TESIS DOCTORAL

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

Valorization of prickly pear

(*Opuntia ficus- indica* (L.) Mill):

Study of its phytochemical, nutraceutical, and functional properties

Lucía Andreu Coll

Director: Francisca Hernández García

Co-director: Pilar Legua Murcia

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UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE

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This doctoral thesis, entitled "Valorization of prickly pear (*Opuntia ficus-indica* (L.) Mill: study of its phytochemical, nutraceutical and functional properties", is classified in a compendium of publication category to qualify as Doctor Degree by Universidad Miguel Hernández de Elche. The following nine publications have been included:

Andreu, L., Nuncio-Jáuregui, N., Carbonell-Barrachina, Á. A., Legua, P., & Hernández, F. (2018). Antioxidant properties and chemical characterization of Spanish *Opuntia ficus-indica* Mill. cladodes and fruits. *Journal of the Science of Foodand Agriculture*, *98*(4), 1566-1573. https://doi.org/10.1002/jsfa.8628

Mena, P., Tassotti, M., **Andreu, L.**, Nuncio-Jáuregui, N., Legua, P., Del Rio, D., & Hernández, F. (2018). Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis. *Food Research International*, *108*, 301-308 https://doi.org/10.1016/j.foodres.2018.03.062

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Kolniak-Ostek, J., Kita, A., Miedzianka, J., **Andreu-Coll, L.**, Legua, P., & Hernandez, F. (2020). Characterization of Bioactive Compounds of *Opuntia ficus-indica* (L.) Mill. Seeds from Spanish Cultivars. *Molecules*, *25*(23), 5734. https://doi.org/10.3390/molecules25235734

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Andreu-Coll, L., Cano-Lamadrid, M., Noguera-Artiaga, L., Lipan, L., Carbonell-Barrachina, Á. A., Rocamora-Montiel, B., ... & López-Lluch, D. (2020). Economic estimation of cactus pear production and its feasibility in Spain. *Trends in Food Science & Technology*. *103,* 379-385. https://doi.org/10.1016/j.tifs.2020.07.003

Andreu-Coll, L., García-Pastor, M. E., Valero, D., Amorós, A., Almansa, M. S., Legua, P., & Hernández, F. (2021). Influence of Storage on Physiological Properties, Chemical Composition, and Bioactive Compounds on Cactus Pear Fruit (*Opuntia ficus-indica* (L.) Mill.). *Agriculture*, *11*(1), 62. https://doi.org/10.3390/agriculture11010062

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Book chapter:

Hernández, F.; **Andreu-Coll, L.**; Cano-Lamadrid, M.; López-Lluch, D.; Carbonell-Barrachina, Á.A.; Legua, P. Valorization of Prickly Pear [*Opuntia ficus-indica* (L.) Mill]: Nutritional Composition, Functional Properties and Economic Aspects. In Invasive Species-Introduction Pathways, Economic Impact, and Possible Management Options; IntechOpen: Rijeka, Croatia, 2020; pp. 1–5. http://dx.doi.org/10.5772/intechopen.92009

La Dra. Dña. *"Francisca Hernández García"*, directora, y la Dra. Dña. *"Pilar Legua Murcia"*, codirectora de la tesis doctoral titulada **"Valorization of prickly pear (*Opuntia ficus-indica* (L.) Mill): Study of its phytochemical, nutraceutical and functional properties"**

INFORMA/N:

Que Dña. *"Lucía Andreu Coll"* ha realizado bajo nuestra supervisión el trabajo titulado **"Valorization of prickly pear *(Opuntia ficus-indica* (L.) Mill): Study of its phytochemical, nutraceutical and functional properties"** conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

Lo que firmo/firmamos para los efectos oportunos, en Orihuela a febrero de 2022

Directora de la tesis

Dra. Dña. *"Francisca Hernández García"*

Codirectora de la tesis

Dra. Dña. *"Pilar Legua Murcia"*

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

CERTIFICA:

Que la Tesis Doctoral titulada "***Valorization of prickly pear (Opuntia ficus-indica (L.) Mill: study of its phytochemical, nutraceutical and functional properties"*** de la que es autora la Máster en Agroecología, Desarrollo Rural y Agroturismo **Dña. Lucía Andreu Coll**, ha sido realizada bajo la dirección de la **Dra. Francisca Hernández García** y la codirección de la **Dra. Pilar Legua Murcia**, profesoras de la UMH, actuando como tutora de la misma la Dra. María Asunción Amorós Marco (UMH). Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA por tanto, apta para su exposición y defensa pