., 2014; Juroszek, Lumpkin, Yan, Ledesma & Ma, 2009) found more carotenoids in organic than conventional tomatoes and strawberries, too. However, the effects of the farming type were not significant in the β-carotene and anthocyanin contents in plums (Lombardi-Boccia, Lucarini, Lanzi, Aguzzi & Cappelloni, 2004) and passion fruit (De Oliveira et al., 2017).

In general, the differences between organic and conventional products are justified by the different moisture content of the products, with lower moisture being expected for the organic ones. However, if this was the case, the differences in weight, total chlorophylls and total carotenoids in jujube fruits should have been only 7.7% (which was the moisture difference between both fruit types), and not 35.7, 45.9, and 30.4%, respectively. Thus, the differences reported here for these 3 parameters can be attributed to the farming type (organic or conventional). As a final conclusion, it can be stated that organic jujube fruits have an equivalent market quality to the conventional ones,

because although they were smaller, they presented higher chlorophylls and carotenoids contents, and had a higher color intensity (*C* parameter).

3.2. Flavor and nutritional quality

The TSS were higher in the organic jujubes (Table 3), and this was supported by higher contents of sugars (sucrose, glucose, and fructose) and also organic acids (ascorbic and succinic).

These data seemed to indicate that organic jujubes should be sweeter than those in conventional grown. These data agreed with those obtained in other organic fruits, such strawberry (Conti et al., 2014), passion fruit (De Oliveira et al., 2017), and grapefruit (Lester et al., 2007). However, no significant effect of the farming type on these flavor parameters were reported in other studies, such as those conducted on tomatoes (Juroszek et al., 2009), apples (Roussos & Gasparatos, 2009), eggplant (Raigón et al., 2010), and black currant berries (Anttonen & Karjalainen, 2006).

The content of organic acids (Table 3) was also higher in organic grown jujubes, but with the predominant compounds (succinic and ascorbic) not presenting significant differences. Lester et al., (2007) also found more acidity in organically than conventionally grown grapefruit, with greater amounts of ascorbic acid. However, Juroszek et al. (2009) in tomatoes, Raigón et al. (2010) in eggplant fruit and Lombardi-Boccia et al. (2004) in yellow plums did not find significant differences in the total content of organic acids.

The volatile compounds found, by HS-SPME and GC-MS, were summarized in the Table 5. Eighteen volatile compounds were found in jujube fruits 'Grande de Albatera' cultivar regardless of the farming technique. The low number of volatile

compounds (18) seemed to indicate that the odor and aroma (perception of volatile compounds outside or inside the mouth, respectively) of these fruits is not one of the most relevant sensory attributes. The predominant compound was benzaldehyde (mean of 1333 μg kg^{-1} fw), followed by α-phellandrene, hexanoic acid, and *p*-cymene. The quantitative analysis showed that organic jujubes had higher total concentration of volatile compounds, which is it normally positively correlated with a higher odor and aroma intensities. Similarly, Picchi et al. (2012) found that the cauliflower 'Magnifico' cultivar had more than twice the content of volatile compounds when it was organically grown; however, the 'Emeraude' cultivar showed less volatiles in organic than in conventional products. A positive effect of organic farming on volatiles was also observed for other fruits and fruit-based products, such as mandarin organic juice (Pérez-López, López-Nicolás & Carbonell-Barrachina, 2007).

The protein content of jujubes in both types of culture was within the normal concentration range (Almansa et al., 2016). However, the protein content was significantly lower in the organic jujubes, which could be linked to the different type of fertilizers used (Table 4). However, other authors did not find significant differences in the protein content of eggplant (Raigón et al., 2010) or yellow plum (Lombardi-Boccia et al., 2004) between conventional and organic productions.

As for the minerals, no significant differences were found (Table 4). Other authors have reported a similar trend in yellow plums (Lombardi-Boccia et al., 2004), grapefruit (Lester et al., 2007), strawberry (Conti et al., 2014) or pepper (López et al., 2013). However, Raigón et al. (2010) found a higher mineral content in organic eggplant.

If it is considered that these differences in contents between organic and conventional products were only due to the moisture content, the differences in sugars,

organic acids, minerals, proteins and volatile compounds, should around 7.7%; however, was not the case of sugars (21.8%), proteins (27.8%) and volatile compounds (31.2%).

Therefore, organic jujube fruits showed a good flavor potential/quality, because they had more sugars, organic acids, and volatile compounds, which is normally reflected in more intense odor, aroma and flavor.

3.3. Nutraceutical compounds

The total antioxidant activity (TAA) as described by the DPPH$^{\bullet}$ test showed that the peel values were about 1.6-1.7 times higher than those of the pulp, in both organic and conventional jujube fruits (Table 6). These results agreed with those obtained by Xue, Feng, Cao, Cao & Jiang, (2009), who also found values of DPPH$^{\bullet}$ between 1.5-1.8 times greater in peel than in jujube pulp of four Chinese varieties. These data were also in line with those obtained by other authors in the pulp of Chinese (Li, Ding & Ding, 2005) and Korean (Choi et al., 2011) jujube varieties; however, the experimental DPPH$^{\bullet}$ values were inferior to those obtained by Kamiloğlu, Ercisli, Şengül, Toplu & Serçe, (2009) in Turkish varieties. The variation of antioxidant capacity between peel and pulp extracts may be due to differences in antioxidant compounds and their effectiveness (Robards, Prenzler, Tucker, Swatsitang & Gloer, 1999).

In the same way, the FRAP test also demonstrated that the peel had higher TAA than the pulp of jujube fruits. Xue et al., (2009) also observed significantly higher FRAP in peel than in pulp but the differences were less clear in their antioxidant activity in four Chinese varieties.

The $ABTS^{\bullet+}$ assay, however, did not show significant differences between peel and pulp. However, Xue et al., (2009) did find differences between 1.3 and 2.0 times in peel and pulp of Chinese jujubes, respectively. They also found higher $ABTS^{\bullet+}$ values than in current the Spanish variety, which could be because they modified the reaction by changing the medium to an acidic pH. The TAA in the jujube peel was always greater than in the pulp, both in hydrophylic (H-TAA) and lipophylic (L-TAA) fractions. However, the differences were higher in the case of L-TAA (15.6 times greater in peel than in pulp) as compared to H-TAA (only 1.5 times). This could be due to the fact that the peel contains a greater part of lipids than the pulp which is mostly watery.

In general, no significant effects of the farming type were observed on the antioxidant activity with any of the methods used. Other researchers did not find differences in the TAA content of fruits grown in organic and conventional systems, such as apple (Valavanidis, Vlachogianni, Psomas, Zovoili & Siatis, 2009), tomatoes (Juroszek et al., 2009) or passion fruit (De Oliveira et al., 2017). Similarly to what happened in the current study, Valavanidis et al. (2009) also quantified a higher concentration of TAA in apple peel than in pulp in both organic and conventional grown, but without finding differences between the two agricultural practices. In this case, these researchers studied five varieties of apples and found more important differences between the varieties than between the agricultural practices.

The contents of total phenols, flavonoids and flavonols were significantly higher in jujube peel than in pulp (Table 7), but the farming system only influenced the content of total flavonoids and flavonols, with conventional jujubes fruits having higher peel flavonoids and pulp flavonols while organic peel had higher total flavonols.

The content of total phenols was 1.6 times higher in the peel than in the pulp of jujube fruits, independently of the farming system. Xue et al. (2009) also found higher values in peel than pulp extracts in Chinese jujube varieties. Tomás-Barberán et al. (2001) also found that the peel of nectarines, peaches and plums had more total phenols than the pulp. Phenolic compounds tend to accumulate in the plant epidermal tissues because they have a potential role in the protection against ultraviolet rays, also because they act as attractants in the dispersion of fruits and as defensive chemicals against pathogens and predators (Xue et al., 2009).

Faller & Fialho (2010) also found similar contents of total phenols in organic and conventional banana, orange and apple pulp, although they found higher amounts in organic papaya and mandarin and smaller in organic mango. Similarly, no significant effect of the farming system was reported in eggplant fruits (Raigón et al., 2010), tomatoes (Anttonen & Karjalainen 2006; Juroszek et al., 2009). However, Lombardi-Boccia et al. (2004) found higher total phenols in conventional than in organic yellow plums. Therefore, it was not clear whether the farming system has a significant and consistent effect on the total content of phenolic compounds accumulated in fruits and vegetables.

In general, the total flavonoids were 4.5-5.5 times higher in peel than in pulp. It was also unclear whether the cropping system influences the flavonoids content of the fruits. Thus, De Oliveira et al. (2017) found equivalent flavonoid concentrations in passion fruit under organic and conventional conditions, while Anttonen & Karjalainen (2006) found that the profile of flavonoids in black currant dependent more on where they have been grown than whether they were grown under organic or conventional farming conditions.

Total flavonols have been also higher in peel than in pulp and this difference was 2.7 and 3.8 times in conventional and organic jujubes, respectively, although total flavonols content was higher in the peel of organic jujubes and in the pulp of conventional jujubes. Mitchell et al. (2007) and Chassy, Bui, Renaud, Horn & Mitchell, (2006) found higher concentrations of flavonols in organic tomatoes than in conventional ones, while Lombardi-Boccia et al. (2004) found that organic yellow plums had less content of total flavonols than the conventional ones.

4. Conclusions

In general, organic farming led to higher contents of (i) chlorophylls, (ii) carotenoids, (iii) sugars, (iv) organic acids, (v) total volatile compounds; similar contents of (i) minerals, total phenols, and antioxidant activity; but lower contents of proteins. Considering that the difference on moisture content between organic and conventional jujube fruits was of only 7.7% (which is a low value as compared to those found in the rest of parameters studied), it can be concluded that the observed differences on weight, contents of chlorophylls, carotenoids, sugars, proteins, and volatile compounds were mainly due to the farming type, with organic farming leading to jujube fruits of higher quality and functionality. Besides, it is recommended to consume unpeeled jujubes because their peel has high contents of most of the nutraceutical compounds (total phenols, flavonoids, flavonols and antioxidant activity).

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TABLES

Table 1. Physical parameters and moisture of jujube fruits, 'Grande de Albatera' cultivar, under organic and conventional farming

Farming type	Fruit				Stone		
	Weight (g)	Equatorial diameter (mm)	Length (mm)	Moisture (%)	Weight (g)	Equatorial diameter (mm)	Length (mm)
Organic	23.4 ± 0.4 b[†]	37.5 ± 0.4 b	37.2 ± 0.4 b	73.35 ± 0.74 b	0.71 ± 0.02 a	9.7 ± 0.1 a	20.4±0.2 b
Conventional	36.4 ± 1.3 a	42.8 ± 0.7 a	41.8 ± 0.5 a	79.43 ± 1.21 a	0.80 ± 0.06 a	9.8 ± 0.3 a	23.5± 0.4 a

[†]*Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95 % confidence level (n=30 per year; n=60).*

Table 2. External color of jujube fruits, 'Grande de Albatera' cultivar, under organic and conventional farming

Farming type	$L^{*\ddagger}$	$a^{*\ddagger}$	$b^{*\ddagger}$	$C^{\ddagger}$	$H^{o\ddagger}$
Organic	71.70 ± 0.64 a	1.84 ± 0.48 a	33.82 ± 0.41 a	33.97 ± 0.40 a	86.82 ± 0.82 a
Conventional	70.63 ± 0.46 a†	0.65 ± 0.36 b	32.07 ± 0.33 b	32.19 ± 0.33 b	88.79 ± 0.65 a

†Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95 % confidence level. ‡L, lightness; a*, green/red coordinate; b*, blue/yellow coordinate; C, chroma; H°, hue angle (n=60 per year; n=120).*

Table 3. Total soluble solids (TSS), sugars and organic acids (g 100 g^{-1}) profiles of jujube fruits, 'Grande de Albatera' cultivar, under organic and conventional farming

Farming type	TSS	Sucrose	Glucose	Fructose	Citric acid	Malic acid	Ascorbic acid	Succinic acid
	(ºBrix)				(g 100 g^{-1})			
Organic	24.00 ± 0.06 a[†]	8.82 ± 0.04 a	5.95 ± 0.06 a	7.43 ± 0.09 a	0.47 ± 0.01 a	0.25 ± 0.01 a	0.49 ± 0.01 a	1.02 ± 0.02 a
Conventional	23.41 ± 0.17 b	7.04 ±0.44 b	4.61 ± 0.08 b	5.71 ±0.09 b	0.42 ± 0.01 b	0.21 ± 0.01 b	0.50 ± 0.01 a	0.99 ± 0.11 a

[†]*Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95 % confidence level (n=6 per year; n=12).*

Table 4. Protein and minerals content of jujube fruits, 'Grande de Albatera' cultivar, under organic and conventional farming

Farming type	Macro-elements (g kg^{-1} dw)				Micro-elements (mg kg^{-1} dw)			Protein
	Potassium	Magnesium	Calcium	Zinc	Copper	Manganese	Iron	mg g^{-1} fw
Organic	3.37 ± 0.20 a	0.95 ± 0.06 a	2.22 ± 0.2 a	5.64 ± 1.39 a	0.98 ± 0.00 a	3.26 ± 0.29 a	5.85 ± 1.27 a	0.26 ± 0.03 b
Conventional	3.95 ± 0.16 a	0.79 ± 0.03 a	1.83 ± 0.2 a	4.89 ± 0.34 a	0.98 ± 0.00 a	2.54 ± 0.14 a	7.53 ± 2.37 a	0.36 ± 0.02 a

†Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95 % confidence level (n=6 per year; n=12).

Table 5. Volatile compounds in jujube fruits, 'Grande de Albatera' cultivar, under organic and conventional farming.

Compound	Retention time (min)	Retention Indexes		ANOVA[†]	Concentration (µg kg^{-1} fw)	
		Experimental	Literature		Organic	conventional
Hexanal	7.24	1071	1075	NS	15.7	21.6
α-Phellandrene	9.42	1148	1158	**	189 a[‡]	33.7 b
β-Myrcene	9.55	1152	1156	*	28.7 a	4.9 b
Heptanal	10.29	1177	1176	NS	5.3	4.3
Limonene	10.48	1183	1189	**	62.2 a	18.4 b
β-Phellandrene	10.76	1192	1196	*	106 a	18.7 b
trans-2-Hexenal	11.30	1210	1211	**	104 a	49.4 b
p-Cymene	12.80	1260	1268	**	175 a	49.1 b
Octanal	13.50	1284	1288	NS	9.4	9.0
1-Octen-3-one	13.84	1295	1300	NS	10.6	10.9
2-Heptenal	14.49	1317	1320	NS	36.6	21.0
6-Methyl-5-hepten-2-one	14.92	1331	1337	NS	22.8	5.1
Nonanal	16.58	1387	1389	NS	35.8	31.1
2-Octenal	17.63	1422	1416	NS	59.2	60.8
Benzaldehyde	20.39	1515	1508	***	1412 a	1253 b
6-Methyl-1-heptanol	20.84	1530	1520	NS	10.7	14.3
Hexanoic acid	30.05	1846	1843	*	177 a	91.6 b
Methyl hexadecanoate	39.54	2210	2217	NS	23.9	12.6
Total				**	2483 a	1709 b

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

[‡]*Values (means ± standard error) followed by the same letter, within the same row, were not significantly different according to Fisher's least significant difference (LSD) (n=6 per year; n=12).*

Table 6. Antioxidant activity of the jujube fruits, 'Grande de Albatera' cultivar under organic and conventional farming

Farming type		H-TAA	L-TAA	$ABTS^{+}$	FRAP	$DPPH^{\bullet}$
		(mmol Trolox kg^{-1} fw)		(mmol Trolox kg^{-1} fw)		
Organic	Peel	23.27 ± 0.28 a[†]	24.21 ± 1.41 b	35.8 ± 11.0 a	312.2 ± 30.4 a	68.2 ± 0.9 a
	Pulp	5.87 ± 0.49 c	2.44 ±0.06 c	31.4 ± 0.3 a	6.5 ± 1.2 b	41.2 ± 1.9 b
Conventional	Peel	24.75 ± 1.41 a	31.22 ± 1.78 a	35.5 ± 1.7 a	370.0 ± 34.8 a	67.9 ± 0.3 a
	Pulp	16.50 ± 0.58 b	2.00 ± 0.07 c	31.2 ± 0.4 a	14.7 ± 2.3 b	38.9 ± 0.5 b

[†]*Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95 % confidence level (n=6 per year; n=12).*

Table 7. Total content of phenols, flavonoids and flavonols of the jujube fruits, 'Grande de Albatera' cultivar, under organic and conventional farming

Farming type		Total phenols (mg GAE 100 g^{-1} fw)	Total flavonoids (mg eq. rutin 100 g^{-1} fw)	Total flavonols (mg eq. rutin 100 g^{-1} fw)
Organic	Peel	452.2 ± 1.4 a[†]	83.1 ± 4.7 b	61.7 ± 3.1 a
	Pulp	269.3 ± 4.2 b	17.8 ± 1.6 c	0.83 ± 0.6 d
Conventional	Peel	433.7 ± 11.8 a	111.3 ± 3.2 a	54.6 ± 0.8 b
	Pulp	279.8 ± 8.5 b	20.1± 1.8 c	16.2 ± 0.2 c

[†]*Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95 % confidence level (n=6 per year; n=12).*

FIGURE

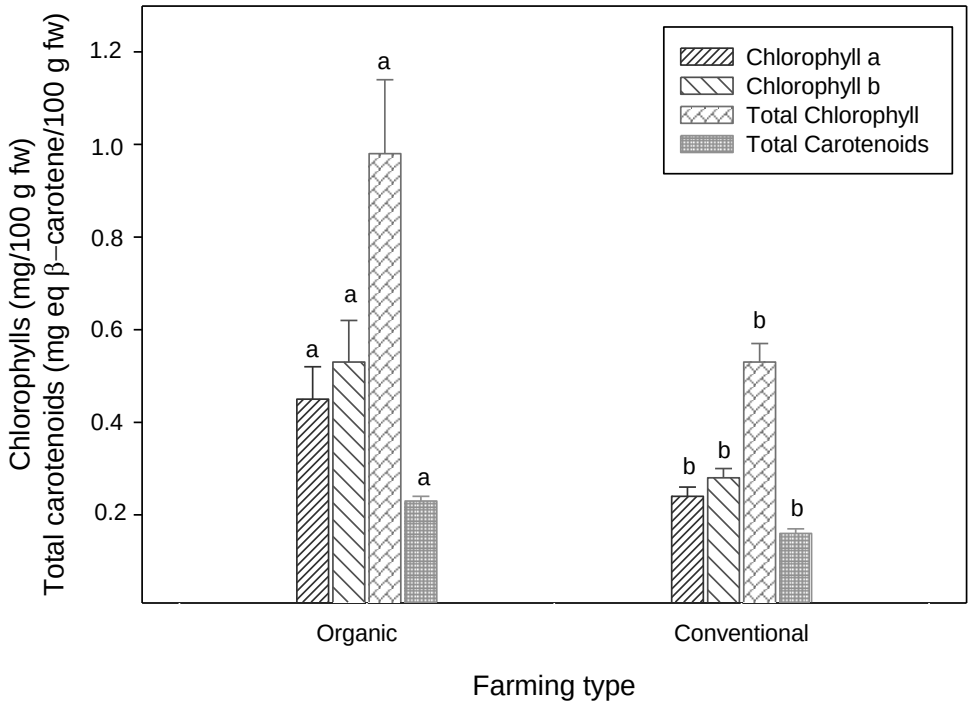

Fig. 1 Chlorophylls and carotenoids in jujube fruits, 'Grande de Albatera' cultivar, under organic and conventional farming. Different letters on top of bars indicate significant differences according to Fisher's least significant difference (LSD) procedure at 95 % confidence level (n=6 per year; n=12).

4.3. Publicación 3

PUBLICACIÓN 3
Relatioships between physico-chemical and functional parameters and genetic analysis with ISSR markers in Spanish jujubes (*Ziziphus jujuba* Mill.)
Juana Reche, Santiago García-Martínez, Pedro Carbonell, María Soledad Almansa, Francisca Hernández, Pilar Legua y Asunción Amorós. ***Scientia Horticulturae 2019, 253, 390-398*** ***doi:* 10.1016/j.scienta.2019.04.068**

PUBLICACIÓN 3: TRANSCRIPCIÓN LITERAL

Relationships between physico-chemical and functional parameters and genetic analysis with ISSR markers in Spanish jujubes (*Ziziphus jujuba* Mill.) cultivars

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Abstract

In this work we have used ISSR molecular markers in Spanish jujube cultivars, for the first time, for study the phylogenetic relationships among cultivars and the genetic diversity assessment. The results of this study showed that the physico-chemical and bioactive compounds were different in the cultivars of Spanish jujubes studied. The PCA grouped the four type of cultivars into 3 groups: (i) the first group formed exclusively by the cultivar 'Da', (ii) the second group formed by the largest and round cultivars, 'Gam' and 'Gab', and (iii) and the third group formed by the medium 'Pe' and 'Me' cultivars. The genetic analysis with ISSR markers showed that was able to differentiate the 5 cultivars studied, so they are useful for genetic studies of *Ziziphus jujuba* specie. The similarity between the genetic dendrogram and the PCA, made with the physical-chemical and functional traits, had been very high, since in both cases the grouping between 'Gam' and 'Gab' cultivars was obtained and 'Da' cultivar was the furthest from the rest.

Keywords:

Flavonoids

Flavonols

Phenols

ISSR markers

PCA

Jujube (*Ziziphus jujuba*)

1. Introduction

Jujube (*Ziziphus jujuba* Mill.) trees belong to the Rhamnaceae family and has its origin in China, where it is popular for its high nutritional value and medicinal uses (Reche et al., 2018). In China, there are more than 700 cultivars of Chinese jujube (Ma et al., 2011). These different cultivars and varieties contain large variation in genetic traits, such as fruit shape, flavor, color and botany traits (Ma et al., 2011). Despite being a marginal crop in Spain and its low consumption, its demand is increasingly (Hernández et al., 2016). However, there is scarce information on the physico-chemical and functional properties of Spanish jujubes as affected by cultivar (Hernández et al., 2016; Almansa et al., 2016; Reche et al., 2018; 2019) and there is not information on genetic studies of Spanish cultivars.

Several molecular markers have been used to study the diversity in jujube. Among these markers, simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) have been the predominating markers during the last years (Ma et al., 2011; Li et al., 2014; Wang et al., 2014; Zhao et al., 2014, Xiao et al., 2015; Zhang et al., 2015; Fu et al., 2016; Zhang et al., 2016). SNPs are the most abundant genetic markers in the genome (Mammadov et al., 2012). SSRs, also known as microsatellites, have many advantages, including high abundance, codominant inheritance, hypervariability and extensive genomic coverage (Tautz, 1989). However, sequence availability in less-studied plant species and necessary technical facilities are the limiting factor for the discovery of SNPs and SSR in minor plant species. Inter-simple sequence repeats, also known as ISSRs (Zietkiewicz et al., 1994) is a quick and simple technique with low running costs and requiring only small quantities of template DNA. The production of large number of fragments and the reproducibility are other

advantages of these markers (Martín and Sánchez-Yélamo, 2000). ISSRs have been recognized as useful molecular markers in the analysis of genetic diversity in various species, as *Diplotaxis* (Martín and Sánchez-Yélamo, 2000), buffalo grass (Budak et al., 2004), *Capparis* (Saifi et al., 2011; Al-Safadi et al., 2014) and *Musa* (Lamare and Rao, 2015). These genetic markers differ with respect to important features, such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements and financial investment. The most appropriate genetic marker will depend on the specific application, the presumed level of polymorphism, the presence of sufficient technical facilities and know-how, time constraints and financial limitations. To our knowledge, ISSR has not been used to study the jujube. Therefore, the objectives of this work were: (i) to study the physicochemical and functional properties of four types of Spanish jujubes, (ii) to carry out their genetic analysis using ISSR markers, and (iii) to study the relationship between the genetic profile and the physicochemical and functional properties, to be able to discriminate between them.

2. Materials and methods

2.1.Experimental conditions and plant material

The experiment was carried out in a commercial farm with 22-years-old jujube trees (latitude 38º10’22, 29’’N x longitude 0º51’36,138’’W, 19 m above sea level) in Albatera (Alicante) from Spain. Trees were trained as a vase and spaced 4 m × 4 m. The jujube fruit belong to five cultivars called: Grandes de Albatera' ('Gab') and 'Grandes de Alhama' ('Gam') [type Large], 'Medianos' ('Me') [type Medium], 'Pequeños' ('Pe') [type Small] and 'Dátil' ('Da') [type Date]. These cultivars belong to the four most important jujube cultivar types in Spain (Reche et al. 2018, 2019). All the cultivars were cultivated

in the same area and were cultivated under the same agronomic conditions. All cultivars of the Large, Medium and Small types are harvested between the second half of August and the first of September, while the Date type is harvested in the second half of October. During the vegetative growth stage of the tree, forty leaves from four trees (ten per tree) of each cultivar were hand-harvested. They were immediately taken to the laboratory and frozen at -80ºC until they were used for genetic analysis. Subsequently, one hundred fruits from four trees (twenty five fruits per tree) of each cultivar were hand-harvested at commercial maturity. This stage corresponds to the stage in which the farmers harvest the fruit in the area the cultivars cited to sell. The fruits were recollected when presented > 15 ºBrix. After they were immediately transported under ventilated conditions to the laboratory. Thirty fruits were taken for the physical analyses, while the other seventy fruits were frozen at -80ºC in an ultra-low temperature freezer, premium range (New Brunswick Scientific, Edison, New Jersey US) and later used for the biochemical parameters analyses.

2.2. Physical parameters

The following physical parameters were measured in 30 fruits: equatorial diameter and fruit length (mm) using a digital caliper (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK); fruit weight (g) was measured using a digital balance (model BL-600; Sartorius, Madrid, Spain).

2.3.Chemical and biochemical parameters

Thirty fruits of each cultivar were chopped and subdivided in 3 subsamples that were used to perform the following analyses that were measured in duplicate each subsample (n = 6).

The total soluble solids (TSS) were analyzed in juice of the fresh jujubes. These analyses were measured using a refractometer (model ATAGO N20; Minato-Ku, Tokyo, Japan). Titratable acidity (TA) was determined by automatic titration (model pH-Matic 23, Crison Instruments, S.A., Barcelona, Spain) with 0.1 mol L^{-1} NaOH up to pH 8.1 using 1 mL of juice diluted in 25 mL of distilled H_2O, and the results were expressed as g malic acid equivalent 100 g^{-1} fresh weight (fw). The maturity index (MI) was calculated as the relationship between TSS and TA.

Chlorophylls *a* and *b* were extracted from each sample using 85% acetone in a relation 1:2 (w:v) (AOAC, 1990). The fresh subsamples were crushed with sea sand in a mortar and then centrifuged at 12,000 rpm for 20 min at 4°C. Absorbance of the supernatant was read at 664 and 647 nm, using a Helios Gamma spectrophotometer (model UVG 1002E; Helios, Cambridge, UK). The results were expressed as mg 100 g^{-1} fw. Total carotenoids were extracted according to Valero et al. (2011), with acetone and diethyl ether; 10% NaCl was used to promote the phases separation. The lipophilic fraction was used to estimate the total carotenoids content, by reading the absorbance at 450 nm, and the results were expressed as mg of β-carotene equivalent 100 g^{-1} fw, taking into account the the $\varepsilon_{cm}^{1\%} = 2560$.

To carry out the following analyses, another 30 fruits were taken for each cultivar and separated into 3 subsamples of 10 fruits each. The fruits of each subsample were chopped, mixed and frozen at -80ºC until their later use for the measurements of proteins, phenols and antioxidant activity. The first step was to make an extract of each

subsample. Briefly, one gram of jujube fruit were homogenized with 5 ml of 50 mM Tris-acetate buffer pH 6.0, 20 mM CaCl, and 6 mL of ethyl acetate, centrifuged, and, then, the aqueous (hydrophilic fraction) and organic (lipophilic fraction) phases were separated and frozen at -80ºC.

The protein content was determined in the hydrophilic fraction by the Bradford (1976) method using the Bio-Rad reactive. A standard curve of pure bovine serum albumin (BSA) was used for quantification according to Almansa et al. (2016). The results were expressed as mg 100 g^{-1} fw.

Total phenolic compounds were quantified according to Singleton et al. (1999), and using the Folin–Ciocalteu reagent. Briefly, 25 µL of aqueous extracts were mixed with 2.5 mL of Folin–Ciocalteau. The mixture was incubated for 2 min at room temperature and 2 mL of sodium carbonate (75 g L^{-1}) were added and vortexed. Finally, the mixture was incubated at 50ºC for 5 min and the absorbance was measured at 760 nm. A calibration curve was performed with gallic acid and the results were expressed as mg GAE 100 g^{-1} fw.

Flavonoids and flavonols were extracted using 80% methanol from jujubes following the method of Zhuang (1992). The analysis of total flavonoids (mg rutin equivalents 100 g^{-1} fw) was performed by spectrophotometry after using 5% $NaNO_2$, 10% $AlCl_3$ and 1 M NaOH; absorbance was measured at 512 nm on a Helios Gamma spectrophotometer (model UVG 1002E; Helios, Cambridge, UK). Quantification of total flavonols (mg rutin equivalents 100 g^{-1} fw) was done according to Kumaran et al. (2007), using $AlCl_3$ (2 mg mL^{-1}) and sodium acetate (50 mg mL^{-1}), and absorbance was measured at 440 nm.

The activity of H-TAA (hydrophilic-TAA) and L-TAA (lipophilic-TAA) of jujube fruit were determined in the aqueous and organic phases, respectively. The reaction mixture contained 10 mM ABTS, 1 mM hydrogen peroxide, and 10 mM peroxidase in a total volume of 1 mL of 50 mM glycine-HCl buffer (pH 4.5) for H-TAA, or ethyl acetate for L-TAA. The reaction was monitored at 730 nm until a stable absorbance was obtained using a UNICAM Helios spectrophotometer (Cambridge, UK). After that, a suitable amount of jujube fruit extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with Trolox as antioxidant standard for both H-TAA and L-TAA (Arnao et al., 2001). The results were expressed as mg Trolox equivalent 100 g^{-1} fw.

For the total antioxidant activity determination, a methanol extract was prepared as described by Wojdyło et al. (2013). The free radical scavenging capacities were determined by three methods, $ABTS^{\bullet+}$ method (Re et al., 1999), $DPPH^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams et al., 1995), and FRAP (ferric reducing antioxidant power) method (Benzie and Strain, 1996). Calibration curves, in the range 0.5-5.0 mmol Trolox L^{-1}, were prepared for all three methods and showed good linearity (R^2= 0.998). Results were expressed as mmoles Trolox kg^{-1} fw.

2.4. Genetic study

2.4.1. *DNA extraction*

Genomic DNA was extracted from young leaves, following the CTAB method with slight modifications (Doyle and Doyle, 1990). The extracted DNA was dissolved in double distilled water and the final concentration was adjusted to 15 ng μl^{-1}, using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, USA).

2.4.2. PCR optimization and ISSR selection

We used 6 markers of the UBC primer set #9 of the University of British Columbia Biotechnology Laboratory (Vancouver, Canada), and 12 markers from the work of Al-Safadi et al. (2014) (Table 1 supplementary material). These markers were the more polymorphic in previous studies (Al-Safadi et al., 2014; Singh et al., 2009). Annealing temperature was optimised by running a gradient PCR between 45 and 60ºC. Annealing temperature of 53ºC obtained the best results. Amplification with each arbitrary primer was repeated twice and only those primers that produced reproducible and consistent bands were selected for data generation.

2.4.3. PCR amplifications

Reactions were carried out in 25 µl volume containing 30 ng template DNA, 0.5 U TaqDNA polymerase, 10 mM dNTP, 10 µM primer in 1× reaction buffer that contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl and 2.5 mM $MgCl_2$. The temperature profile used was: a denaturation for 2 min at 94ºC, then 35 cycles consisting each of a denaturation step for 30 sec at 94ºC; an annealing step for 30 sec at 53ºC; an extension step for 1 min at 72ºC and the final extension for 5 min at 72ºC, using a Eppendorf Mastercycler Gradient (Hamburg, Germany).

2.4.4. Electrophoresis conditions

Amplified products were loaded on 1.5% agarose gel and separated in 1× TAE buffer at 100 V. The gels were visualized under UV after staining with ethidium

bromide and documented using a gel documentation and image analysis system (Vilber Lourmat, Collégien, France).

2.4.5. Data analysis

The banding patterns were scored as present (1) or absent (0). Only clear and repeatable fragments were considered in the genetic analysis. Band size determination was carried out using the molecular weight marker GeneRuler100 bp Plus DNA Ladder (ThermoFisher Scientific, Waltham, USA).

Three indexes were calculated: MR (Multiplex Ratio), PIC and RP (Resolving Power). The MR is defined as the number of polymorphic loci found in a reaction (Powell et al. 1996). For dominant (presence/absence) markers the PIC is defined as 1-Faa^2-Fan^2, where Faa^2 is the frequency of the amplified allele and Fan^2 is the frequency of the non amplified allele. The RP is defined as ΣI_b, being I_b =1-(2|0.5-p|), where *p* is the frequency of the genotypes that contain the band. It represents the ability of a marker to discriminate against the different studied accessions.

Phylogenetic relationships among accessions were estimated from the molecular characterization data, using the package NTSYSpc 2.0 (Adams et al. 1998). Dendrogram was constructed using the Unweighted Pair Group Method with arithmetic averaging (UPGMA) clustering analysis based on the genetic similarity coefficient matrices (Nei and Li, 1979). Statistical stability of the branches in the cluster was estimated by bootstrap analysis with 1000 replicates, using the Winboot software program (Yap and Nelson, 1996).

2.5. Statistical analysis

For physical, chemical and biochemical parameters, a basic descriptive statistical analysis was followed by an analysis of variance test (ANOVA) for mean comparisons. The method used to discriminate among the means (multiple range test) was Fisher's LSD (Least Significant Difference) procedure at a 95.0% confidence level. These analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, USA).

Correlation between the different determined parameters was calculated in R using the package 'reshape2' v. 1.4.3 (Wickham, 2007). A Principal Component Analysis (PCA) was performed to determinate which combination of attributes are contributing to phenotypic diversity in our populations. PCA was conducted in R using the package 'FactoMineR' v. 1.41 (Lê et al., 2008). Three different samples from each population were used to perform the PCA, and the mean from values of weight and diameter/length parameters was included in each population in the data matrix.

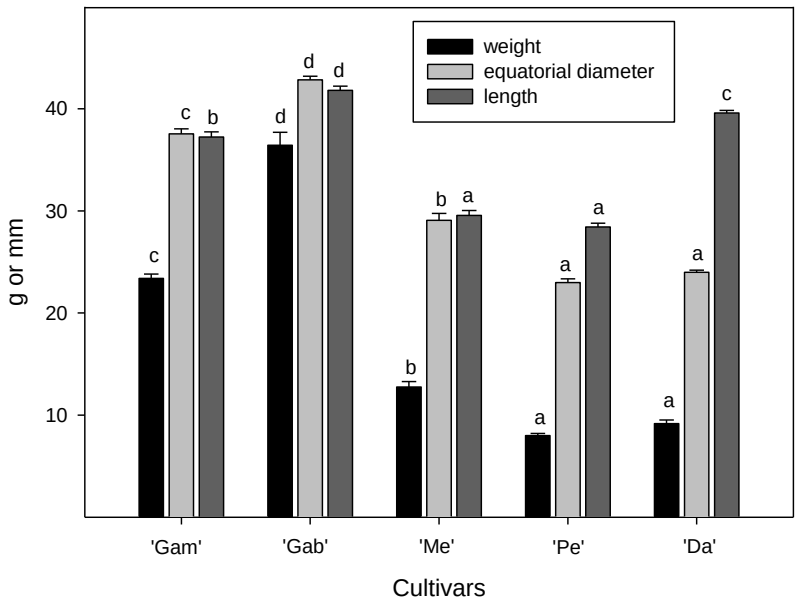

Fig. 1. Physical parameters at harvest time. Bars (means ± SE) with the same letter, for each quality parameter, were not significantly different according to Fisher´s least significant difference (LSD) procedure at 95% significance level (n = 30).

3. Results and discussion

3.1. Physical parameters of jujube fruit

Data showed that there was a significant difference in size among cultivars (Fig. 1). The 'Gab' cultivar had a higher equatorial diameter and length than the other cultivars, however 'Pe' cultivar, was the one with the minor equatorial diameter and length, followed by 'Da', 'Me' and 'Gam'. These differences in the size led to differences in the weight, with the cultivar 'Gab' (thickest fruit) having the highest value and 'Pe' and 'Da' the lowest ones. The shape of the fruits can be estimated by calculating the length/equatorial diameter ratio. This relationship shows that the jujubes 'Gam', 'Gab' and 'Me' presented a practically round shape, with values of 0.99, 0.98 and 1.02, respectively. However, jujubes 'Pe' presented a slightly elongated shape with a value of 1.24 and jujubes 'Da' presented a very elongated shape with a value of 1.66. These weights, dimensions and so varied shapes showed that these jujubes have very different phenotypes that may correspond to different genotypes. These final weights and dimensions were within the normality ranges reported previously in other Spanish cultivars (Almansa et al., 2016; Hernández et al., 2016; Reche et al., 2018; 2019), Korean (Choi et al., 2011), Chinese (Gao et al., 2011; 2012; Wang et al., 2012), Ukrainian (Grygorieva et al., 2014) and Turks (Gündüz and Saraçoğlu, 2014) jujube cultivars.

3.2. Biochemical properties of jujube fruit

The fruits modify their coloration due to the synthesis and degradation of pigments throughout their development and maturation (Almansa et al., 2016; Pék et al., 2010; Reche et al., 2018). All jujubes were harvested at commercial maturity stage; however, slight changes in the contents of pigments were found. Fruits from 'Da' cultivar presented the minimum content of total chlorophylls, both a and b, although they only showed differences with respect to 'Gam' and 'Gab' cultivars (Table 1). Regarding the content of carotenoids, only 'Me' cultivar showed significant differences with respect to the other four cultivars studied, with a maximum content of 0.338 mg eq β-carotene 100 g^{-1} fw.

Table 1

Chlorophylls and carotenoids contents of jujube fruits affected by cultivar

Cultivar	Chlorophyll a (mg 100 g^{-1} fw)	Chlorophyll b (mg 100 g^{-1} fw)	Total Chlorophyll (mg 100 g^{-1} fw)	Carotenoids (mg β-carotene 100 g^{-1} fw)	Protein (mg g^{-1} fw)
'Gam'	0.080 ± 0.014 ab†	0.280 ± 0.044 bc	0.360 ± 0.056 b	0.221 ± 0.008 a	0.34 ± 0.04 a
'Gab'	0.121 ± 0.018 b	0.339 ± 0.031 c	0.427 ± 0.074 b	0.188 ± 0.001 a	0.53 ± 0.01 c
'Me'	0.067 ± 0.015 a	0.246 ±0.022 b	0.312 ± 0.037 ab	0.388 ± 0.039 b	0.49 ± 0.02 c
'Pe'	0.078 ± 0.012 ab	0.214 ± 0.013 ab	0.291 ± 0.024 ab	0.193 ± 0.009 a	0.38 ± 0.02 ab
'Da'	0.049 ± 0.009 a	0.148 ± 0.017 a	0.200 ± 0.023 a	0.247 ± 0.021 a	0.42 ± 0.01 b

†Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) (n=6).

The content of proteins in jujubes ranged from 0.34 ('Gam') to 0.53 mg g^{-1} fw ('Gab'), with differences between cultivars (Table 1). These protein contents were within the normal range for Spanish cultivars (Almansa et al., 2016; Reche et al., 2018), but were lower than those reported in Chinese (Li et al., 2007) and Korean (Choi et al., 2012) cultivars. These differences may be due to different cultivar and environmental conditions.

The 'Pe' cultivar had the lowest value of TSS (17.80ºBrix) with respect to the rest of the cultivars, followed by 'Me' cultivar (22.50ºBrix), 'Gab' and 'Gam' cultivars (23.48 and 24.03ºBrix, respectively) and 'Da' cultivar that presented the highest values (25.08ºBrix) showing differences with all the cultivars (Fig. 2). The reported values of TSS was agree with those found in Spanish (Galindo et al., 2015; Reche et al., 2018; 2019), Chinese (Gao et al., 2011; 2012; Wu et al., 2012) and Turkish cultivars (Gündüz and Saraçoğlu, 2014).

The 'Da', 'Gam' and 'Gab' cultivars had the lowest value of acidity (1.83; 2.12 and 2.23 g 100 g^{-1} fw, respectively) with differences with respect to 'Pe' (3.50 g 100 g^{-1} fw) and 'Me' cultivar which presented the highest value (4.30 g 100 g^{-1} fw) (Fig. 2). The reported values of TA was agree with those found by Hernández et al. (2016) in Spanish cultivar, but were higher than other Spanish (Galindo et al., 2015), Turkish (Gündüz and Saraçoğlu, 2014) and Chinese cultivars (Gao et al., 2011; 2012; Wang et al., 2012).

The 'Da' cultivar showed the highest MI value (13.70 ± 0.25) as it presented the highest TSS content and the lowest TA content, with the jujubes being sweeter with differences compared to the other cultivars studied; however 'Pe' cultivar showed the

lowest MI significant value (5.08 ± 0.20), since it had the lowest TSS content and high TA content. The reported values of TA were agree with those found in Spanish (Hernández et al., 2016) cultivars and lower than other Spanish (Galindo et al., 2015) and Chinese cultivars (Gao et al., 2011).

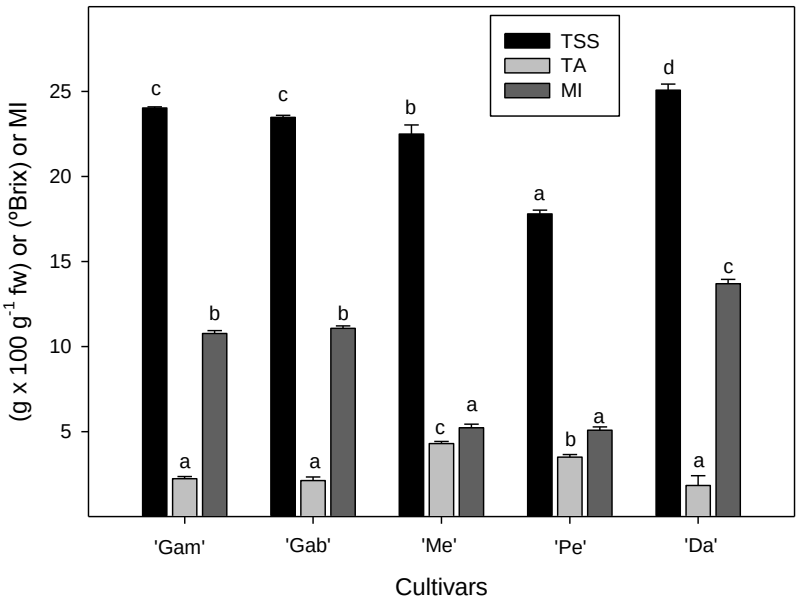

Fig. 2. Main chemical parameters (TSS = total soluble solids (ºBrix), TA = titratable acidity (g 100 g^{-1} fw) and MI = maturity index) at harvest time. Bars (means ± SE) with the same letter, for each quality parameter, were not significantly different according to Fisher´s least significant difference (LSD) procedure at 95% significance level (n = 6).

Table 2

Total phenols, flavonoids and flavonols contents of jujube fruits as affected by cultivar

Cultivar	Total phenols (mg GAE 100 g^{-1} fw)	Total flavonoids (mg rutin eq 100 g^{-1} fw)	Total flavonols (mg rutin eq 100 g^{-1} fw)
'Gam'	371.06 ± 10.12 b†	48.12 ± 2.36 a	28.77 ± 3.67 b
'Gab'	458.23 ± 8.11 c	60.36 ± 1.13 b	23.52 ± 2.54 b
'Me'	469.75 ± 12.88 c	64.45 ± 2.10 b	20.68 ± 4.91 b
'Pe'	351.00 ± 15.72 b	119.56 ± 6.74 c	21.32 ± 3.31 b
'Da'	299.08 ± 9.21 a	46.89 ± 1.35 a	4.21 ± 1.76 a

†Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) (n=6).

3.3. Phenol compounds of jujube fruit

The total phenols content (TPC) showed differences between the cultivars studied. 'Da' cultivar showed the lowest content with 299.08 mg GA 100 g^{-1} fw, followed by 'Pe' and 'Gam' cultivars that presented 351.00 and 371.06 mg GA 100 g^{-1} fw, respectively; whilst 'Gab' and 'Me' cultivars presented the higher contents of TPC, with 458.23 and 469.75 mg GA 100 g^{-1} fw, respectively (Table 2). These values were in the same range as the TPCs found by other authors in jujube fruits (Gao et al., 2011; 2012; Wu et al., 2012; Gündüz and Saraçoğlu, 2014; Reche et al., 2018; 2019) and lower than those obtained by Li et al. (2007). Wojdyło et al. (2016) showed that the predominant compounds among polymeric proanthocyanidins in jujubes cultivars were

the flavan-3-ols phenolics. This study showed higher TPC values in jujube fruits as compared to other fruits, such as cherries (114.56 mg GAE 100 g^{-1} fw), guava (194.11 mg GAE 100 g^{-1} fw), and plum (102.43 mg GAE 100 g^{-1} fw) (Fu et al., 2011). These results have demonstrated the jujubes can be considered a good source of phenolic compounds and, therefore, be good for health, since it was shown that phenols have antimutagenic properties (Tanaka et al., 1998; Valdez-Morales et al., 2014).

The total flavonoids content (TFC) were different in the studied cultivars, being the lowest in 'Da' and 'Gam' cultivars and maximum in 'Pe' cultivar. All the cultivars showed TFC between 12% and 16% of the content of total phenols, except 'Pe' cultivar which TFC was 34% of the TPC (Table 2). These values were similar than other Spanish cultivars (Reche et al., 2018; 2019) and lower than those found in other cultivars of jujubes (Gao et al., 2011; 2012; Wu et al., 2012). Bai et al. (2010) showed that the flavonoids found in *Ziziphus jujuba*, were its main pharmacologically active compounds, and they were responsible for its sedative properties, among other effects.

The total flavonols content (TFoC) was very low in 'Da' cultivar, which was only 8.98% of the TFC content, and presented differences with respect to the other four cultivars studied, whose TFoC were between 17% and 60% the contents of the respective TFC (Table 2). These TFoC contents were in same range of other jujubes cultivars (Reche et al., 2018; 2019) or in greater proportion (Wojdyło et al., 2016).

Table 3

Total antioxidant content of hydrophilic and lipophilic fractions and total antioxidant content for three methods ($ABTS^{\bullet+}$, FRAP and $DPPH^{\bullet}$ of jujube fruits as affected by cultivar

Cultivar	H-TAA (mg Trolox eq 100 g^{-1} fw)	L-TAA (mg Trolox eq 100 g^{-1} fw)	$ABTS^{\bullet+}$, (mmoles Trolox eq kg^{-1} fw)	FRAP (mmoles Trolox eq kg^{-1} fw)	$DPPH^{\bullet}$ (mmoles Trolox eq kg^{-1} fw)
'Gam'	331.31 ± 5.86 a†	149.60 ± 6.20 b	30.01 ± 0.88 a†	54.81 ±3.73 a	217.07 ± 9.43 a
'Gab'	718.22 ± 23.67 d	155.91 ± 3.69 bc	46.52 ± 3.32 ab	79.99 ± 5.40 b	317.17 ± 11.38 b
'Me'	863.79 ± 22.35 e	210.87 ± 4.80 d	33.31 ± 2.43 a	94.23 ± 7.64 bc	231.26 ± 3.84 a
'Pe'	534.11 ± 3.05 c	168.91 ± 3.50 c	56.99 ± 9.23 b	100.92 ± 5.27 c	181.73 ± 29.09 a
'Da'	454.91 ± 7.06 b	96.74 ± 4.52 a	40.94 ± 6.72 ab	54.91 ± 7.48 a	294.79 ± 22.65 b

†Different letters next to a value in each column within cultivar indicate significant differences according to Fisher's LSD test ($p < 0.05$) (n=6).

3.4. Antioxidant activity of jujube fruit

The total antioxidant activity was quantified in jujube fruits by three methods: FRAP, $ABTS^{\bullet+}$, and $DPPH^{\bullet}$. 'Gam' and 'Me' cultivars had the lowest antioxidant activity by the $ABTS^{\bullet+}$ method, being the maximum in 'Pe' cultivar. However, 'Gam' and 'Da' had the lowest FRAP activity, while the 'Pe' presented the maximum value. By $DPPH^{\bullet}$ method, 'Gab' and 'Da' presented the maximum values with differences with respect to the other cultivars (Table 3). These values were in the same range as other Chinese (Gao

et al., 2012; Wu et al., 2012) and Spanish cultivars (Wojdyło et al., 2016). These differences between cultivars were also found by other authors in jujubes (Choi et al., 2012; Gao et al., 2012; Reche et al., 2018; 2019). In this study, the differences observed are due to the genotype, since all the cultivars were cultivated in the same geographical area and agronomic conditions. The H-TAA presented minimum values in 'Gam' cultivar followed by 'Da' while the maximum values were presented by 'Me' cultivar. The L-TAA presented minimum values in 'Da' cultivar, followed by 'Gam' and 'Gab', while 'Me' presented the maximum values (Table 3).

3.5. Correlation between traits and principal coordinates analysis

Those traits that measured chlorophylls content had a very close positive correlation among them, as well as all of them had a positive correlation with physic traits (fruit weight and diameter/length ratio). The diameter/length ratio was correlated positively with fruit weight, but also with phenols, flavonols and L-TAA. H-TAA had important positive correlation with phenols, protein and L-TAA, while L-TAA was positively well correlated with TA, FRAP and phenols. Flavonoids had a negative correlation with TSS and, finally, FRAP was positively well correlated with TA and negatively with TSS (Fig. 3).

In order to gain a better understanding of the results and trends of the variables studied (17 traits and 5 jujube cultivars), the main components analysis (PCA) was applied and the results were showed in Table 4 and Fig. 4.

The first three main components accounted for 77.34% of the total variation for the results obtained in the jujube fruit, and the 58.7% of the variability of the data studied were explained by the first two components. PC1 and PC2 from the PCA explained the

34.7 and the 24.0% of the variability, respectively (Table 4). The first component (PC1) was positively related to diameter/length, phenols, chlorophyll b, total chlorophylls and L-TAA. The PC2 was positively correlated with TA, and was negatively correlated with TSS and $DPPH^{\bullet}$. The PC3 was positively correlated with carotenoids and was negatively correlated with flavonoids, standing out above the other traits (Table 4). PCA clearly distinguished 'Da' cultivar from the others, based in PC2, with negative values. 'Pe' and 'Me' cultivars formed another group, similarly to 'Gam' and 'Gab'. These two groups distinction was based in PC1, and they had positive values for 'Me' and 'Pe' and negative values for 'Gab' and 'Gam' cultivars (Figure 4).

3.6. ISSR analysis

The five jujube cultivars were amplified consistently with 8 of the 18 primers (Table 5 and Figure 1 supplementary material). The number of products generated per primer was found to range from 3 to 8 of different sizes in the range of 0.18 to 2.2 kb (Table 5). The primer UBC807 exhibited the maximum (8) products whereas primers UBC825 and ISSR2 gave the least (3) number of products. A total of 34 amplified products were produced with an average of 4.25 products per primer, of which 16 (47%) were polymorphic and 18 (53%) products were monomorphic (Table 5). The percentage of polymorphic bands ranged from 25% for primer UBC820 to 100% for primer ISSR15. These ISSR primers gave a high PIC value of 0.38 for primer ISSR15 and low PIC value of 0.080 for primer UBC820, with an average PIC value of 0.197 per primer. An average RP of 1.125 per primer was obtained with the highest RP value of 2.00 being that of primers UBC817 and ISSR15, and the lowest value of 0.40 for primer UBC820 (Table 5). The values obtained were lower to those obtained in other works with ISSR, such as those of Paolini et al. (2009) in *Cistus*, Saifi et al. (2011) and Al-

Safadi et al. (2014) in *Capparis* and Lamare and Rao (2015) in *Musa*. This result could be attributed to the low genetic diversity of the jujube cultivars, and the studied samples in each work. The other works included samples of different species, while this work included plants of only one specie. The inclusion of several species in the study usually produces an increase in the genetic variability found with molecular markers. For this reason, some studies distinguish between variability obtained between accessions of different species and the variability obtained among the accessions of the cultivated species (Park et al., 2004; Blanca et al., 2012; Blanca et al., 2015).

The dendrogram obtained by the ISSR data appears in Figure 5. The Nei's similarity coefficient ranged from 0.80 to 1. In the dendogram, all the nodes were supported by bootstrap values near or higher than 50%. In the dendrogram the 'Da' cultivar was clearly distinguished from the rest. 'Gam' and 'Gab' cultivars were the most similar, as showed by their grouping, with a bootstrap of 76.0. 'Pe' and 'Me' were between the previous group and 'Da' cultivar.

Figure 6 shows the PCA obtained with the ISSR results. The first two main principal components (PC1 and PC2) explained the 46.4 and the 30.3% of the variability, respectively. The cultivar distribution was very similar to that of the dendrogram, where 'Da' cultivar was separated from the rest, and 'Gab' and 'Gam' cultivars were the nearest.

3.7. Physico-chemical and bioactive compounds and ISSR markers comparison

Comparing the PCA obtained with physico-chemical and functional compounds and ISSR results (Fig. 4 and 6, respectively), we shall observed that although the cultivar distribution was not identical, figures were very similar. In both cases, 'Da'

cultivar was separated of the rest, while 'Gab' and 'Gam' cultivars were the closest. The 8 ISSR markers studied allow distinguishing the cultivar with greater differences in physico-chemical traits and bioactive compounds, as well as the closest ones. Similar grouping for morphological, composition and molecular markers were obtained previously in *Cistus* (Paolini et al., 2009) and in almond (Kadkhodaei et al., 2011).

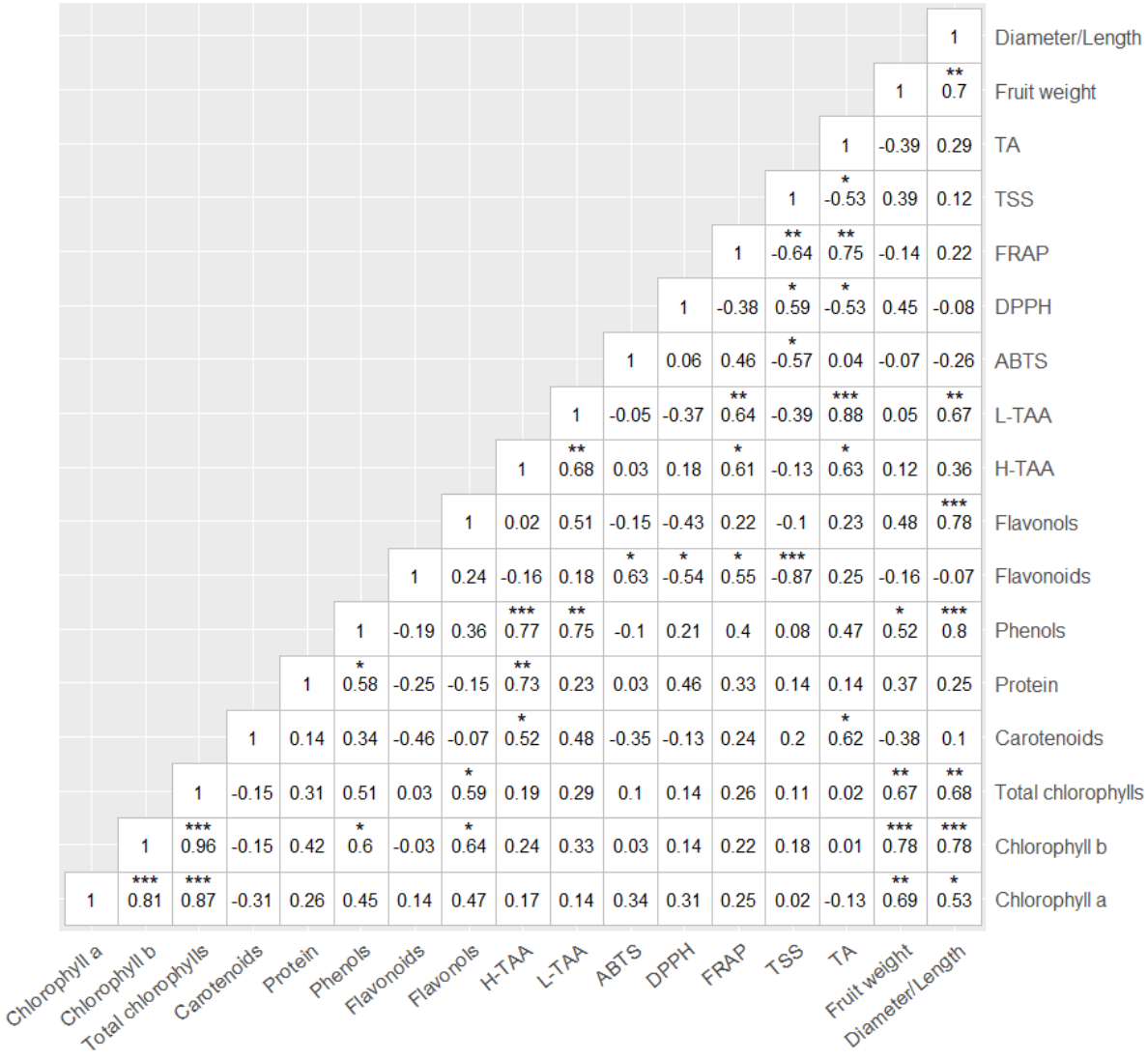

Fig. 3. Pearson coefficient of the correlations between traits. Positive correlations were represented by positive values, and negative correlations by negative values. The

significance level of correlations is represented by * (p-value < 0.5), ** (p-value < 0.01) and *** (p-value < 0.001).

Table 4

Eigenvectors of each variable in the three first Principal Components

	PC1	PC2	PC3
Eigenvalues	5.89	4.18	3.07
Cumulation proportion of variation (%)	34.65	59.26	77.34
Characters		**Eigenvectors**	
Chlorophyll a	0.69	-0.36	-0.46
Chlorophyll b	0.85	-0.37	-0.25
Total chlorophylls	0.79	-0.33	-0.32
Carotenoids	0.16	0.33	0.81
Protein	0.52	-0.16	0.36
Phenols	0.88	-0.02	0.34
Flavonoids	0.04	0.60	-0.75
Flavonols	0.64	0.03	-0.34
H-TAA	0.64	0.28	0.53
L-TAA	0.74	0.55	0.24
$ABTS^{+\bullet}$	0.03	0.29	-0.58
$DPPH^{\bullet}$	0.02	-0.72	0.24
FRAP	0.54	0.71	-0.11
TSS	-0.06	-0.82	0.47
TA	0.44	0.82	0.31
Fruit weight	0.62	-0.68	-0.22
Length/diameter	0.88	-0.14	0.01

4. Conclusion

The results of this study showed that the physico-chemical and bioactive compounds were different in the five cultivars of Spanish jujubes studied in this work. The PCA grouped the four type of cultivars into 3 groups: (i) the first formed exclusively by the cultivar 'Da', (ii) the second formed by the largest and round cultivars, 'Gam' and 'Gab', and (iii) and the third formed by the medium 'Pe' and 'Me' cultivars. The genetic analysis with ISSR markers showed that was able to differentiate the four type cultivars studied, so they were useful for genetic studies in the *Ziziphus jujuba* species. The dendrogram obtained with the ISSR results showed that there was a well-defined group formed by 'Gam' and 'Gab', with a bootstrap of 76.0. 'Da' cultivar was ungrouped with a bootstrap of 62.4 and the other two cultivars were between these two groups. Therefore, the similarity between the genetic dendrogram and the PCA made with the physical-chemical and functional traits has been very high, since the group formed 'Gam' and 'Gab' was genetically confirmed and the distance of 'Da' cultivar from the other groups was also genetically confirmed. The only difference between the PCA based on the 17 traits and the PCA based on the genetic study was the group formed by 'Me' and 'Pe', which in the first PCA were grouped while in the genetic study they were not. This difference could be due to the fact that 8 ISSR markers have been studied and may not be enough to distinguish this group. Therefore, the ISSR markers used in this work were useful to classify cultivars of jujubes and can be used in future breeding programs to select the most interesting plants.

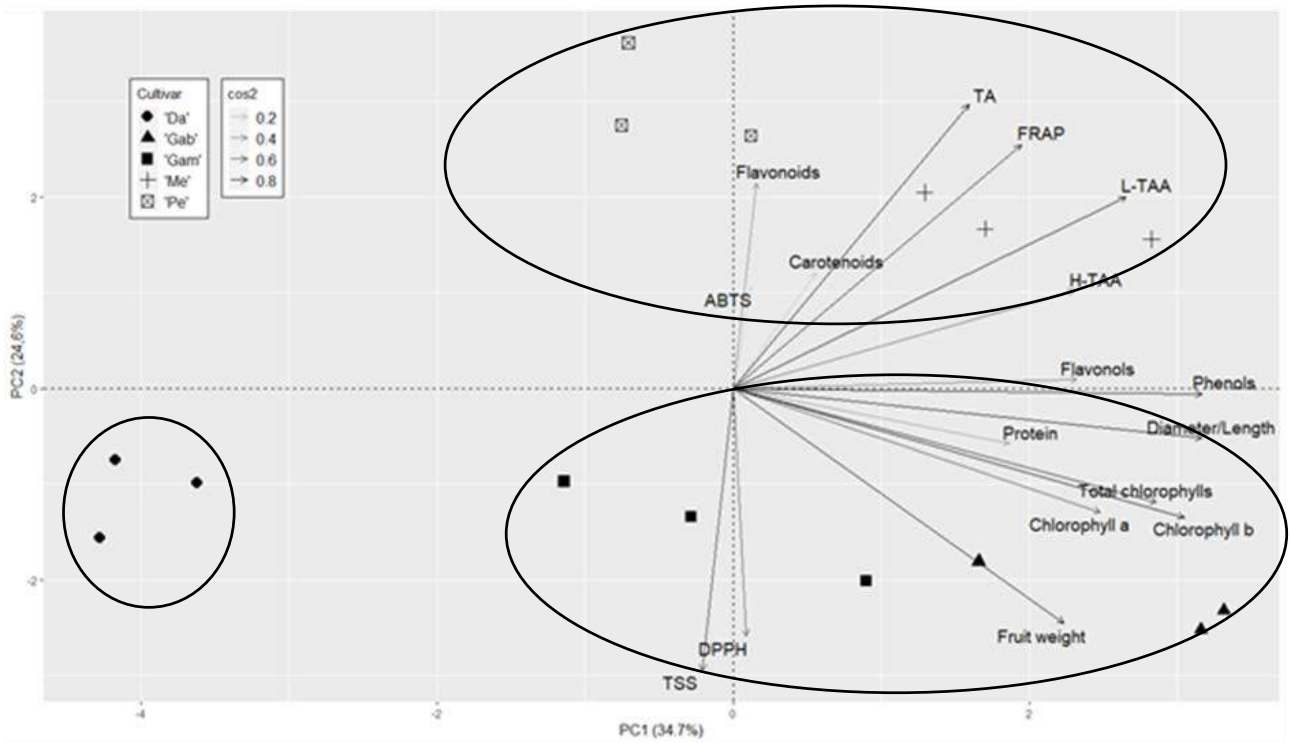

Fig. 4. Principal Component Analysis. Variables were plotted according their Cos2 value, which indicates the quality of representation.

Table 5

Genetic variation obtained with ISSR markers

Marker	Number of bands	Size range (pb)	MR	PIC	RP
UBC807	8	400-1600	2	0.120	1.20
UBC817	4	520-1100	3	0.320	2.00
UBC820	4	1200-2100	1	0.080	0.40
UBC825	3	900-2200	1	0.125	0.50
ISSR2	3	500-680	1	0.125	0.50
ISSR14	4	180-750	1	0.120	0.80
ISSR15	4	310-850	4	0.380	2.00
ISSR43	4	400-750	3	0.280	1.60
Total	34	ND	16	ND	ND
Average	4.25	ND	2.00	0.194	1.125

MR: multiplex ratio; RP: resolving power; PIC: Polymorphic Information Content; ND: Not determined.

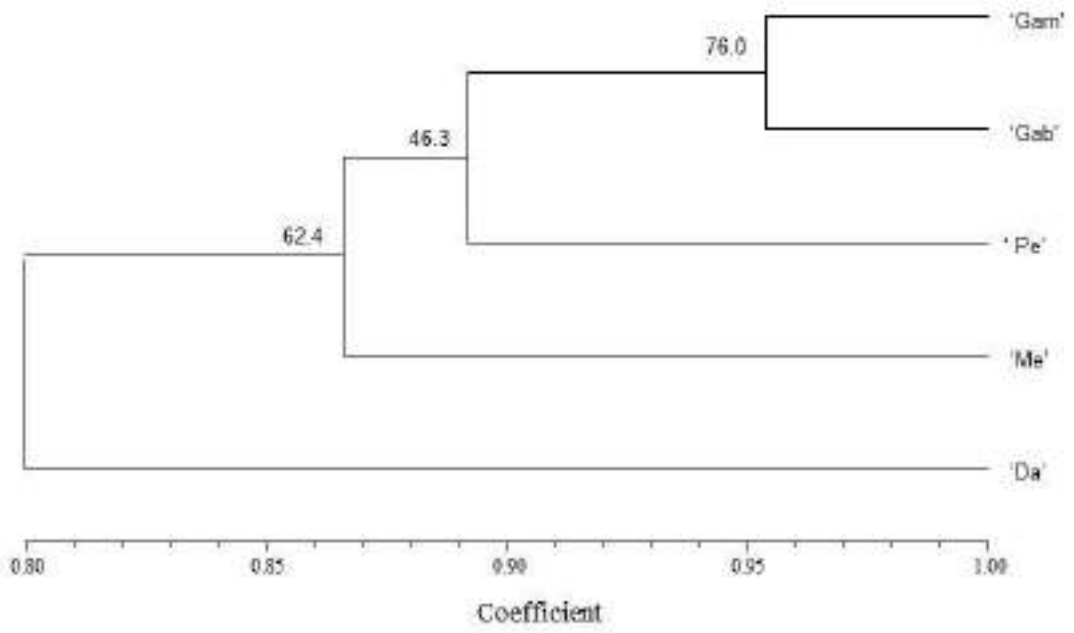

Fig. 5. Dendrogram obtained using ISSR markers based on Nei and Li (1979) distance and UPGMA method. Percentages of 1000 bootstrap replications were given for each node.

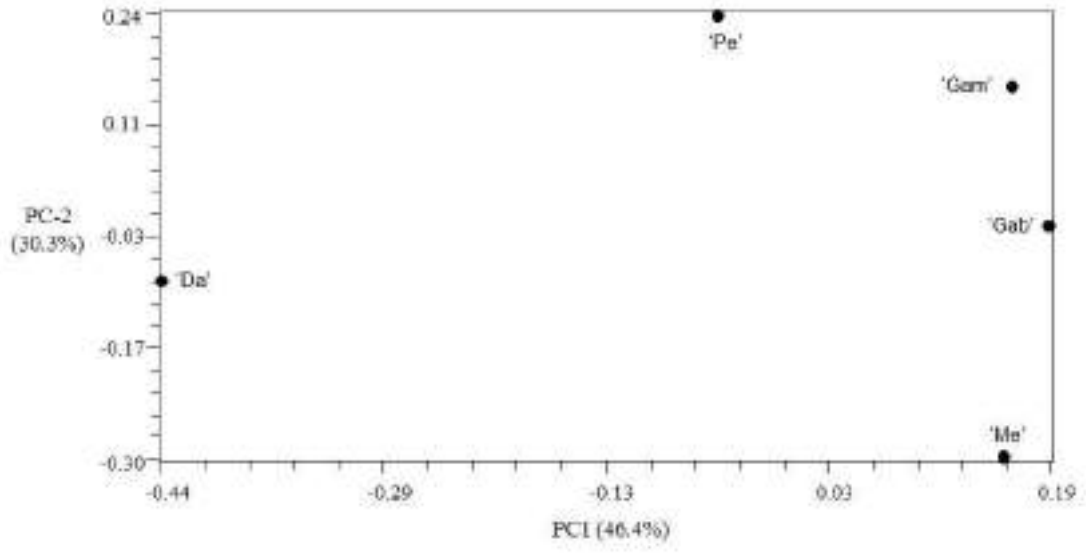

Fig. 6. Principal component analysis (PCA) using the ISSR results.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scientia.2019.04.068.

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4.1. Publicación 4

PUBLICACIÓN 4
Fatty acid profile of peel and pulp of Spanish jujubes (*Ziziphus jujuba* Mill.) fruit
Juana Reche, María Soledad Almansa, Francisca Hernández, Ángel A. Carbonell-Barrachina, Pilar Legua y Asunción Amorós. ***Food Chemistry 2019, 295, 247-253*** ***doi: 10.1016/j.foodchem.2019.05.147***

PUBLICACIÓN 4: TRANSCRIPCIÓN LITERAL

Fatty acid profile of peel and pulp of Spanish jujubes (*Ziziphus jujuba* Mill.) fruit

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Abstract

The fatty acid methyl esters (FAMEs) profiles of fruit peel and pulp of 4 Spanish cultivars of *Ziziphus jujuba* were studied. The FAMEs profile of the cultivar 'GAL' was studied under two farming practices, (i) organic, 'GAL-E' and conventional, 'GAL-T'. Eleven FAMEs were identified, the predominant ones were *cis*-palmitoleic acid (pulp) and oleic and palmitic acid (peel). When comparing the FAMEs profiles between organic and conventional 'GAL' peel jujube fruits, the 'GAL-E' (organic) presented a higher unsaturated/saturated ratio than the 'GAL-T' cultivar (conventional), while this relationship in the pulp was the opposite. The 'DAT' cultivar was interesting for its high palmitoleic acid content while the 'GAL-E' and 'GAL-T' cultivars had high contents of oleic, palmitic and linoleic acids. The LDA (linear discriminant analysis) model showed that the FAMEs contents could be used to discriminate the studied cultivars, and the agricultural practice, because all groups were clearly separated with no overlaps.

Keywords:

Linoleic acid

Oleic acid

MUFAs

Palmitic acid

Palmitoleic acid

PUFAs

1. Introduction

Jujube (*Ziziphus jujuba* Mill.) belongs to the Rhamnaceae family and contains approximately 40 species (San & Yildirim, 2010). This tree was native of China and was widely distributed in the warmer parts of Asia, Australia, Russia, northern Africa, southern Europe, the Middle East and southwestern USA (Almansa, Hernández, Legua, Nicolás-Almansa & Amorós, 2016). The jujube can be consumed fresh, dried and processed (jams, loaf, cakes, etc.) (Elaloui et al., 2014).

Fatty acids are essential, some are synthesized in the body, but others must be ingested through food for a healthy life because they cannot be produced in the human body (Guil-Guerrero, Díaz, Matallana & Torija, 2004). Among the essential fatty acids, the best known are linolenic acid (omega 3) and linoleic acid (omega 6). These acids are not synthesized by the human body and are of vital importance (Guil-Guerrero et al., 2004), and must be ingested through a healthy diet rich in fruit, although their major sources are fruit seeds. They are an important source of energy and for a balanced diet it is advisable to avoid taking fatty acids from the family of "saturated and *trans*" and to eat in a greater quantity those that are polyunsaturated or monounsaturated. Oleic acid is directly related to anti-inflammatory drugs (Massaro & De Caterina, 2002), and together with linoleic acid offer a large amount of pharmacological activities, including playing an important role in cancer treatment (Lee, Lee, Cho & Kim, 2005), increased exercise capacity (Mizunoya, Haramizu, Shibakusa, Okabe & Fushiki, 2005), and also benefits bone structure in menopausal women (Brownbill, Petrosian & Llich, 2005).

Only few studies have evaluated the fatty acid composition in jujubes. Zhao et al. (2006) studied it in the stone of Chinese jujube fruits while Guil-Guerrero et al. (2004), San & Yildirim (2010), Elaloui et al. (2014), Yamamoto, Shibahara, Sajuma, Nakayama & Kajimoto (1990) and Song, Bi, Chen, Wu, Lyu & Meng (2019) studied their profile

in the edible portion of jujube fruits. It seems that there are significant differences among the contents of various bioactive compounds in peel and pulp of the jujube fruits (Xue, Feng, Cao, Cao & Jiang, 2009; Zhang, Jiang, Ye, Ye & Ren, 2010), but this has never proved before for FAMEs (fatty acid methyl esters). Because the content of fatty acids may vary among cultivars, it would be very interesting to expand the available information on nutritional data of *Ziziphus jujuba* fruits by studying the cultivar effect.

On the other hand, organic food is associated, in general, by consumers with improved nutritional properties, as well as with non-contaminating sustainable agricultural practices. Many consumers believe that organic foods are healthier than conventionally products and that they are more environmental friendly (Zanoli & Naspetti, 2002). However, comparison of fatty acid profiles in organic and conventional jujube fruits has not been yet published.

Considering all the above, the aim of this work was to study, for the first time, the fatty acid profile of peel and pulp of jujube fruits. This fruit could be eaten with or without peel, and its functional properties may be different; thus, the profiles of peel and pulp were studied. In addition, the effect of organic farming on the fatty acid profile was also studied. All cultivars studied were from Spain.

2. Materials and methods

2.1 Chemicals and reagents

n-Hexane, methanol, methylene chloride, and boron trifluoride (BF_3) reagent were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and sodium hydroxide and anhydrous sodium sulfate were purchased from Panreac (Castellar del Vallès, Barcelona, Spain). Identification of peaks was made by comparison with FAME standards from Sigma-Aldrich Chemie GmbH (Steinheim, Germany): (9*Z*,12*Z*)-octadeca-9,12-dienoic or linoleic acid (C18:2), (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic or linolenic acid (C18:3), (5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoic or arachidonic acid (C20:4), (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-5,8,11,14,17-pentaenoic or eicosapentaenoic acid (C20:5), (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)- docosa- 4,7,10,13,16,19- hexaenoic or docosahexaenoic acid (C22:6), and heptadecanoic acid (C17:0). All other chemicals and reagents were of analytical grade.

2.2. Plant materials

Fruits of 4 cultivars of *Ziziphus jujuba* Mill., named 'GAL-E' from organic agriculture and 'GAL-T', 'MSI', 'PSI' and 'DAT' from conventional agriculture, were collected in August 2016 and 2017 from a commercial and organic farm located in San Isidro (latitude 38º10’22,29’’N x longitude 0º51’36,138’’W, 19 m above sea level). Jujube trees were 20 years old, were trained in vase structure, spaced at 4 m × 4 m, and were grown under similar conditions of irrigation, fertilization, and pest control, except the 'GAL-E' cultivar whose fertilization was organic and was grown without pest control. For each cultivar, 100 fruits from 5 trees (20 fruits *per* tree) were hand-harvested each year and immediately transported under ventilated conditions to the laboratory. The jujube fruits were randomly selected in the optimum stage of maturity

for commercialization, corresponding to a minimum total soluble solids of 15 ºBrix (Reche et al., 2018), to perform the analytical determinations. For the determination of total soluble solids (TSS), titratable acidity (TA) and maturity index (MI), 3 batches of 10 fruits were made for each cultivar (4 cultivars plus 1 organic = 5) and 3 repetitions *per* batch/year were made by sample in two years (n=18). These main characteristics of the studied cultivars at harvest are shown in **Fig. 1** of supplementary material. The MI was obtained from the relationship between TSS/TA, and results were expressed as the mean ± standard error. On the other hand, 3 samples of peel or pulp from 20 fruit each one were dried in a hot air oven at 50 ºC until reaching constant weight and, then, milled. The results of fatty acids contents (FAMEs) were expressed as the average of the two years (3 replicates x 2 years, n=6).

2.3. Fatty acid extraction

2.3.1. Methylation procedure

Fatty acids were methylated *in situ* using the method described by Trigueros & Sendra (2015), with some modifications. After grinding 0.5 g of dried jujube (peel or pulp) fruit were transferred into a test tube. In each tube, 60 µL of C17:0 in n-hexane solution (20 mg mL^{-1} solution in HPLC grade *n*-hexane) was added as internal standard. At this point, 100 µL of methylene chloride and 1 mL of 0.5 N NaOH in methanol were added, and, the tubes were heated in a water bath at 90 ºC for 10 min. Then, 1 mL of BF_3 in methanol was added and the mixture was left at room temperature (25 ºC) for 30 min. Later, 1 mL of distilled water and 600 µL hexane were added, and then, the FAMEs were extracted by vigorous shaking for about 1 min. Following centrifugation, aliquots were dried with anhydrous sodium sulfate and the top layer was transferred into a vial flushed with nitrogen and stored at -20 ºC until analyzed.

2.3.2. GC-MS: gas chromatography analysis

The FAMEs were identified, and semi-quantified using GC-MS in SCAN mode with a SupraWax-280 column, 100 % polyethylene glycol (Teknokroma S. Co. Ltd., 165 Barcelona, Spain; 30 m × 0.25 mm × 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.1 mL min^{-1}. The oven program was: (i) initial temperature 80 ºC, held for 2 min, (ii) rate of 8.0 ºC min^{-1} to 160 ºC; (iii) rate of 4 ºC min^{-1} from 160 to 220 ºC and held for 13 min, and (iv) rate of 10 ºC min^{-1} from 220 to 260 ºC and held for 6 min. Injector and detector temperatures were held at 230 and 260 ºC, respectively; and, 0.5 µL of the extract was injected. Identification of the FAME peaks was performed by comparing (i) the retention times of the FAME standards from Sigma-Aldrich, and (ii) comparing their mass spectra with those of the Wiley 09 MS library (Wiley, New York, NY, USA) and NIST14 (Gaithersburg, MD, USA) mass spectral databases. The results were expressed as percentage of total fatty acids because this is the first study on this topic, but in later studies full-quantification will be conducted using GC-MS in SIM mode.

2.4. Statistical analysis

Statistical analyses were performed using SPSS 22.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance (ANOVA) test for mean comparisons. The method used to discriminate among the means (Multiple Range Test) was Fisher's Least Significant Difference (LSD) procedure at a 95.0% confidence level. Principal component analysis (PCA) was also performed using SPSS and the linear discriminant analysis (LDA) was carried out to discriminate the fatty acid content according to the cultivars. Each value was the mean of 6 replications, 3 replications *per* year and 2 years of experiment (n=6).

3. Results and discussion

3.1. Fatty acids composition in the pulp of jujube fruits

Table 1 shows the results of the FAMEs profile detected in the jujube pulp. A total of 11 compounds were found: myristic acid, myristoleic acid, palmitic acid, *trans*-palmitoleic acid, *cis*-palmitoleic acid, stearic acid, oleic acid, 11-octadecenoic acid, elaidic acid, linoleic acid and linolenic acid. The most abundant acid in the pulp was the *cis*-palmitoleic acid and was found in three cultivars (27.51% in 'DAT', 32.17% in 'GAL-E' and 32.47% in 'PSI' cultivars, respectively), followed by oleic acid (22.64% in 'GAL-T' and 20.36% in 'MSI') and palmitic acid with 21.47% in 'GAL-E' cultivar. The values of these FAMEs in the Spanish cultivars were almost the double of those reported by Guil-Guerrero et al. (2004) in the cultivar 'Lang' from Ciudad Real (Spain) and by San & Yildirim (2010) in '20-Ç-51' cultivar, which showed values very similar to those obtained for 'GAL-T' and 'MSI' cultivars, with a mean value of approximately 18%.

The oleic acid appeared in all four cultivars, and was the second most abundant acid; its content was 22.64% and 20.36% in 'GAL-T' and 'MSI' cultivars, respectively. The third most abundant compound in the pulp of jujube was the palmitic acid, although it had the highest content in the 'GAL-E' cultivar (21.47%), followed by the rest cultivars with decreasing contents until reaching the lowest value, 8.45% in the 'DAT' cultivar. These values were similar to those obtained by San & Yildirim (2010). The 'DAT' cultivar had a very similar content to that obtained by Guil-Guerrero et al. (2004) for the 'Li' variety cultivated in Ciudad Real. In contrast, the rest of cultivars showed very close contents to those found by Elaloui et al. (2014) when studying Turkish cultivars.

Table 1. Fatty acid composition of the pulp of jujube fruits as percentage of total fatty acid profile

Fatty acid	'GAL-E'	'GAL-T'	'MSI'	'PSI'	'DAT'
Tetradecanoic acid or myristic acid, C14:0	0.91±0.01Aa[†]	Nd	Nd	1.37±0.57Aa	0.89±0.19Aa
(*Z*)-Tetradec-9-enoic acid or myristoleic acid, C14:1	3.54±2.09Aa	Nd	Nd	4.57±1.82BCa	6.2±0.66Ba
Hexadecanoic acid or palmitic acid, C16:0	21.47±1.86Dc	16.4±0.71Cb	14.89±1.40BCb	17.65±1.15Ebc	8.45±1.85Ba
(*E*)-Hexadec-9-enoic acid or *trans*-Palmitoleic acid, C16:1	10.13±1.79Ba	11.07±0.15Ba	16.16±0.49BCb	19.67±0.67Eb	17.82±1.82Db
(*Z*)-Hexadec-9-enoic acid or *cis*-palmitoleic acid, C16:1	32.17±1.19Dc	17.77±0.71CDa	18.56±1.58CDa	32.47±1.36Fc	27.51±0.54Eb
Octadecanoic acid or stearic acid, C18:0	3.17±0.56Aab	4.46±0.83Ab	4.8±1.41Ab	3.76±0.15ABab	1.79±0.14Aa
(*Z*)-Octadec-9-enoic acid or oleic acid, C18:1n9c	10.52±1.43Ba	22.64±1.02Eb	20.36±0.57Db	8.42±1.17Da	7.51±0.49Ba
(*Z*)-Octadec-11-enoic acid 11-Octadecenoic acid, C18:1n7	Nd	3.9±0.43Aa	6.85±1.25Ab	3.33±1.15ABa	12.03±0.45Cc
(*E*)-Octadec-9-enic acid or elaidic acid, C18:1n9t	Nd	Nd	Nd	Nd	7.01±0.01Ba

(9*Z*,12*Z*)-Octadeca-9,12-dienoic acid or linoleic acid, C18:2	17.07±1.51Cb	19.49±0.60Db	4.04±1.90Aa	7.17±1.07CDa	7.67±0.19Ba
(6*Z*,9*Z*,12*Z*)-Octadeca-6,9,12-trienoic acid or linolenic acid, C18:3	Nd	4.25±0.57Ab	12.71±1.14Bc	1.59±0.01ABa	2.91±0.19Aab
Total PUFA	17.07	23.74	16.75	8.76	10.58
Total MUFA	57.36	55.38	61.93	68.46	78.08
Total SFA	25.55	20.86	19.69	22.78	11.13
USFA/SFA	2.91	3.79	4.00	3.39	7.97

[†]Values (means ±SE) followed by the same letter, within the same row, were not significantly different according to Fisher´s least significant difference (LSD) procedure at 95% significance level (n = 6). Different capital letters showed differences between fatty acids in the same cultivar; different lowercase letters showed differences in the percentage of each fatty acid between the different cultivars. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. Nd: not detected. U/S, unsaturated/saturated fatty acids.

trans-Palmitoleic acid also presented a high percentage in 'PSI' cultivar (19.67%), followed by 'DAT' cultivar (17.82%) and 'MSI' (16.16%). These last two cultivars presented values very close to those of '20-Ç-52' cultivar (San & Yildirim, 2010). In contrast, 'GAL-E' and 'GAL-T' cultivars presented much lower values, ~10-11% in 'GAL-E' and 'GAL-T'; the similarity in these last values was due to the fact that they are the same cultivar but growing under different farming systems. These values were very similar to those reported in '20-Ç-10' and '20-Ç-51' cultivars (San & Yildirim, 2010).

The 'GAL-T' cultivar had a high linoleic acid (precursor of the omega 6 acids) content with a value of 19.49% followed by the 'GAL-E' cultivar with a value of 17.07%. These values presented for 'GAL' cultivars were practically the same as those reported in the 'Choutrana' cultivar, with a values of 18.1% (Elaloui et al., 2014), but they were slightly lower than those obtained by San & Yildirim (2010) in '20-Ç-10', '20-Ç-22' and '20-Ç-51' cultivars, with contents ranging between 17% and 34%. The rest of the analyzed cultivars presented lower values, 4.04% for 'MSI' cultivar, which was similar to that obtained by Guil-Guerrero et al. (2004) for an unknown jujube cultivar (Castilla-La Mancha, Spain), with had a value of 4.97%. The 'PSI' and 'DAT' cultivars presented linolenic values of 7.17% and 7.67%, respectively; these results were very similar to those obtained by Guil-Guerrero et al. (2004) for 'Li' and 'Gorda' cultivars from Ciudad Real and Castellón, respectively.

The stearic acid content for the jujube pulp was similar for all analyzed cultivars and close to the content of the jujube fruits reported by San & Yildirim (2010). In 'GAL-E' cultivar, 11-octadecenoic acid was not detected; however, for the rest of the cultivars it was detected with values ranging between 3.33% for 'PSI' cultivar and 12.03% for 'DAT' cultivar. The linolenic acid (the acid base for the synthesis of all

omega 3) showed its highest content in the 'MSI' cultivar, with a percentage of 12.71%; in contrast, the rest of cultivars showed significant lower contents until being not detected in the 'GAL-E' cultivar. Most of these linolenic contents in the Spanish jujube pulp were higher than those reported by Elaloui et al. (2014), which values ranged between 0.56-0.85%. The 'DAT' cultivar was the only one in which the elaidic acid was detected in the jujube pulp. Elaloui et al. (2014) only found this acid in 'Sfax' cultivar but with a lower value than in this Spanish cultivar. On the other hand, Guil-Guerrero et al. (2004) and San & Yildirim (2010) did not found it in the analyzed cultivars. The 'DAT' cultivar presented similar values to 'PSI' but not for 11-octanodecenoic acid, which had showed a higher value of this acid than in the rest of the cultivars. The value for 'DAT' was 12.03% and it was quite higher than those reported in other similar studies of jujube (Guil-Guerrero et al., 2004; San & Yildirim, 2010). On the other hand, myristic and myristoleic acids were only detected in 'GAL-E', 'PSI' and 'DAT' cultivars but with very low values, and were not detected in 'GAL-T' and 'MSI' cultivars. Myristoleic acid was detected in jujube in greater percentage by Yamamoto et al. (1990) and with similar values and Elaloui et al. (2014) in 'Mahres' cultivar.

On other hand, when comparing the FAMEs profiles of organic and conventional 'GAL' jujube fruits, 'GAL-E' (organic) presented a higher percentage of palmitic and *cis*-palmitoleic acid than the 'GAL-T' cultivar. The acid omega 7, (*cis*-palmitoleic acid) is interesting for their participation in controlling cholesterol and triglyceride levels, as well as in maintaining glucose levels (Mozaffarian et al., 2010). However, the oleic acid content was significantly higher in the 'GAL-T' fruits. The highest PUFAs content was presented in the 'GAL-T' cultivar; thus, this cultivar was interesting for its high oleic and linoleic acid contents.

On the other hand, the results showed a wide range of saturated fatty acids (SFAs) from 11.13% to 25.55%, corresponding the highest value to 'GAL' and 'PSI' cultivars. Monounsaturated fatty acids (MUFAs) ranged from 55.38% to 78.08%, with the values being the lowest for the 'GAL' cultivar and the highest for the 'DAT' cultivar. Polyunsaturated fatty acids (PUFAs) values ranged from 8.76% to 23.74% in the pulp of jujube fruit. The most important SFAs were palmitic and stearic acids, while, oleic and *cis*-palmitoleic acids were the predominant MUFAs. Finally, only two PUFAs were found, linoleic and linolenic acids. This high abundance of unsaturated (MUFAs and PUFAs) fatty acids (74.43% to 88.66%) was also observed in jujube fruits of 'Choutrana', 'Mahres', 'Mahdia' and 'Sfax' cultivars (68.5% to 72.4%) (Elaloui et al., 2014).

The jujube pulp had a high MUFA content, with the cultivar 'DAT' having the highest value and one of the lowest values of both PUFA and SFA. The 'GAL-E' cultivar with a low MUFA value (57.36%), presented a similar content to that of 'Mahres' cultivar and the same of 'GAL-T'. The percentage of PUFAs in the pulp of the Spanish jujube fruits was similar to those reported by Elaloui et al. (2014). On the other hand, the percentage of MUFAs and SFAs had similar values to those determined by San & Yildirim (2010). These results were very similar to those obtained by Yamamoto et al. (1990), where the fresh jujube cultivars analyzed had a high content of monounsaturated acids. As a simple comparison, PUFAs in avocado and olives were predominant as compared to MUFAs (Martínez & Moreno, 1995). The differences observed could be due to plant origin, genetic factors, ripening of fruits, environmental conditions, and temperature during the time elapsed between flowering and ripening (Sánchez-Salcedo, Sendra, Carbonell-Barrachina, Martínez & Hernández, 2016). The MUFAs are more stable than PUFAs and show great health benefits; they strengthen the

immune system, help in preventing diabetes and even help in cancer prevention processes (San & Yildirim, 2010).

The unsaturated/saturated (USFA/SFA) ratio took high values in the pulp of jujube fruits, ranging between 2.91% for 'GAL-E' and 7.97% for 'DAT'. These values were much higher than those obtained by Elaloui et al. (2014) in Tunisian cultivars, which values ranged between 1.68% to 2.37%, and those obtained by San & Yildirim (2010) in selections from Turkey, which values ranged between 2.27% to 2.73%. These values of the USFA/SFA ratio supported the healthy potential of the Spanish jujube pulp according to their fatty acid composition.

3.2. Fatty acids content in the peel of jujube fruit

Ten fatty acids were detected in the peel (Table 2). The same fatty acids were detected as in the pulp except elaidic acid that was not presented in the peel. The values obtained for the peel were somewhat similar to those of pulp, because the predominant FAME was *cis*-palmitoleic, with values in the interval 15.43-33.12%, similar to the contents reported by San & Yildirim (2010); the 'DAT' cultivar presented the highest value with 33.12%.

Oleic acid was the next most abundant acid contained in the peel of the jujube cultivars tested with values of 29.00% for 'GAL-E' cultivar followed by 'MSI' cultivar with 22.51%, 'GAL-T' and 'PSI' cultivars with very similar values (17.5 and 16.7%, respectively) and the lowest value (8.86%) in 'DAT' cultivar. These values were higher than those found in the fruit pulp. *trans*-Palmitic acid also showed a high percentage with values of 20.51% in 'PSI' cultivar and very similar values for 'GAL-T' and 'DAT' cultivars with 16.41% and 16.79%, respectively. In the peel, the linolenic acid was only

detected in 'MSI' and 'DAT' cultivars, with values of 5.63% and 3.94%, respectively. There were higher percentages of both MUFAs and PUFAs in the peel of the 'GAL-E' cultivar as compared to the same cultivar but under organic farming ('GAL-T'). The 'MSI' cultivar presented the highest PUFA content. In terms of MUFA content, all the cultivars had similar values, but the 'DAT' cultivar stood out because of the high content in *trans*-palmitoleic acid. This cultivar was harvested in the month of October; this is two months later than the rest of the cultivars, because it is a late harvesting cultivar. This fact could be behind the differences in fatty acid content, or at least in part, as compared to the other cultivars. Therefore, the peel of 'DAT' was interesting for its high content of *trans*-palmitoleic acid, obtaining health benefits from its consumption.

Table 2. Fatty acid composition of peel of jujube fruits as % of total fatty acid profile

Fatty acid	'GAL-E'	'GAL-T'	'MSI'	'PSI'	'DAT'
Tetradecanoic acid or myristic acid, C14:0	2.28±0.44Aa †	2.00±0.71Aa	Nd	2.82±0.76Aa	1.67±0.08Aa
(*Z*)-Tetradec-9-enoic acid or myristoleic acid, C14:1	7.67±1.46BC ab	2.00±0.40Aa	Nd	9.42±3.26BC b	7.00±0.78DE ab
Hexadecanoic acid or palmitic acid, C16:0	13.95±2.22D ab	17.27±1.12 Db	15.02±1.13C Dab	15.11±1.82D Eab	10.73±0.79F a
(*E*)-Hexadec-9-enoic acid or *trans*-Palmitoleic acid, C16:1	9.57±1.01Ca	16.41±0.55 Dc	15.52±0.01D b	20.51±2.83EF c	16.79±0.54G c
(*Z*)-Hexadec-9-enoic acid or *cis*-palmitoleic acid, C16:1	21.24±1.31E b	22.9±0.45Eb	15.43±0.54C Da	25.51±0.05Fc	33.12±0.54H d
Octadecanoic acid or stearic acid, C18:0	4.21±0.74AB a	8.78±1.11Cb	3.94±1.01Aa	3.78±1.16AB a	2.33±0.21AB a
(*Z*)-Octadec-9-enoic acid or oleic acid, C18:1n9c	29.00±1.24F d	17.47±1.27 Dbc	22.51±1.89Ec	16.67±2.32Eb	8.86±0.85EF a

(*Z*)-Octadec-11-enoic acid 11-Octadecenoic acid, C18:1n7	3.79±0.85Aa	5.17±1.16Ba	10.78±1.01Bb	5.11±0.78AB a	10.37±0.82F b
(9*Z*,12*Z*)-Octadeca-9,12-dienoic acid or linoleic acid, C18:2	8.3±0.69Cab	9.35±0.99Ca b	11.89±2.09B Cb	10.96±1.71C Db	5.36±0.20C Da
(6*Z*,9*Z*,12*Z*)-Octadeca-6,9,12-trienoic acid or linolenic acid, C18:3	Nd	Nd	5.63±0.04Aa	Nd	3.94±1.68BC a
Total PUFA	8.3	9.35	17.52	10.96	9.3
Total MUFA	71.27	63.95	64.24	66.81	76.14
Total SFA	20.44	28.05	18.96	23.09	14.14
USFA/SFA	3.90	2.61	4.31	3.37	6.04

[†]Values (means ±SE) followed by the same letter, within the same row, were not significantly different according to Fisher´s least significant difference (LSD) procedure at 95% significance level (n = 6). Different capital letters showed differences between fatty acids in the same cultivar; different lowercase letters showed differences in the percentage of each fatty acid between the different cultivars. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. Nd: not detected. U/S, unsaturated/saturated fatty acids.

The SFA content was similar to that obtained in pear peel (*Opuntia ficus-indica*) (Ramadan & Mörsel, 2003) where myristic and stearic acid had values very similar to those found in the jujube peel.

The unsaturated/saturated (USFA/SFA) ratio was also very high in peel of jujube fruits with values between 2.61% for 'GAL-T' and 6.04% for 'DAT' cultivars. These values of the USFA/SFA ratio were similar to those previously discussed for the jujube pulp and much higher than those obtained by Elaloui et al. (2014) in Tunisian cultivars (between 1.68% to 2.37%) and by San & Yildirim (2010) in selections from Turkey (between 2.27% to 2.73%). The high values of the USFA/SFA indicate that the Spanish

jujube cultivars have high interest due to the potential health benefits of the fatty acids profile of their peel and pulp.

The results obtained showed that jujube fruits are an interesting source of fatty acids, with the composition being significantly affected by the cultivar, and other factors such as the genetic material, ripeness stage and edaphological growth conditions. This last statement is supported by the different results obtained for the 'GAL-E' and 'GAL-T' cultivars, where the farming conditions were different.

3.3. Principal components analysis

In order to gain a better understanding of the whole data produced in this study and relationships among the studied variables (11 FAMEs for the pulp, 10 FAMEs for the peel and 5 jujube cultivars), main components analysis (PCA) was applied and results are showed in Fig. 1 and Table 3.

The first three main components accounted for 85.03% of the total variation for the results obtained in the jujube pulp, and the 72.14% of the variability of the data studied were explained by the first two components (Table 3). The first component (PC1), represented 41.64 % of the total variation and was positively related to the contents of myristic (C14:0), myristoleic (C14:1), *trans*-palmitoleic (C16:1) and oleic (C18:1n9c) acids and negatively related to the contents of stearic (C18:0) and 11-oxtadecenoic (C18:1n7) acids (Fig. 1A and Table 3). The PC2 accounted for 30.49 % of the total variation, and was positively correlated with the contents of palmitic (C16:0), *trans*-palmitoleic (C16:1), and linoleic (C18:2) acids and was negatively correlated with elaidic (C18:1n9t) acid in the jujube pulp.

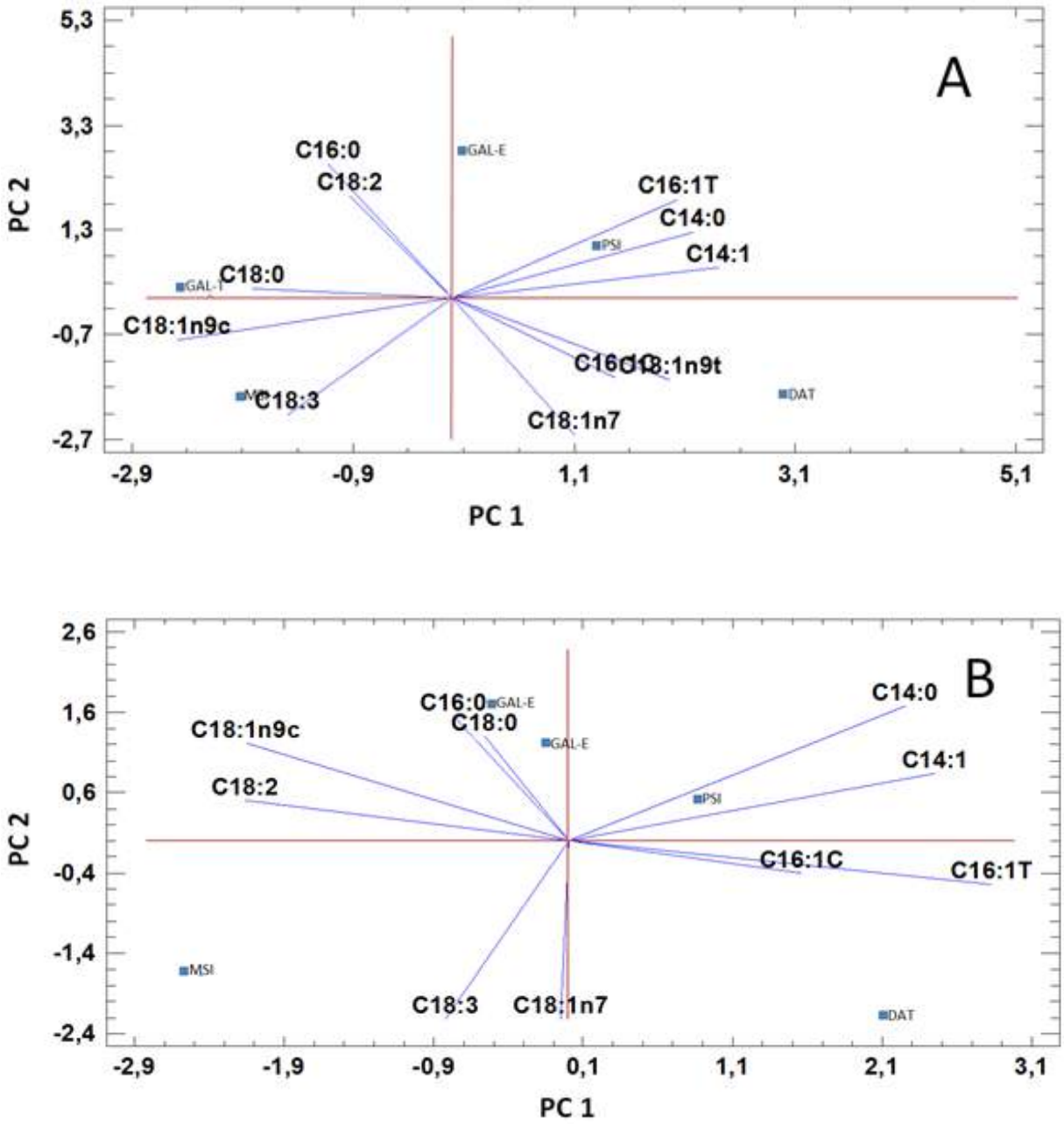

Figure 1. Principal component analysis (PC1 and PC2) of pulp (A) and peel (B) of jujube fruits.

The principal component PC1 (Fig. 1A) of the pulp of the jujube fruit allowed separation of 'MSI' and 'GAL-T' cultivars, which were located in the negative axis, and 'GAL-E', 'PSI' and 'DAT' in the positive axis and clearly differentiated the 'DAT'

cultivar with a higher positive value. This indicated that the 'MSI' and 'GAL-T' cultivars were characterized by a high content of oleic acid (C18:1n9c) and low content of myristic (C14:0), myristoleic (C14:1) and *cis*-palmitoleic (C16:1) acids. These data suggested that 'GAL-T' and 'MSI' cultivars were a good source of fatty acid because they had a high percentage of oleic acid. The 'DAT' cultivar, that presented opposite behavior in the principal component PC1, was characterized by a low content of oleic acid (C18:1n9c) and high contents of *cis*-palmitoleic (C16:1), 11-octadecenoic (C18:1n7) and elaidic (C18:1n9t) acids. The principal component PC2 (Fig. 1A) allowed a clearly separation in positive axis of the 'GAL-E', 'PSI' and 'GAL-T' cultivars. The 'GAL-E' cultivar was different from the 'PSI' cultivars by the null content in 11-octadecenoic (C18:1n7) acid; and differs from the 'GAL-T' cultivar by the high content in oleic acid (C18:0).

Table 3. Eigenvalues, proportion of variation and eigenvectors associated with each principal component for the pulp and peel of jujube fruit.

Principal components	**PULP**			**PEEL**		
	1	**2**	**3**	**1**	**2**	**3**
Eigenvalues	4.58072	3.35422	1.41876	3.20057	2.83616	1.51954
Cumulation proportion of variation (%)	41.643	72.136	85.034	32.006	60.367	75.563
Characters	**Eigenvectors**					
	1	**2**	**3**	**1**	**2**	**3**
Tetradecanoic acid or myristic acid, C14:0	0.362571	0.216953	0.215494	0.396323	0.377552	0.0269523
(*Z*)-Tetradec-9-enoic acid or myristoleic acid, C14:1	0.402631	0.100371	-0.0145367	0.431174	0.189773	-0.140279
Hexadecanoic acid or palmitic acid, C16:0	-	0.444873	0.245199	-0.134413	0.350623	0.464858

	0.187910					
(*E*)-Hexadec-9-enoic acid or *trans*-Palmitoleic acid, C16:1	0.341090	0.328932	0.213894	0.496794	-0.121749	-0.107242
(*Z*)-Hexadec-9-enoic acid or *cis*-palmitoleic acid, C16:1	0.246957	-0.262787	0.481523	0.273382	-0.0906275	0.617167
Octadecanoic acid or stearic acid, C18:0	-0.302778	0.0335173	0.391876	-0.0979674	0.294652	0.36765
(*Z*)-Octadec-9-enoic acid or oleic acid, C18:1n9c	0.329268	-0.274056	-0.360765	-0.378175	0.275746	-0.373045
(*Z*)-Octadec-11-enoic acid 11-Octadecenoic acid, C18:1n7	-0.416198	-0.138388	-0.0827631	-0.009326	-0.50109	0.165833
(*E*)-Octadec-9-enic acid or elaidic acid, C18:1n9t	0.184984	-0.455737	-0.110726	-	-	-
(9*Z*,12*Z*)-Octadeca-9,12-dienoic acid or linoleic acid, C18:2	-0.154792	0.338404	-0.484725	-0.379159	0.112834	0.240637
(6*Z*,9*Z*,12*Z*)-Octadeca-6,9,12-trienoic acid or linolenic acid, C18:3	-0.249596	-0.387424	0.278974	-0.144723	-0.498788	0.106632

The first three main components accounted for 75.56 % of the total variation for the results obtained in the jujube peel (Table 3). The 60.37% of the variability of the data studied were explained by the first two components (Fig.1B and Table 3). The first component, PC1, representing 32.01% of total variation that was positively related to myristic (C14:0), myristoleic (C14:1), *trans*-palmitoleic (C16:1) and linoleic (C18:2) acids, and was negatively correlated with oleic (C18:1n9c) and elaidic (C18:1n9t) acids (Fig. 1B and Table 3).

The second component, PC2, represented 28.36% of the total variation that was positively related to myristic (C14:0), palmitic (C16:0) and linolenic (C18:3) acids and was negatively correlated with 11-octadecenoic (C18:1n7) and linoleic (C18:2) acids (Fig. 1 and Table 3).

The principal component PC1 (Fig. 1B) allowed separation of the jujube peel cultivars 'MSI', 'GAL-T' and 'GAL-E', which were located in the negative axis, while

'PSI' and 'DAT' were located in the positive axis and clearly differentiated of 'DAT' cultivar with a high positive value. This separation was similar to than previously discussed in the jujube pulp. 'MSI' presented the more negative value in PC1.

These data suggested that 'GAL-T', 'GAL-E' and 'MSI' cultivars were a good source of oleic acid. With peel of 'DAT' cultivar happened the same as in the pulp where the 'DAT' cultivar was characterized by a low content of oleic acid (C18:1n9c) and high contents of *cis*-palmitoleic (C16:1). The principal component PC2 (Fig.1B) allowed a clearly separation in positive axis of the 'GAL-E', 'GAL-T' and 'PSI' cultivars, while 'MSI' and 'DAT' were located in the negative axis. The 'MSI' cultivar was different from the 'DAT' cultivar by the high content in linoleic (C18:2) and oleic (C18:1n9) acids. The 'GAL-E' and 'GAL-T' cultivars presented a close position in PC, indicating that they were the same cultivar but were grown under different farming systems (organic or conventional).

3.4. Discriminant analysis

The linear discriminant analysis (LDA) was carried out to establish a model to discriminate the 5 Spanish jujube cultivars ('GAL-E', 'GAL-T', 'MSI', 'PSI' and 'DAT'), according to the contents of the 11 FAMEs found (C14: 0, C14: 1, C16: 0, C16: 1c, C16: 1t, C18: 0, C18: 1n9c, C18: 1n7, C18: 1n9t, C18: 2, C18: 3). The elements that contributed to variate function 1 and 2 were myristic (C14:0), palmitic (C16:0), *trans*-palmitoleic (C16:1), *cis*-palmitoleic (C16:1), oleic (C18:1n9c) and 11-octadecenoic (C18:1n7) acids (Table 4). Classification of the cultivar by the LDA model showed 100% accuracy. In other words, 60 of the total 60 samples [3 samples x 5 cultivars x 2 (peel and pulp) x 2 years = 60 samples] were correctly classified. In the model, each

group was well separated as shown in Fig. 2. Therefore, it could be stated that the fatty acid contents could be used to discriminate the studied cultivars, because all the groups have been successfully separated and there had been no overlaps. It could be observed in Fig.2, that 'GAL-E' and 'GAL-T' cultivars were very close, but without overlaps. Therefore, these results would also indicate that with the analysis of fatty acids the type of crop used could be identified. The cultivar 'DAT' was the one that showed a greater difference with respect to the other cultivars, because it had the scores of the both functions 1 and 2.

After performing the LDA, in order to check if there were differences in the fatty acids according to the origin, peel or pulp, a multifactorial analysis of the multivariate variance of ANOVA, MANOVA, was performed (Table 4). The variance of the 30 samples (15 from each of the two studied years) of each acid contained in the peel or in the pulp of the jujube, fruit was calculated. To check if there were significant differences among the means distinguishing between peel and pulp, and it was found that there was significant differences for myristic (C14: 0), myristoleic (C14:1), oleic (C18:1n7), elaidic (C18:1n9t) and linolenic (C18:3) acids.

4. Conclusions

The results of this study showed that the FAME profile of jujube fruits was influenced by the cultivar, the farming type (organic or conventional), and the part of the fruit (pulp or pulp). The predominant fatty acids in the jujube pulp and peel were *trans*-palmitoleic, oleic and palmitic acids. It has been proven that there were differences between the same cultivar grown under organic or conventional farming, showing a higher percentage content in some fatty acids in the peel of the fruit with conventional farming for the 'GAL' cultivar; besides, the ratio USFA/SFA was higher in

the pulp of conventional fruits while it was higher in peel in organic jujubes. The 'DAT' cultivar was interesting and different from the others because of its high *trans*-palmitoleic acid percentage in both pulp and peel, which makes their U/S ratio to be high (pulp 7.97% and peel 6.04%). 'MSI' was the cultivar that presented the highest percentage of PUFAs in peel, although in pulp the highest percentage was in 'GAL-T'; thus, they are important natural sources of essential fatty acids omega 3 and omega 6, which human body is not capable of producing. The fatty acids of Spanish jujube cultivars have great health potential for both fresh consumption and/or industrial processing, and the peel can be used as a co-product in the pharmaceutical and food industry because it contained interesting FAMEs profiles.

Declaration of Competing Interest

None.

Acknowledgements

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Table 4. Elemental concentrations of fatty acids cultivars and function 1 and 2 scores, and MANOVA, multivariate analysis of the variance of 30 samples of the acids contained in peel and pulp of the jujube fruit.

Fatty acid	Function 1 score (peel)	Function 2 score (pulp)	MANOVA Pulp	MANOVA Peel
Tetradecanoic acid or myristic acid, C14:0	-6.10638	-7.87943	0.666 a	1.870 b
(*Z*)-Tetradec-9-enoic acid or myristoleic acid, C14:1	-0.343295	-0.717448	2.782 a	5.086 b
Hexadecanoic acid or palmitic acid, C16:0	3.73774	4.21045	15.614 a	14.545 a
(*E*)-Hexadec-9-enoic acid or *trans*-Palmitoleic acid, C16:1	6.15807	6.75597	14.648 a	15.637 a
(*Z*)-Hexadec-9-enoic acid or *cis*-palmitoleic acid, C16:1	3.69977	3.84472	25.841 a	23.417 a
Octadecanoic acid or stearic acid, C18:0	0.943841	0.754195	3.565 a	4.821 b
(*Z*)-Octadec-9-enoic acid or oleic acid, C18:1n9c	8.51695	11.8146	13.715 a	18.641 b
(*Z*)-Octadec-11-enoic acid 11-Octadecenoic acid, C18:1n7	5.70414	5.78371	5.393 a	6.953 a
(*E*)-Octadec-9-enic acid or elaidic acid, C18:1n9t	Nd	-0.107554	1.401 a	0.000 a
(9*Z*,12*Z*)-Octadeca-9,12-dienoic acid or linoleic acid, C18:2	1.6529	1.93744	10.994 a	9.119 a
(6*Z*,9*Z*,12*Z*)-Octadeca-6,9,12-trienoic acid or linolenic acid, C18:3	2.79112	4.19965	4.263 b	1.893 a

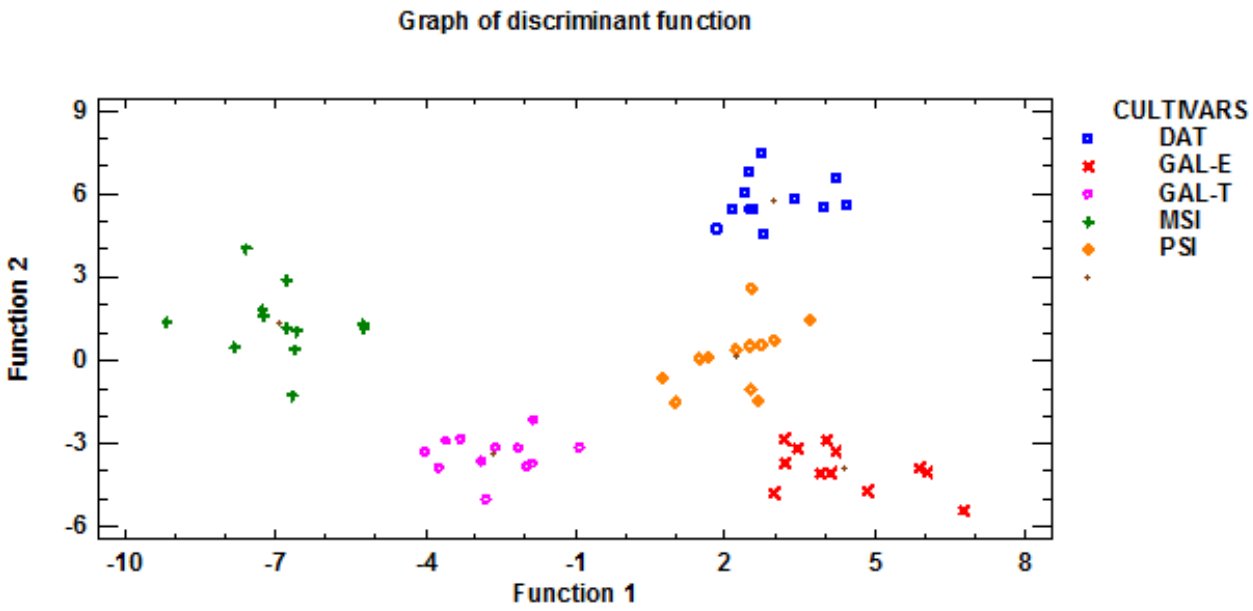

Figure 2. Graph of discriminant functions in scatter plots of 60 samples subjected to the LDA model using concentrations of 10 fatty acids for discrimination of cultivar groups ('GAL-E', 'GAL-T', 'MSI', 'PSI' and 'DAT') of jujube Spanish fruits.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.food chem.2019.05.147.

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4.2. Publicación 5

PUBLICACIÓN 5
Effect of modified atmosphere packaging on the physiological and functional characteristics of Spanish jujubes (*Ziziphus jujuba* Mill.) cv 'Phoenix' during cold storage
Juana Reche, María Emma García-Pastor, Daniel Valero, Francisca Hernández, María Soledad Almansa, Pilar Legua y Asunción Amorós. Scientia Horticulturae ***2019,*** 258, 108743 ***doi: 10.1016/j.scienta.2019.108743***

PUBLICACIÓN 5: TRANSCRIPCIÓN LITERAL

Effect of modified atmosphere packaging on the physiological and functional characteristics of Spanish jujube (*Ziziphus jujuba* Mill.) cv 'Phoenix' during cold storage

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Abstract

Jujube fruits cv 'Phoenix' were stored in modified atmosphere packaging (MAP) using a polyester (12 µm)-polypropylene (60 µm) film at 5 °C and 90% RH during 49 days. Jujube fruits stored without packaging and in normal air and same temperature and RH served as control. The atmosphere composition at the steady state was at 35 days with 14.50 kPa O_2 and 3.86 kPa CO_2. The atmosphere packaging showed an almost zero amount of ethylene during all storage days. The jujubes at MAP have been very effective as they presented the same appearance throughout the 49 days of storage. On the other hand, the fruits in control showed a wrinkled and non-commercial appearance at day 21. Treatment with MAP caused a significant delay in the ripening of the fruit after harvest. It caused less weight loss, more firmer and more intense color. Improved total carotenoids, total phenols, hydrophilic-total antioxidant activity (H-TAA), lipophilic-total antioxidant activity (L-TAA). Meanwhile, the maturity index (MI) was reduced compared with control jujubes.

Keywords:

MAP

Ethylene

Respiration

Total antioxidant activity

Phenols

1. Introduction

Jujube fruits are widely consumed in Asian countries as a food and food additive due to its high nutritional value (Almansa et al., 2016). Different parts of the jujube plant are used as pharmacological agents (Jiang et al., 2007). There are many jujube cultivars, each one with different physico-chemical, physiological and functional characteristics. Most studies have been done on Asian cultivars; however, the Spanish cultivars are still very poorly studied (Reche et al., 2019). The 'Phoenix' cultivar is characterized by an elongated appearance, is very sweet with a total soluble solids content of 24 °Brix at commercial maturity (Reche et al., 2018). This cultivar is usually harvested mid-October, and thus considered as late- Then, the extension of the postharcest period is vcery interesting for expanding the commercial time for consuming raw jujubes in Spain.

The jujube is a non-climacteric fruit (Almansa et al., 2016; Zhang et al., 2018), but at room temperature spoils quickly, especially by dehydration and increments in brown index and rot rate (Wu et al., 2016). On these way the storage life of jujube fruits is extremely short and the rapid perishability of the fruits is the main postharvest problem (Siddiq and Uebersax, 2012). So it would be very interesting to study the postharvest behavior of these fruits to increase their shelf life.

The decrease in storage temperatures reduces the metabolic activity of the fruits, which extends its postharvest life. These temperatures can be between -2 and 10 °C but temperatures of -1 °C or lower for a long time produce chilling injury (Wu et al., 2016). MAP is a method of extending the shelf-life of fresh produce. The technology involves the modification of the air inside the package with a beneficial mixture of elevated carbon dioxide and reduced oxygen. It is achieved by the natural interplay between the

respiration of the produce and the transfer of gases through the packing material. The effectiveness of MAP on extending shelf-life is dependent on several factors, such as the type of produce, gas mixture, storage temperature, packing material and hygiene during handling (Valero and Serrano, 2010). MAP technology has already been used successfully in other fruits such as peach and nectarines (Akbudak and Eris, 2004; loquat (Amorós et al., 2008); plum (Díaz-Mula et al., 2011a); grape (Martínez-Romero et al., 2003); mango (Pesis et al., 2000), pomegranate (Selcuk and Erkan, 2014), among others. The MAP has been little studied in jujubes. Only one article has been published that showed the efficacy of MAP in decreasing weight loss and delayed the reduction of sulphur firmness by 20 days (Lu et al., 2014). On the other hand, jujubes have higher total phenol contents as compared to other fruits, such as persimmon, pomegranates, and apples (Fu et al., 2011). In this sense, it is worthy to study the evolution of bioactive compounds and antioxidant activity under MAP packages. For this reason, the objective of this work was to study, for the first time, a MAP study with cv 'Phoenix' Spanish jujubes stored at lower temperature to extend their postharvest life. The evolution of physicochemical and phytochemical parameters and bioactive compounds of the control and MAP jujubes stored at 5°C were studied.

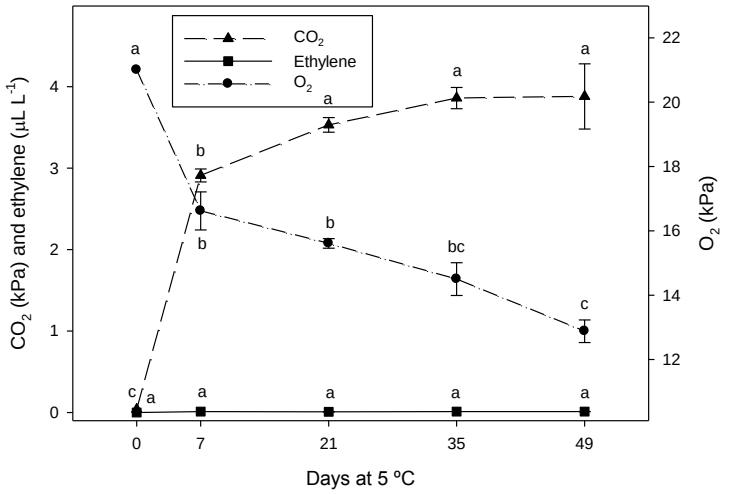


Figure 1: Changes in O_2, CO_2 and ethylene concentration inside MAP packages containing jujube fruits 'Phoenix' cultivar during storage at 5 °C. Data are the mean ± SE (n=6). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p<0.05$) during each storage time.

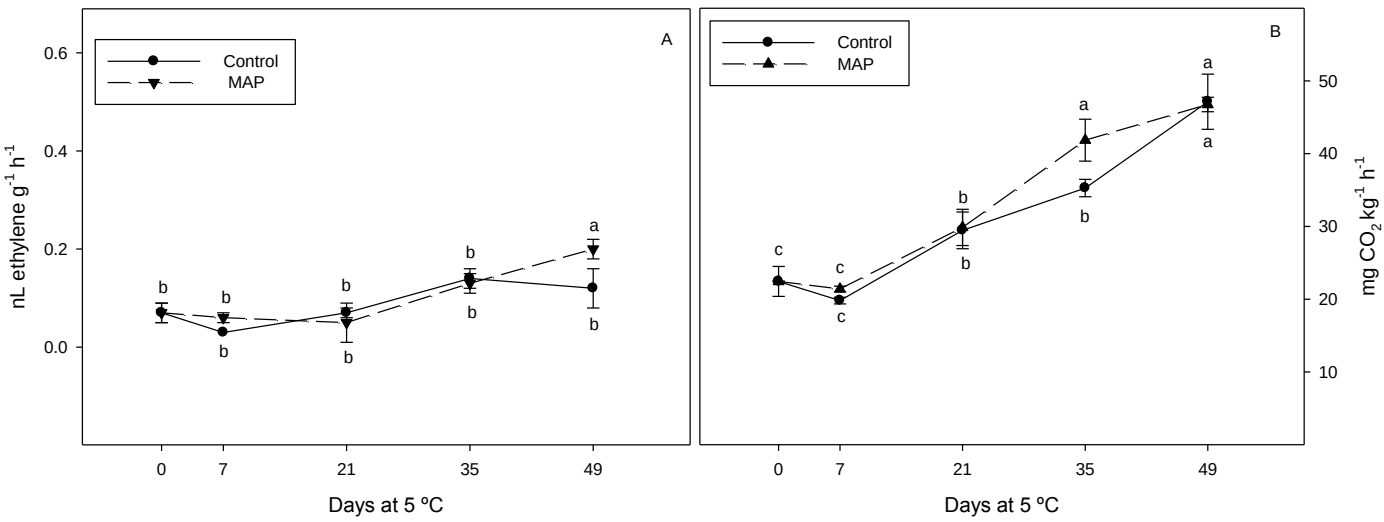
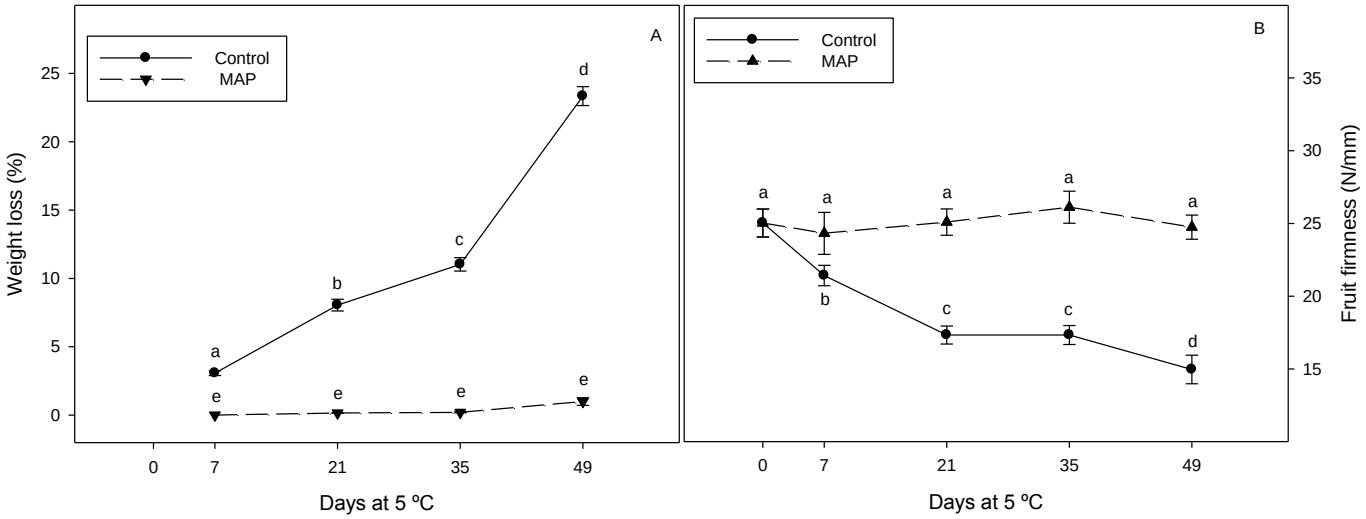


Figure 2: Ethylene and CO_2 production rates during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n=6).

Least significant differences (LSDs) test at 95% confidence level are shown. Different



letters indicate significant differences ($p<0.05$) during each storage time and treatment.

Figure 3: Weight loss and fruit firmness changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n=45). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p<0.05$) during each storage time and treatment.

2. Materials and methods

2.1. *Plant material and experimental design*

The jujubes were harvested from a commercial farm with 21-years-old jujube trees (latitude 38°10'22,29''N x longitude 0°51'36,138''W, 19 m above sea level) in Albatera (Alicante, Spain). Trees were trained as a vase and spaced 4 m × 4 m. The

jujube fruits under study belonged to cv 'Phoenix'. Nine hundred fruits from nine trees (100 fruits per tree) were hand-harvested at commercial ripening stage (above 15°Brix). Fruits were immediately transported (30 minutes), under cold conditions (at 5 ºC), to the laboratory. Then, 405 fruits were selected based on homogeneous colour and size, and absence of visual defects, and distributed at random into 27 lots of 15 fruit. Three lots were used to determine physicochemical properties at harvest (day 0). The remaining lots were individually deposited in polypropylene baskets. 12 lots were left in the refrigeration chambers without film, which would serve as control, while the other 12 lots were used for MAP treatment. These 12 baskets were thermos-sealed on top with the film with a total area of 336 cm^2, 14 cm x 24 cm. The film was composed of polyester (12 µm)-polypropylene (60 µm) (Amcor Flexibles, Barcelona, Spain) with permeability to O_2 = 75 mL O_2 m^{-2} day^{-1} atm^{-1}, CO_2 = 350 mL CO_2 m_2 day^{-1} atm^{-1} and water vapour = 75 mL H_2O mL m^{-2} atm^{-1}. All baskets, control and MAP, were stored at 5 °C and 90% RH. Four samples were taken at 7, 21, 35 and 49 days after collection. In each sampling, 3 control lots and 3 MAP lots were taken in which all analyzes below mentioned were carried out.

2.2. *Gas composition inside the packages*

Firstly, the gas composition of each MAP sample was analysed. The CO_2 and O_2 concentrations of each package were quantified in duplicate by extracting 1 mL of headspace atmosphere using an airtight syringe. Subsequently, the following were injected into a gas chromatograph GC 14B (Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD), with the characteristics explained in Díaz-Mula et al. (2011a). Results were expressed as kPa CO_2 and kPa O_2 inside the baskets (n=6).

The ethylene content inside the packages was determined for quantification (in duplicate) of 1 mL of the atmosphere that was withdrawn with a gas syringe and injected into a Shimadzu TM GC-2010 gas chromatograph (Kyoto, Japan), with the characteristics explained in Díaz-Mula, et al. (2011a). Results were expressed as µL L^{-1} into the baskets (n=6).

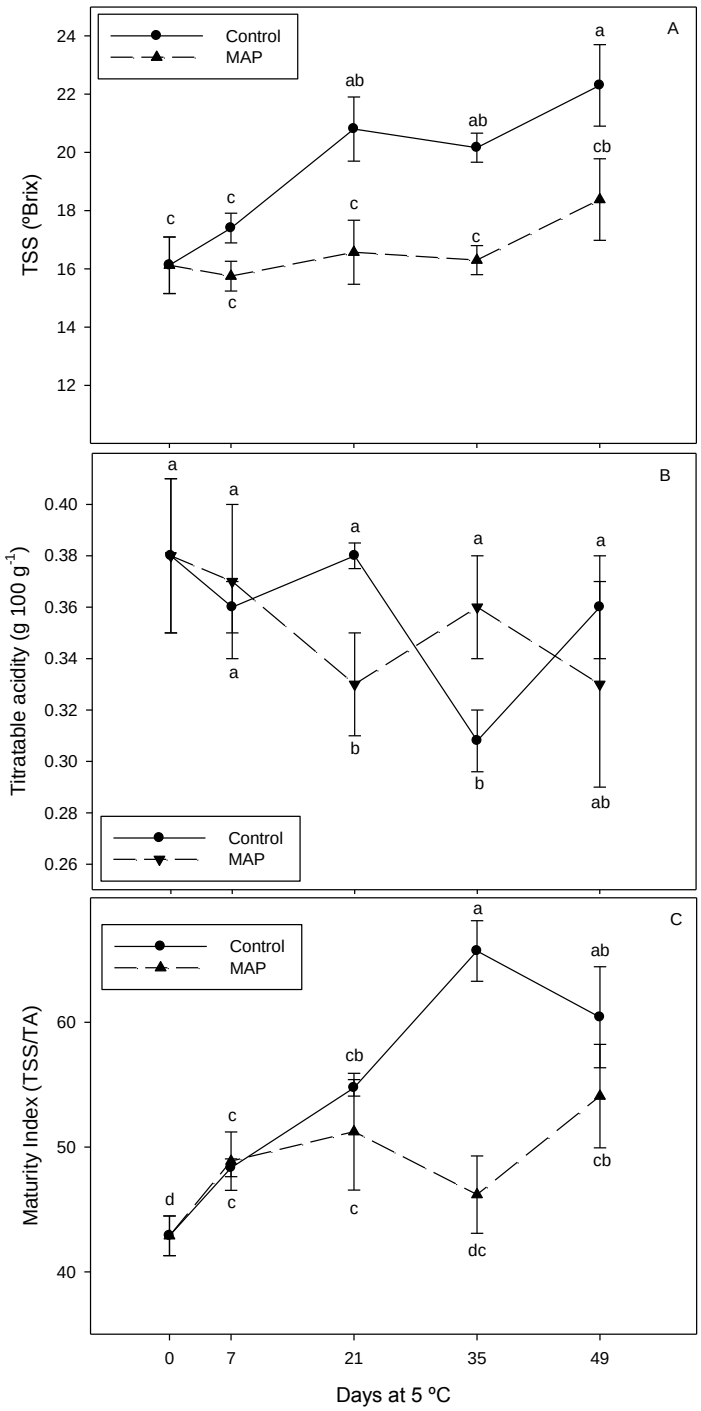


Figure 4: Total Soluble Solids (TSS) (A), Total Acidity (B) and Maturity Index (C) changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n=6). Least significant differences (LSDs) test at

95% confidence level are shown. Different letters indicate significant differences ($p<0.05$) during each storage time and treatment.

2.3. *Respiration rate and ethylene production of fruits*

Secondly, for each sampling date, the packs were opened and the jujubes were placed in a 750 mL glass jar hermetically sealed with a rubber cap for 30 min to measure the production of cold temperature ethylene (5 °C). For ethylene quantification (in duplicate) 1 mL of the atmosphere was withdrawn with a gas syringe and it was injected into the gas chromatograph as explained in the previous section. Results were the mean ± SE of determinations for six replicate and expressed as nL g^{-1} h^{-1}. The rate of respiration as CO_2 emission was quantified in a similar way, injecting 1 mL of the glass jar atmosphere into the gas chromatograph GC 14B described above. Results were expressed as mg kg^{-1} h^{-1} and were the mean ± SE. For day 0, respiration and ethylene rates were measured at 20 °C. These parameters were also measured in the control fruits.

Subsequently the parameters involved in points 2.4 and 2.5 were analyzed for all samples, control and MAPs in each time storage.

2.4. *Fruit quality parameters*

The fruits of each lot were weighed using a digital balance (model BL-600; Sartorius, Madrid, Spain) to calculate the weight loss. Fruit firmness was measured with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK) with a force that

achieved a 3% deformation of the jujube diameter. Results were expressed as the force-deformation (N mm^{-1}) and were the mean ± SE (n = 45). Jujube peel color was assessed at two equidistant points of the equatorial region of individual fruit in each 45 fruits using a Minolta colorimeter CR200 model using D65 illuminant (Minolta Camera Co., Japan). The result were expressed using the CIE L*a*b* system. The fruit was then cut into pieces and mixed to have a homogeneous juice sample for each replica. The total soluble solids (TSS) and titratable acidity (TA) were determined in duplicate. The TSS concentration was determined with a digital refractometer Atago Pocket PAL-1 (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and expressed as °Brix. TA (g of malic acid equivalent per 100 g^{-1} fresh weight) was determined by automatic titration (TitraLab AT1000 series, Hach) with 0.1N NaOH up to pH 8.1, using 1mL of diluted juice in 30 mL distilled H_2O. The maturity index (MI) was calculated as the ratio between the TSS and the titratable acidity.

2.5. *Phytochemical parameters*

Vitamin C was measured by titration with iodine in acid medium with a titrant HachTitraLab AT 1000 series.

The method of Tomás-Barberán et al. (2001) was used for total phenolic extraction by using water: methanol (2:8) containing 2 mM NaF. The phenolic content was quantified using the Folin-Ciocalteu reagent and results (mean ± SE) were expressed as mg gallic acid equivalent g^{-1} fresh weight.

Total antioxidant activity (TAA) was quantified as described by Arnao et al. (2001). This medology allows the determination of TAA due to both hydrophilic (H-TAA) and

lipophilic (L-TAA) compounds in the same extraction using 50 mM phosphate buffer pH 7.8 and ethyl acetate. The upper fraction was used for L-TAA while the lower fraction for H-TAA quantification using the enzymatic system composed of the chromophore 2,2´-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horseradish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide). The reaction was monitored at 730 nm until a stable absorbance was obtained using a UNICAM Helios spectrophotometer (Cambridge, UK). After that, a suitable amount of jujube fruit extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with Trolox as standard antioxidant for both H-TAA and L-TAA. The results were expressed as mg Trolox equivalent 100 g^{-1} fw. Total carotenoids were estimated in the lipophilic extract (Arnao et al., 2001). The absorbance was measured at 450 nm in a UNICAM Helios- spectrophotometer (Cambridge, UK). The result were expressed as mg of β-carotene equivalent 100 g^{-1} fresh weight, taking into account the $\varepsilon^{1\%}_{cm}$ = 2560 and the results were the mean ± SE.

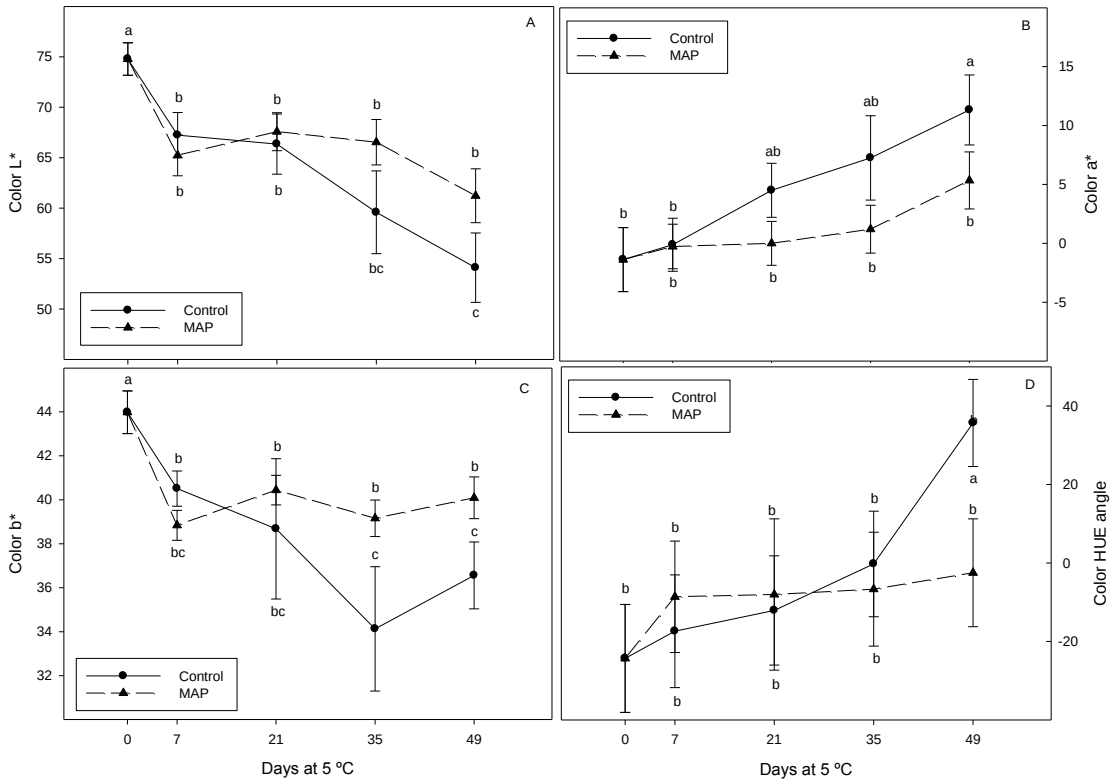


Figure 5: Color parameters L* (A), a* (B), b* (C) and HUE angle (D) changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n=90). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p<0.05$) during each storage time and treatment.

2.6. *Statistical analysis*

Statistical analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance test (ANOVA) for mean comparisons. The method used to discriminate among the means (multiple range test) was Fisher's LSD (Least Significant Difference) procedure at a 95.0% confidence level.

3. Results and discussion

3.1. *Gas composition and ethylene production*

The low storage temperature decreased jujube respiration. The O_2 concentration decreases in a concentration of 12.88 kPa and the CO_2 concentration increased to 3.6 kPa at day 49 of storage at 5 ºC (Figure 1) with significant differences. Therefore, a steady-state atmosphere was reached, as in other fruits like loquat (Amorós et al., 2008) or plum (Díaz-Mula et al., 2011a). These results are similar to those obtained by Jat et al. (2012) with Indian jujube fruit stored in MAP at room temperature. This is due to the effect of the low permeability of the film used together with the decrease in respiration due to the low storage temperature. As a result, the concentration of O_2 and CO_2 was slightly modificated. On the other hand, the accumulation of ethylene in the packages was very low at 5 °C, with concentration being 0.01 µL L^{-1} in all the samples inside the packages until day 49. The low ethylene emission rate of the jujube fruit is due to the fact that they are non-climacteric fruits (Almansa et al., 2016; Zhang et al., 2018), and to the decrease of metabolism at low temperatures. Indian jujube (*Z. mauritiana*), a climacteric specie (Abbas and Fandi, 2002), also decreased ethylene production when stored in MAP at room temperature (Jat et al., 2012). Any inhibitory effect on ethylene accumulation due to CO_2 concentration was observed. This may have been due low ethylene production rate of jujube fruit and the low CO_2 concentration reached inside packages. This rate could not be high enough to inhibit the biosynthesis of ethylene. However, in apricot MAP, ethylene production rate was inhibited, especially when CO_2 concentration inside packages was higher than 20 kPa (Pretel et al., 1993).

The jujubes in MAP and control maintained at 5 ºC, showed low ethylene emission and a low respiratory level. There were no significant differences in ethylene

during the 49 days of storage (about 0.1 nL ethylene g^{-1} h^{-1}) (Figure 2A and 2B). This behavior of the fruits once removed from the package and measuring their respiration and ethylene emission was similar to their evolution of gas pressures within the packages. The maintenance of the fruit ethylene synthesis corroborated that they are a climacteric fruits.

3.2. *Fruit quality parameters*

The MAP jujube fruit showed a weight loss very low during the 49 days of storage (Figure 3A). However, when the jujubes were stored without film, the weight loss increased dramatically since 7 day of storage, and increased proportionally to storage days. Significant differences were shown and weight loss of 23.3% was achieved within 49 days of storage. Therefore, the jujube MAPs were well hydrated at the end of the storage period, while the jujubes control showed wrinkles already in the third week of storage. This is a direct consequence of the film protective effect that causes a maintenance of the humidity of the fruits. This may have mainly been due to the effect of polyester-polypropylene film on increasing water vapor pressure around the fruit, and, in turn, reduction of the transpiration rate (Amorós et al., 2008). This has been observed as a general effect of MAP on fruit and vegetables, and proven in jujubes (Lu et al. 2014), loquat (Amorós et al., 2008), apricot (Pretel et al., 1993), sweet cherry (Serrano et al., 2005), among others. This indicated that the film used in this work achieved the expected result for this parameter.

This moisture maintenance of the MAP fruits is also observed to maintain the jujube firmness, which did not vary compared with respect to the control jujubes

firmness of day 0 (Figure 3B). However, control jujubes showed a significant decrease in the fruit firmness from day 7 of storage, which decreased proportionally to the stored days. This MAP effect was also corroborated by Lu et al. (2014) in MAP jujubes during 20 days of storage. Pretel et al. (1999) reported that low O_2 concentration is more effective at inhibiting fruit softening than high CO_2 concentration, conditions that were found in the atmosphere of the jujube MAP.

The MAP jujube maintained the TSS content about 16 °Brix with respect to control jujube after 49 days of storage at 5 °C (Figure 4A). While control jujubes significantly–enhanced their TSS from day 21 (20.2°Brix). The increase of this parameter being proportionally to the days stored, showing an increase in the maturation of control fruits with respect to the MAP jujubes. These TSS values are normal for this cultivar (Reche et al., 2018). The TA remained very similar in jujubes both control and MAP during the entire storage period. With values of TA close to 0.35 g 100 g^{-1} (Figure 4B). This caused that the maturity index (MI) remained in the jujubes MAP about MI≅50, while in the controls it increased significantly from the day 21 of storage up to day 49 (Figure 4C). Also, MAP peach and nectarine delayed the decrease in TA and the increase in TSS with respect to controls (Akbudak and Eris, 2004). This difference was also found in plums (Díaz-Mula et al., 2011a).

Jujube cv 'Phoenix' vary from green in immature stage to red and finally dark brown color in a ripening stage, as other Spanish jujube cultivars (Almansa et al., 2016). The jujube fruit external color varied significantly respect to day 0 during the storage cold period (Figure 5). The jujubes were collected on day 0 with a slight green color (a* -1.37), with yellow background color (b* 44) that gave it a very high luminosity (L* 74.78). The color parameter values reported here were very similar to those previously

reported in Spanish (Collado-González et al., 2014; Galindo et al., 2015; Almansa et al., 2016; Reche et al., 2018; 2019), and Chinese jujube cultivars (Wang et al., 2012). The MAP jujubes showed a color more similar to day 0 color than the controls, without significant differences in a* and HUE angle color. The yellow color measured by the coordinate b* is due to the content of carotenoids (Figure 6A). There was a similar behavior of this figure and 5C, where the yellow color, and the total carotenoids, which decreased during the conservation were higher in the MAP jujubes, at the end of the conservation period. The decrease of the yellow color is parallel to the increase of the brown color of the jujubes when they mature. This decreased the luminosity (decrease of the L* parameter), of the fruit that was greater in the control jujubes than in the MAP (Figure 5A). These effects can be attributed to a delay in the degradation of carotenoids induced by cold storage, and the effect of MAP at the end of the treatment. The delay in color change associated with the postharvest ripening process in MAP storage has also been shown in other fruits, such as mango (Pesis et al., 2000), table grape (Martínez-Romero et al., 2003) and loquat (Amorós et al., 2008) under MAP conditions. All these changes indicated that the fruits matured slightly during the cold conservation, less the MAP than the control.

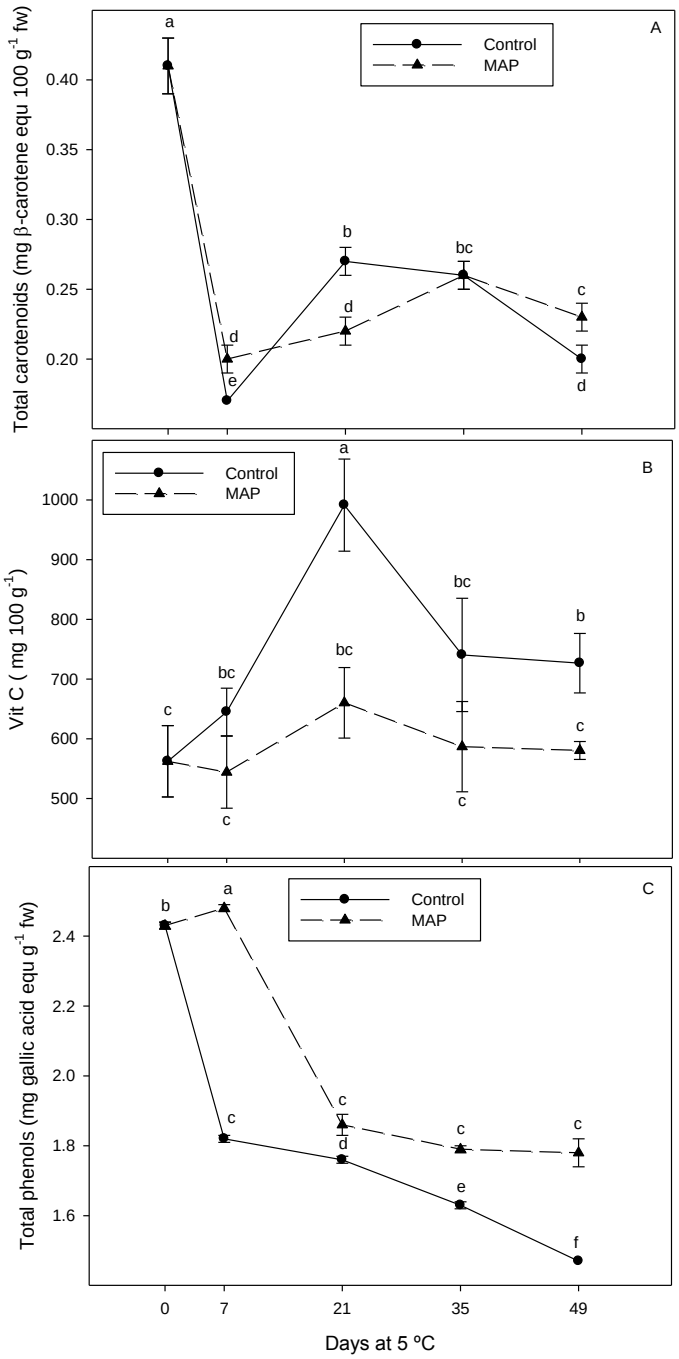
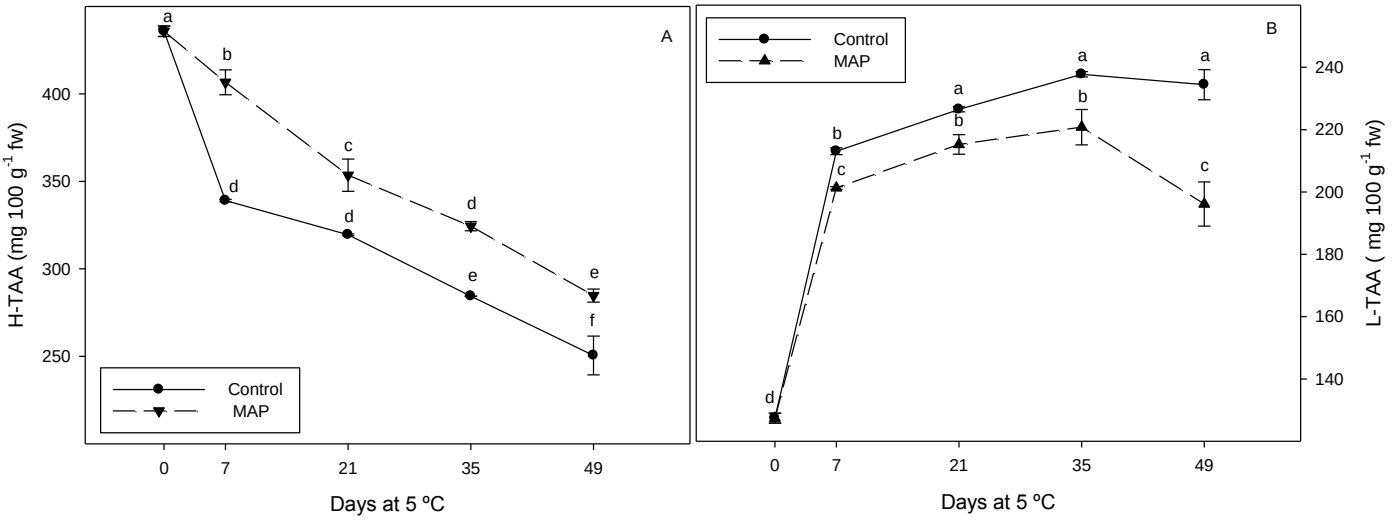


Figure 6: Total carotenoids (A), Vitamin C (B) and total phenols contents (C) changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n=6). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p<0.05$) during each storage time and treatment

Figure 7: H-TAA (A) and L-TAA (B) changes during storage at 5 °C of jujube fruits 'Phoenix' cultivar under control and MAP conditions. Data are the mean ± SE (n=6). Least significant differences (LSDs) test at 95% confidence level are shown. Different letters indicate significant differences ($p<0.05$) during each storage time and treatment.



3.3. *Phytochemical parameters*

The vitamin C content of the jujube fruit was very high, with 562.4 mg 100 g^{-1}, similar to those found by Collado-González et al. (2014). This content was increased by the effect of cold storage of the control fruits with a maximum on day 21 of storage, which subsequently decreases until day 49. The increase in this content may be due in part to the weight loss that occurred during the cold storage of the jujubes control and that does not occur in the jujubes MAP (Figure 6B). From day 21 of storage there was a decrease in the vitamin C content probably due to its degradation due to aging of the fruits. However, in the MAP fruits there were no significant differences in the vitamin C content. The maintenance of the vitamin C content by MAP effect was also observed in other fruits such as loquat (Amorós et al., 2008).

The results obtained of the total phenols content of day 0 were in accordance with the obtained by Reche et al. (2018). During the cold storage period the jujube control total phenol content decreased significantly respect to day 0 (Figure 6C). However, in the MAP jujubes this decrease was significantly delayed with respect to the jujubes control. This decrease during the 49 days of storage at 5 °C was 39.90% in the control fruits and 26.75% in the jujubes MAP with respect to the content of total phenols from day 0. Also, this decrease during the cold storage was not in agreement with other fruits results such as plum (Díaz-Mula et al., 2011b), sweet cherry (Serrano et al., 2009), peach and nectarine (Di Vaio et al., 2008), in which there was an increase in the total phenols content with cold storage. The delay in the degradation of total phenols in the MAP jujubes may be due to the fact that the decrease of O_2 and increase of CO_2 in the atmosphere of the package caused a delay in the ripening of the fruits. This atmosphere could reduce polyphenol oxidase (PPO) or peroxidase activities. These are the main enzymes responsible for the degradation of phenols, as Selcuk and Erkan (2014) observed in pomegranates.

H-TAA and L-TAA day 0 were similar to Reche et al. (2018) in this cultivar. Cold storage had a similar effect on the content of total phenols and H-TAA of the jujubes (Figures 6C and 7A), since phenols are the main antioxidant compounds of jujubes (Choi et al., 2012). The jujube H-TAA decreased significantly respect to day 0. However, in the MAP jujube this decrease was significantly delayed with respect to the jujubes control. During the 49 days of cold storage the decrease was 42.52% in the control fruits and 34.67% in the jujubes MAP with respect to the content of total phenols from day 0. However, L-TAA presented the opposite behavior, since the increase of the cold storage period increased the L-TAA, and in greater quantity in the

control than in the MAP jujubes, with significant differences (Figure 7B). This behavior was the opposite in plums (Díaz-Mula et al., 2011b), since cold storage caused an increase in total phenols and H-AAT. While L-AAT decreased, although MAP caused delays effects, as in this study. In any case, both our results and those of Díaz-Mula et al. (2011b) suggest that MAP causes a delay in the maturation of the treated fruits.

4. Conclusion

The cold storage of jujube fruits (5 °C and 90% of RH) together with a MAP treatment achieved a significant delay of postharvest fruit ripening. This resulted in lower weight loss, lower IM, and greater firmness and color intensity. Interestingly, the content of total carotenoids, total phenols and H-TAA and L-TAA was greater than control jujubes. The package atmosphere was enriched in CO_2, and the content of O_2 decreased. The control jujubes had a non-commercial wrinkled aspect at day 21 of cold storage. While the MAP jujubes showed a normal visual aspect at day 49 of storage. Therefore, the film used increased the jujube shelf life more than twice as long as compared with the jujubes stored without this film (control jujubes).

Acknowledgment

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5. RESUMEN DE LOS RESULTADOS, DISCUSIÓN Y CONCLUSION

5. RESUMEN DE LOS RESULTADOS, DISCUSIÓN Y CONCLUSIONES

a. Publicación 1

PUBLICACIÓN 1

Physicochemical and nutritional composition, volatile profile and antioxidant activity differences in Spanish jujube fruits

Juana Reche, Francisca Hernández, María Soledad Almansa, Ángel A. Carbonell-Barrachina, Pilar Legua y Asunción Amorós.

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El **objetivo** de esta investigación fue evaluar las propiedades físicas y bioquímicas, de 3 cultivares diferentes de jínjol, así como las propiedades funcionalesde la piel y la pulpa de los frutos . Este es el primer estudio que refleja la capacidad antioxidante de jínjoles en la piel y en la pulpa por separado.

i. Resumen de los resultados y discusión

Las propiedades **físicas** mostraron que el cultivar 'Isidro' era el de mayor tamaño y peso, al igual que su hueso, seguido del cultivar 'Rate'. 'Phoenix' obtuvo el menor peso y dimensiones. Esto puede ser debido al genotipo del cultivar y a la carga de cultivo (Gao et al., 2012). El rendimiento de la pulpa en cambio fue mayor en 'Rate' con un 94.90%, con un color superficial más rojizo y oscuro que los otros cultivares, pero con menor luminosidad. En cambio, 'Isidro' y 'Phoenix' presentaron una mayor luminosidad en la piel y unos valores más altos en amarillo e 'Isidro' presentó el contenido más alto en clorofilas. Los carotenoides fueron equivalentes en los tres cultivares.

En cuanto a las **propiedades bioquímicas**, el cultivar 'Rate' presentó los valores más bajos en **sólidos solubles totales (SST)** y los cultivares 'Isidro' y 'Phoenix' mostraron unos valores más altos en **azúcares** totales, glucosa y fructosa, que junto con sacarosa fueron los principales azúcares. Estos valores fueron mayores que los obtenidos para otros cultivares de jínjoles españoles (Almansa et al., 2016) pero menores que en jínjoles chinos en los que apenas se encontró sacarosa (Wu et al., 2012), pero con valores muy altos de glucosa y fructosa (Gao et al., 2012). Esto puede ser porque durante el desarrollo del fruto, se degrada la sacarosa y produce un aumento de glucosa y fructosa (Almansa et al., 2016), por lo que el perfil de azúcares de los jínjoles recolectados en diferentes espacios de tiempo, pueden variar considerablemente.

El cultivar 'Isidro' fue el que mayor cantidad de **ácidos orgánicos** obtuvo y 'Phoenix' obtuvo una concentración muy baja de ácidos. El ácido que se encontró en mayor cantidad fue el succínico. Estos cultivares presentaron un elevado contenido en ácido ascórbico, que es un gran antioxidante, que osciló entre 0,33 g 100 g^{-1} para 'Phoenix' y 0,65 g 100 g^{-1} para 'Isidro', en comparación con otras frutas comunes como las fresas y naranjas (0,046 g 100 g^{-1} y 0,031 g 100 g^{-1} respectivamente) (Roberts y Gordon, 2003).

Los jínjoles estudiados presentan una buena relación azúcares/ácidos orgánicos lo que implica que tengan un sabor dulce pero también equilibrado. 'Phoenix' es el que mayor dulzor obtuvo, por lo que es un cultivar interesante para su consumo en fresco.

Las **proteínas** fueron mayores en el jínjol 'Phoenix', pero el resto, a pesar de tener un menor contenido, se encuentran dentro de los rangos normales. 'Rate' fue el cultivar menos jugoso por su alto contenido en materia seca. El azufaifo es una buena fuente de **vitamina C**, y **minerales** como el K y Ca. Estos cultivares obtuvieron contenidos medios para los minerales Mg y Na, y fueron algo más bajos en Zn, Cu, Mn y Fe, lo que demuestra que tienen buen contenido en macroelementos y microelementos.

En el análisis de los **compuestos volátiles** se encontraron 18, lo que supone un número bajo, indicador del bajo olor y aroma de estos frutos, siendo 'Rate' el que mayor cantidad obtuvo con diferencias significativas respecto a los otros cultivares. El hexanal fue el volátil más abundante.

El contenido en **fenoles totales** fue mucho mayor en la piel del fruto en los cultivares 'Isidro' y 'Rate', pero en la pulpa los fenoles fueron mayores en 'Phoenix', lo que puede ser debido a diferencias genéticas. El que sea mayor el contenidode los compuestos fenólicos en la piel puede ser debido a que se acumulan en el tejido epidérmico para una mayor protección del fruto frente a las radiaciones solares (Xue et al., 2009). El contenido de fenoles totales se ha mostrado mayor en el jínjol que en otras frutas como la granada y el caqui (Fu et al., 2011) por lo que consumir jínjoles es muy saludable frente a la prevención de ciertas enfermedades.

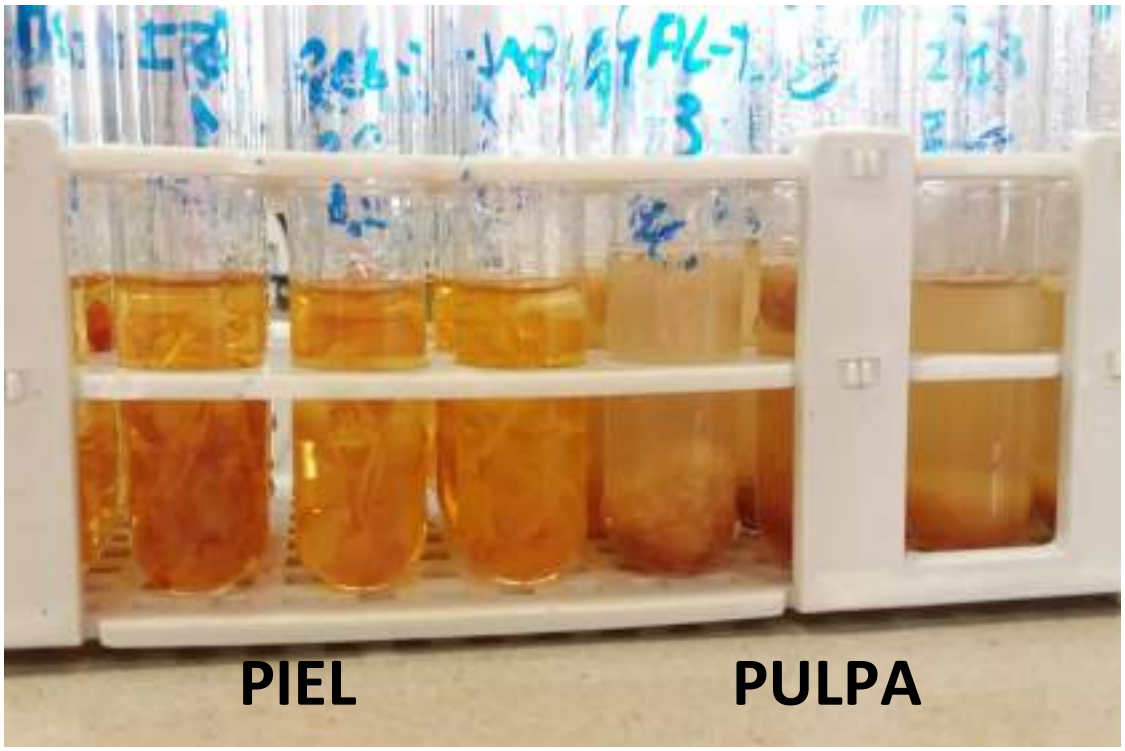

Imagen 32: Extracción en piel y pulpa previa al análisis de la AAT.

El contenido en **flavonoides totales**, responsable de las propiedades sedantes del jínjol (Bai et al., 2010), a pesar de ser bajo, también fue significativamente mayor en la piel que en la pulpa en el cultivar 'Isidro', en cambio, para los cultivares 'Rate' y 'Phoenix', el contenido en **flavonoles** fue mayor en la pulpa.

La **actividad antioxidante total**, analizada en piel y pulpa (Imagen 32), se determinó por tres métodos diferentes, FRAP, $ABTS^{\bullet+}$ y $DPPH^{\bullet}$. En los cultivares 'Isidro' y 'Rate', fue mayor en la piel que en la pulpa, y no mostró diferencias significativas con 'Phoenix'. La actividad antoxidante total en la fase hidrosoluble fue

mayor en la pulpa en los tres cultivares. Se obtuvo una correlación positiva entre el contenido en fenoles totales y la actividad antioxidante total en la fase hidrosoluble, tanto en la piel como en la pulpa.

ii. Resumen de la conclusión

Por primera vez se evaluó la actividad antioxidante; fenoles, flavonoides y flavonoles totales en las fases hidro y liposolubles, y la AAT por los métodos FRAP, $ABTS^{\bullet+}$ y $DPPH^{\bullet}$ en la piel y en la pulpa del jínjol. Los tres cultivares estudiados presentaron un buen contenido en compuestos bioactivos y propiedades antioxidantes, por lo que son de gran interés como cultivo alternativo en el sureste español. El cultivar 'Isidro' puede ser el más interesante por tener una gran actividad antioxidante y ser el fruto de mayor tamaño, para su consumo en fresco y con la piel.

b. Publicación 2

PUBLICACIÓN 2

Effects of organic and conventional farming on the physicochemical and functional properties of jujube fruit

Juana Reche, Francisca Hernández, María Soledad Almansa, Ángel A. Carbonell-Barrachina, Pilar Legua y Asunción Amorós.

LWT- Food Science and Technology 2019, 99, 438-444
doi: **10.1016/j.lwt.2018.10.012**

El **objetivo de este artículo** fue estudiar el cultivar de jínjol 'GAL', y mostrar las diferencias producidas del cultivo del árbol en agricultura ecológica y en convencional o tradicional, valorando los parámetros de los frutos relacionados con la calidad comercial, la composición nutritiva y los compuestos nutracéuticos. Estos últimos se evaluaron en la piel y en la pulpa.

i. Resumen de los Resultados y Discusión

Los frutos se recogieron en la misma fecha, y los procedentes de agricultura convencional mostraron un mayor **peso** y un mayor **tamaño**. Esto puede ser debido a que en cultivo orgánico los abonos son de liberación más lenta y tienen una disponibilidad más baja para la planta, pero son más sostenibles. El **color** también fue diferente, siendo más rojizo y más amarillo el orgánico, además de los contenidos en **clorofilas y carotenoides**, que también fueron mayores en el fruto ecológico. Lo mismo sucede con las fresas y tomates en cultivo ecológico que presentan mayor coloración en comparación con los frutos convencionales (Lombardi-Boccia et al., 2004).

El contenido en **sólidos solubles totales** fue mayor en cultivo ecológico que se correspondió con un mayor contenido no sólo en **azúcares,** sino también en **ácidos orgánicos**, lo que indicó que la fruta cultivada bajo la normativa ecológica fue más dulce que la fruta convencional y más sabrosa.

Se identificaron 18 **compuestos volátiles, con benzaldehído como compuesto principal,** que indicaron que el aroma de los jínjoles no es su principal característica, aunque los cultivados en ecológico obtuvieron un valor mayor que en convencional. Por el contrario, el **contenido proteíco**, a pesar de estar dentro de los valores normales, fue más bajo en los jínjoles ecológicos. En **el contenido mineral** no se encontraron diferencias significativas. Por lo tanto, los jínjoles ecológicos tienen una buena calidad sensorial, ya que tenían más azúcares, ácidos orgánicos y compuestos volátiles, lo que se reflejó en un color, aroma y dulzor más intensos.

La **actividad antioxidante total** no demostró diferencias significativas entre los dos tipos de jínjoles cultivados con diferentes prácticas agrícolas. Se observó que la actividad antioxidante fue mayor en la piel del jínjol por los métodos DPPH$^{\bullet}$, FRAP y en la fase hidrófila y lipófila (Imagen 33). Lo mismo ocurrió con el contenido en **fenoles, flavonoides y flavonoles,** que fueron mayores en la piel del fruto independientemente del tipo de cultivo. Puede ser debido a la acumulación de fenoles en la parte epidérmica ya que la piel está preparada como barrera de protección frente a plagas, radiaciones y productos químicos (Xue et al., 2009) y los fenoles le proporcionan esas características.

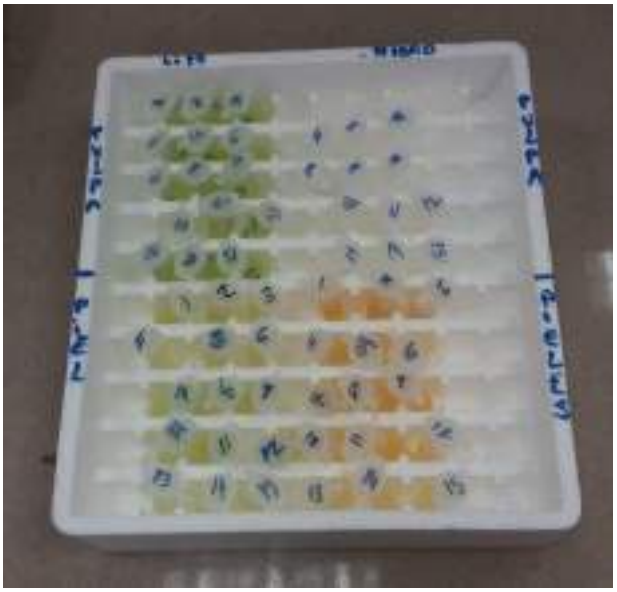

Imagen 33: Muestras de piel y pulpa de la fracción hidrófila y lipófila para cuantificar la AAT.

ii. Resumen de la conclusión

Los jínjoles procedentes de agricultura ecológica son de mejor calidad y funcionalidad. Contienen más azúcares, ácidos orgánicos, carotenoides, clorofilas y compuestos volátiles, por lo que son más dulces y olorosos. La capacidad antioxidante es mayor en piel que en pulpa, sin diferencias entre los jínjoles ecológicos y convencionales.

c. Publicación 3

PUBLICACIÓN 3

Relatioships between physico-chemical and functional parameters and genetic analysis with ISSR markers in Spanish jujubes (*Ziziphus jujuba* Mill.)

Juana Reche, Santiago García-Martínez, Pedro Carbonell, María Soledad Almansa, Francisca Hernández, Pilar Legua y Asunción Amorós.

Scientia Horticulturae 2019, 253, 390-398
***doi:* 10.1016/j.scienta.2019.04.068**

El **objetivo** del trabajo se compone por una parte de la caracterización física, nutricional y funcional, de 5 cultivares de jínjoles españoles recogidos en su estado óptimo de madurez y, por otro lado, obtener el perfil genético de estos cultivares utilizando los marcadores ISSR y así relacionar el perfil físico-químico con el genético.

i. Resumen de los Resultados y Discusión

Los cultivares estudiados tienen fenotipos muy diferentes que pueden corresponder a genotipos diferentes, pues se observó una gran variabilidad en la **caracterización física** de los jínjoles. El fruto de mayor tamaño fue 'Gab', que junto con 'Gam' y 'Me', son redondeados, en cambio 'Da' y 'Pe' tenían forma alargada y pequeña. Este último cultivar, 'Pe', también presentó el menor contenido en **SST**, con un valor de 17,80 ºBrix frente al valor más alto para 'Da', de 25,08 ºBrix. 'Da' presentó el valor más bajo de TA, lo que conllevó que obtuviera el valor más elevado del **Índice de Madurez**, siendo los más dulces y 'Pe' el más ácido y el de valor menor de IM. El resto de cultivares estuvieron comprendidos entre los valores de estos dos cultivares, dentro de un rango normal de valores para los jínjoles españoles (Reche et al., 2018).

El mayor contenido en **fenoles totales** lo presentaron los cultivares 'Gab' y 'Me', con un valor de 458,23 y 469,75 mg de AG 100 g^{-1} pf respectivamente, que en

comparación con otras frutas como cerezas y ciruelas (194,11 y 102,43 mg de AG 100 g^{-1} pf) hace que los jínjoles sean una buena fuente de compuestos fenólicos y que sean buenos para la salud. Todos los cultivares mostraron un contenido total de **flavonoides totales** entre el 12% y el 16% a excepción del cultivar 'Pe', que tenía un 34% de los fenoles totales.

La **actividad antioxidante total** mostró su máximo valor mediante el método $ABTS^{\bullet+}$ y FRAP para el cultivar 'Pe'. Por el método $DPPH^{\bullet}$ (Imagen 34), los cultivares 'Gab' y 'Da' mostraron la actividad más alta, pero en cambio, los cultivares 'Gam' y 'Me' mostraron los valores más bajos. Estas diferencias se deben al genotipo ya que los jinjoleros se cultivaron en la misma área geográfica y condiciones similares.

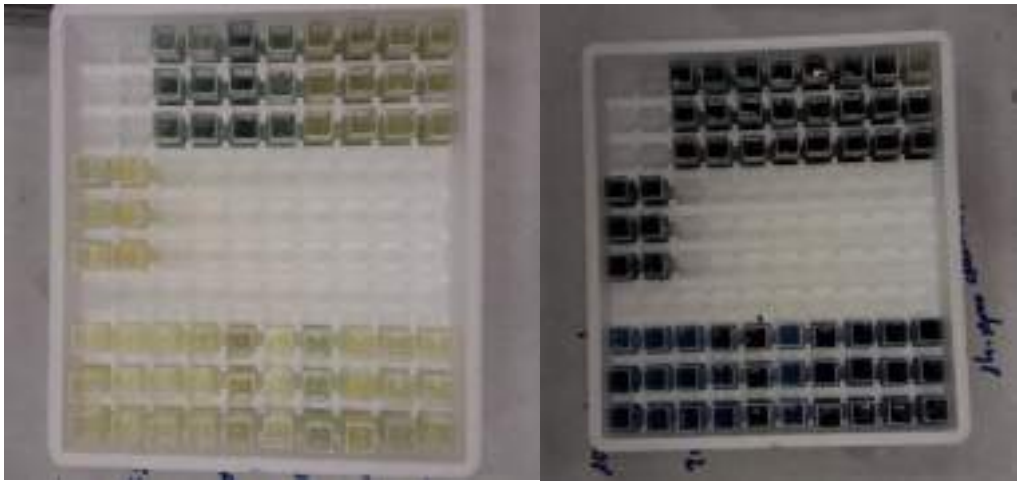

Imagen 34: Cubetas con las muestras y los reactivos para cuantificar la AAT.

El **análisis de componentes principales** (PCA) y **la correlación entre rasgos** mostró correlación positiva de la relación diámetro/longitud con el peso, y los fenoles totales, flavonoles totales y la actividad antioxidante total en la fase hidrófila y lipófila con fenoles totales, proteínas y L-AAT. La PCA, de 17 rasgos y 5 cultivares, mostró una representación del 77,34% de la variación total de los valores obtenidos del jínjol explicados por los tres primeros componentes, y el 58,7% fue explicada por los dos primeros componentes. En cuanto a los cultivares, destacó claramente 'Da' del resto de cultivares, por otro lado estaban 'Pe' y 'Me' y un tercer grupo similar fue formado por 'Gam' y 'Gab'. Esta distinción fue por el primer componente principal.

En el **análisis ISSR** el análisis del ADN de los cultivares se amplificaron con 8 de los 18 cebadores. Cada marcador generó productos entre 3 y 8 tamaños en el rango

de 0,18-2,2 kb, produciendo un total de 34 productos de los cuales 16 resultaron polimórficos y 18 monomórficos. El marcador ISSR15 obtuvo un valor muy alto de PIC con un 0,38 y de RP con un valor de 2.00 junto con el cebador UBC817. En cambio, el valor más bajo de 0,080 lo dió el cebador UBC820, que también resultó tener el valor más bajo de RP con un 0,40, siendo el promedio por cebador de PIC de 0,197 y de RP de 1,125. Estos valores explican la baja diversidad genética de los azufaifos. Otros trabajos dan una mayor diversidad, pero puede ser debido a que incluyen más de una especie en el estudio genético, lo que conlleva una mayor variabilidad genética.

El **dendograma** resultante muestra como se puede distinguir significativamente el cultivar 'Da' del resto, y los cultivares más parecidos fueron 'Gam' y 'Gab'. Los cultivares 'Pe' y 'Me' se sitúan entre estos dos grupos. Esta separación de grupos también se refleja en el análisis de componentes principales.

La relación existente entre las propiedades bioquímicas y los marcadores ISSR demostró valores similares, distribuyendo los cultivares no de igual forma, pero sí parecida. El cultivar 'Da' fue el más diferente genética y bioquímicamente, explicado por los 8 marcadores del análisis ISSR.

ii. Resumen de la conclusión

El análisis genético de los marcadores ISSR mostró una clara diferenciación entre 4 grupos de jinjoleros. Por un lado, destacó el grupo con un único cultivar, 'Da', aislado y definido. Después se distintinguió el grupo formado por 'Me' y 'Pe', y por último el grupo de 'Gam' y 'Gab', también muy diferenciado del resto. La relación entre las propiedades bioquímicas y genéticas es muy alta. Por ello, los marcadores ISSR que se utilizaron para realizar este estudio pueden servir para futuros programas de reproducción y mejora para selección de jinjoleros.

d. Publicación 4

PUBLICACIÓN 4

Fatty acid profile of peel and pulp of Spanish jujubes (*Ziziphus jujuba* Mill.) fruit

Juana Reche, María Soledad Almansa, Francisca Hernández, Ángel A. Carbonell-Barrachina, Pilar Legua y Asunción Amorós.

Food Chemistry 2019, 295, 247-253
doi: 10.1016/j.foodchem.2019.05.147

Dada la importancia de los ácidos grasos en el organismo y la salud, el **objetivo** de este artículo consistió en identificar y cuantificar los ácidos grasos presentes en la piel y en la pulpa, por separado, de 5 cultivares de jínjoles españoles, y observar también los perfiles de ácidos grasos entre azufaifos cultivados en ecológico y en convencional.

i. Resumen de los resultados y discusión

En la pulpa se encontraron 11 ácidos grasos (Imagen 35):

1. Ácido mirístico
2. Ácido miristoleico
3. Ácido palmítico
4. Ácido trans-plamitoleico
5. Ácido cis-palmitoleico
6. Ácido esteárico
7. Ácido oleico
8. Ácido 11-octadecenoico
9. Ácido elaídico
10. Ácido linoleico
11. Ácido linolénico

El ácido más abundante fue el cis-palmitoleico en los cultivares 'GAL-E', 'PSI' y 'DAT', seguido del ácido oleico en los cultivares 'GAL-T' y 'MSI'. Estos valores fueron mucho mayores que los encontrados en otros cultivares españoles de Ciudad Real (Guil-Guerrero et al., 2004). El cultivar 'GAL-T' y 'GAL-E' obtuvieron alto contenido en ácido linoleico, que es el precursor del ácido omega 6, tan necesario para el organismo. El cultivar 'MSI' obtuvo el mayor contenido en ácido linolénico, que es la base para la síntesis de ácido omega 3.

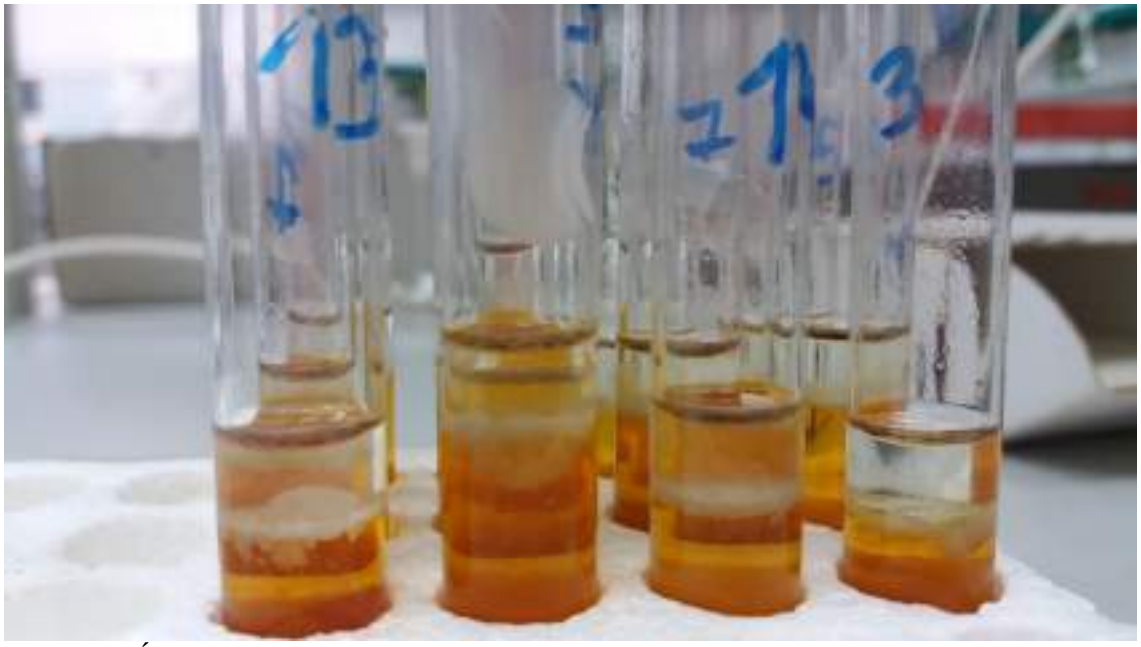

Figura 35: Ácidos grasos del jínjol en la parte superior.

Los cultivares 'PSI' y 'DAT', presentaron valores similares y 'DAT' fue el único en el que se detectó el ácido elaídico. El ácido cis-palmitoleico, que participa en el control del colesterol, triglicéridos y glucosa, fue más alto en el cultivar 'GAL' en agricultura ecológica, pero el ácido oleico fue mayor en los frutos cultivados en agricultura convencional. El cultivar 'DAT' fue el que más ácidos grasos monoinsaturados (MUFA) tenía y el que obtuvo el valor más bajo de ácidos grasos poliinsaturados (PUFA) y ácidos grasos saturados (SFA). La relación de ácidos grasos insaturados/saturados fue máxima para el cultivar 'DAT' y mucho mayor que los jínjoles de otros países como Turquía (San y Yildirim, 2010).

En la piel del jínjol se detectaron 10 ácidos grasos, los mismos que en la pulpa a excepción del ácido elaídico. Los valores obtenidos en la piel y en la pulpa fueron algo similares, y continuó siendo el ácido cis-palmitoleico el más abundante, que en el

cultivar 'DAT' obtuvo el valor más alto con un 33,12%, seguido del ácido oleico con valores de 29,00% en el cultivar 'GAL-E'. Estos valores sí fueron más altos en la piel que en la pulpa del azufaifo, al igual que el ácido trans-palmítico en el cultivar 'PSI', con un valor del 20,51%, seguido de 'GAL-T' y 'DAT'.

El contenido en **MUFA** fue muy similar en todos los cultivares, pero destacó el cultivar 'DAT' con el valor más alto y ligeramente mayor en el jínjol en ecológico que en convencional. Lo mismo ocurrió con el contenido en **PUFA**. La relación de ácidos insaturados /saturados (**USFA /SFA**) también fue muy alta en la piel del jínjol con valores entre el 2,61% en el cultivar 'GAL-T' y 6,04% en el cultivar 'DAT'. Estos altos valores hacen que el azufaifo sea muy interesante y tenga un alto potencial de beneficios para la salud. La diferencia de valores con el cultivar 'DAT' puede ser debido a que la cosecha es de aproximadamente dos meses más tarde en este cultivar, y puede ser muy interesante por su alto contenido en ácidos grasos.

Se realizó un análisis de los componentes principales (**PCA**) donde la variabilidad fue explicada por los tres primeros componentes principales con un 85,03% del total y el 72,14% por los dos primeros componentes. El PC1 del análisis de la pulpa permitió la separación de los cultivares 'GAL-E', 'PSI' y 'DAT' en el eje positivo y 'GAL-T' y 'MSI' en el eje negativo, indicando que son una buena fuente para la salud por su contenido en ácido oleico. Los tres primeros componentes de la PCA del perfil graso en la piel del jínjol, fue representada por el 75,56% del total de la varianza y el 60,37% fue explicada por los dos primeros componentes. Los cultivares 'GAL-E', 'GAL-T' y 'MSI' pudieron ser separados en el eje negativo por medio del primer componente, diferenciándolos claramente del cultivar 'DAT' con un alto valor positivo. El cultivar 'MSI' presentó el valor más negativo en PC1. Estos datos indicaron que 'GAL-E', 'GAL-T' y 'MSI' fueron una buena fuente de ácido oleico en la piel del jínjol, y 'DAT' fue una buena fuente de cis-palmitoleico.

El **análisis discriminante** que se realizó a los datos obtenidos del contenido en ácidos grasos de los 5 cultivares de jínjoles, mostró una buena separación de todos los cultivares estudiados. Los cultivares 'GAL-E' y 'GAL-T' están cercanos, pero no muestran solapamientos, por lo que también se podría separar el tipo de cultivo por su contendio en ácidos grasos.

ii. Resumen de la conclusión

El contenido en ácidos grasos de los frutos cultivados en agricultura ecológica y convencional mostró diferencias significativas en la piel y en la pulpa. La relación USFA/SFA fue mayor en la pulpa del fruto en convencional mientras que en la piel fue mayor en ecológico. Los ácidos grasos mayoritarios tanto en la pulpa como en la piel fueron el trans-palmitoleico, oleico y palmítico. El cultivar 'DAT' fue el que mostró una alta relación de ácidos saturados/insaturados. El cultivar 'MSI' presentó el mayor porcentaje de PUFAs en la piel del fruto y 'GAL-T' lo mostró en la pulpa. Por todo ello, los jínjoles son una buena fuente natural de ácidos grasos esenciales para el organismo, como el ácido omega 3 y ácido omega 6, y tienen un elevado potencial para su consumo en fresco, así como el posible uso de la piel del jínjol como sub-producto en la industria farmacéutica y alimentaria.

e. Publicación 5

PUBLICACIÓN 5

Effect of modified atmosphere packaging on the physiological and functional characteristics of Spanish jujubes (*Ziziphus jujuba* Mill.) cv 'Phoenix' during cold storage

Juana Reche, María Emma García Pastor, Daniel Valero, Francisca Hernández, María Soledad Almansa, Pilar Legua y Asunción Amorós.

Scientia Horticulturae *2019*, 258, 108743
doi: 10.1016/j.scienta.2019.108743

El **objetivo** de este trabajo fue realizar un estudio **MAP**, para mostrar su eficacia en jínjoles del cultivar 'Phoenix', que se almacenaron a 5 ºC para alargar su vida poscosecha. Se estudió la evolución de parámetros fisicoquímicos y fitoquímicos, además de compuestos bioactivos de las azufaifas control y MAP almacenadas.

i. Resumen de los resultados y discusión

Con el tratamiento MAP se alcanzó una atmósfera estable dentro del paquete en la que **la respiración** disminuyó con la bajada de temperatura de almacenaje, y propició una disminución en la concentración de O_2 hasta 12,88 kPa y un aumento de la concentración de CO_2 hasta 3,6 kPa el día 49 de almacenaje a una temperatura de 5 ºC, tal como se ha visto en otras frutas como el níspero (Amorós et al., 2008) o la ciruela (Díaz-Mula et al., 2011a). Estos resultados fueron similares a los obtenidos por Jat et al. (2012) con fruta india de azufaifa almacenada en MAP a temperatura ambiente debido al efecto de la baja permeabilidad de la película utilizada, lo que propició una disminución de la respiración (Imagen 36).

En la concentración de etileno no hubo cambios significativos, lo que confirma que los jínjoles son frutos no climatéricos (Almansa et al., 2016).

Imagen 36: Conservación de frutos día 28 y día 28 más Self Life, en MAP y control.

Entre los parámetros de calidad de los frutos se encuentra el **peso,** que mostró diferencias significativas, produciéndose una bajada de peso del 23,3% durante los 49 días de almacenaje de los frutos control frente al 0,99% de los frutos MAP. La piel de los jínjoles control ya se mostró arrugada desde la tercera semana de almacenamiento, mientras que los frutos MAP no mostraron ningún síntoma en los 49 días de almacenamiento. Esto se debe a que los frutos estuvieron hidratados por el efecto protector de la película de poliéster-polipropileno, que provocó un aumento de la presión de vapor de agua alrededor de la fruta y, a su vez, a la reducción de la tasa de transpiración de los jínjoles (Amorós et al., 2008).

La **firmeza** del fruto se mantuvo durante los 49 días de almacenamiento de los frutos MAP, mientras que hubo una disminución significativa desde el día 7 de almacenamiento en los frutos control, que disminuyó proporcionalmente a los días almacenados (Imagen 37). Este efecto MAP también fue corroborado por Lu et al. (2014) en azufaifas MAP durante 20 días de almacenamiento.

Imagen 37: Jínjoles en MAP y en control.

El aumento de los **SST** fue proporcional a los días almacenados, mostrando un aumento en la maduración de las frutas control con respecto a las azufaifas MAP, en las que se mantuvo los SST durante los 49 días de almacenamiento, con valores entre 16 y 22 ºBrix, que son normales para este cultivar (Reche et al., 2018). La **AT** permaneció similar en azufaifas control y MAP durante los 49 días de almacenamiento, alrededor de 0,35 g 100 g^{-1}. Estos parámetros produjeron un índice de madurez alrededor de 50 en los frutos MAP, mientras que en los controles aumentó significativamente desde el día 21 de almacenamiento hasta el día 49 alcanzando valores superiores a 60. Este comportamiento de los jínjoles MAP es similar a otros frutos como melocotones y nectarinas (Akbudak y Eris, 2004), y también en ciruelas (Díaz-Mula et al., 2011a) en los que el tratamiento MAP también retrasó la disminución de la AT y el aumento de SST con respecto a los frutos control.

Los valores de los parámetros de **color** de los jínjoles de este trabajo fueron muy similares a los mostrados anteriormente en otros cultivares españoles (Collado-González et al., 2014; Galindo et al., 2015; Almansa et al., 2016; Reche et al., 2018), y de China (Wang et al., 2012). El color amarillo (parámetro b* del color) y los carotenoides totales, disminuyeron durante la conservación y fueron mayores significativamente en jínjoles MAP a partir del día 21 y hasta al final del período de conservación. La disminución del color amarillo es paralela al aumento del color marrón (parámetro a* del color) de las azufaifas cuando maduran, lo que provocó una

disminución de la luminosidad (parámetro L* del color). Todos estos cambios fueron más rápidos en los frutos control y más lentos en los frutos MAP (Imagen 38), lo que indicó que las frutas maduraron ligeramente durante la conservación en frío, menos en MAP que en control.

Imagen 38: Imagen superior jínjoles en el día 7 de conservación, y en la imagen inferior jínjoles en el día 42 desde su recolección.

El contenido de **vitamina C** fue muy alto, con 562,4 mg 100 g^{-1} en los jínjoles el día 0 de conservación y similares a los encontrados por Collado-González et al. (2014) en otros cultivares españoles. Este contenido se incrementó con el efecto del

almacenamiento en frío de las frutas control llegando a 726,7 mg 100 g^{-1} el día 49 de almacenamiento. Sin embargo, en las frutas MAP no hubo diferencias significativas en el contenido de vitamina C durante todo el periodo de almacenamiento. El mantenimiento del contenido de vitamina C por efecto MAP también se observó en otras frutas como el níspero (Amorós et al., 2008).

Los resultados obtenidos del contenido de **fenoles totales** del día 0 estuvieron de acuerdo con los obtenidos por Reche et al. (2018; 2019) en este cultivar. Durante el período de almacenamiento en frío, el contenido total de fenoles totales disminuyó tanto en los frutos control como en los MAP. Sin embargo, en las azufaifas MAP, esta disminución se retrasó significativamente con respecto a los frutos control. Esto puede deberse al hecho de que la disminución de O_2 y el aumento de CO_2 en la atmósfera del paquete causaron un retraso en la maduración de los jínjoles. Esta atmósfera podría reducir las actividades de la polifenol oxidasa (PPO) o peroxidasa, que son las principales enzimas responsables de la degradación de los fenoles, como Selcuk y Erkan (2014) observaron en granadas.

La actividad antioxidante total, tanto de la fracción lipo (AAT-L) como hidrosoluble (AAT-H), en el día 0, fueron similares a Reche et al. (2018) en este cultivar. La AAT-H de la azufaifa, disminuyó significativamente con respecto al día 0. Sin embargo, en la azufaifa MAP esta disminución se retrasó significativamente con respecto a las azufaifas control. En cambio, L-TAA presentó un comportamiento opuesto, aumentando su valor en el período de almacenamiento en frío y más significativamente en los frutos control que en los azufaifos MAP. Tanto nuestros resultados como los de Díaz-Mula et al. (2011b) en ciruelas sugieren que el tratamiento MAP causa un retraso en la maduración de las frutas tratadas.

ii. Resumen de la conclusión

El almacenamiento en frío de las frutas de azufaifa (5 °C y 90% de HR), junto con un tratamiento MAP, logró un retraso significativo de la maduración de la fruta una vez recolectada, obteniendo frutos que presentaron una menor disminución en la pérdida de peso, un índice de madurez menor y una mayor fimeza e intensidad de color. En

cambio, los contenidos de carotenoides totales, vitamina C y AAT-L fueron mayores en los jínjoles control. La atmósfera del paquete se enriqueció en CO_2 y el contenido de O_2 disminuyó. Las azufaifas control tenían un aspecto arrugado no comercial en el día 21 de almacenamiento en frío mientras que las azufaifas MAP mostraron un aspecto visual normal en el día 49 de almacenamiento. Por lo tanto, el plástico utilizado aumentó la vida útil de los jínjoles más del doble de días en comparación con los jínjoles almacenados sin este plástico.

6. CONCLUSIONES FINALES E INVESTIGACIONES FUTURAS

6. CONCLUSIONES GENERALES E INVESTIGACIONES FUTURAS

6.1. Conclusiones generales

- ✓ Los jínjoles españoles estudiados, 'GAL-E', 'GAL-T', 'MSI', 'PSI' y 'DAT', tienen un alto contenido en vitamina C, minerales, compuestos fenólicos y una alta actividad antioxidante, lo que conlleva que sea un fruto saludable nutritiva y funcionalmente. Estos cultivares mostraron diferencias significativas entre sí.
- ✓ El contenido en ázucares y ácidos orgánicos hace que el jínjol sea una buena alternativa como fruto comercial, y por su gran adaptabilidad al clima árido del sureste español.
- ✓ El cultivo del azufaifo en manejo ecológico hace que éstos sean de mejor calidad y más saludables, aunque más pequeños que los cultivados tradicionalmente.
- ✓ En el perfil graso destaca el contenido en ácido oleico y otros ácidos esenciales para el organismo. El contenido de ácidos grasos en la piel es mayor que en la pulpa.
- ✓ El estudio genético permitió diferenciar claramente tres grupos de jínjoles: por un lado, el cultivar 'Da' que fue el más diferente y alejado de todos, los cultivares 'Gab' y 'Gam' que fueron cercanos ente sí y, entre estos dos grupos estarían los cultivares 'Me' y 'Pe'.
- ✓ Los jínjoles, una vez recolectados y almacenados en MAP, muestran mejores características y una vida útil más del doble de tiempo que los frutos almacenados sin ninguna película protectora.

6.2. Investigaciones futuras

El jínjol español tiene un escaso valor comercial en España, pero se presenta como una gran alternativa a la agricultura del sureste peninsular, por ello, esta Tesis Doctoral, por ser pionera en el estudio de varios aspectos del azufaifo, deja abiertas varias líneas de investigación:

- Estudiar las propiedades bioquímicas del jínjol bajo diferentes fórmulas de secado, en el horno con aire seco, secado al vacío, secado por congelación, secado al sol…para poder aplicarlo en la industria alimentaria y farmacéutica para obtener un mayor rendimiento de este fruto en España.
- El estudio más detallado de la piel del jínjol por sus buenas y mayoritarias propiedades funcionales que en la pulpa.
- El estudio de reproducción y mejora de los cultivares para mejorar el rendimiento agronómico, así como las propiedades físico-químicas de los nuevos cultivares obtenidos.
- El estudio del gran número de las propiedades funcionales del jínjol en la salud humana.
- El estudio de las propiedades nutracéuticas de la semilla.
- Uso de los marcadores ISSR utilizados para programas de mejora genética.
- El aumento de los días en almacenaje del fruto en MAP y control, ya que queda reflejado que después de 49 días de almacenaje, los jínjoles aún siguen en un estado óptimo de calidad comercial.

7. REFERENCIAS

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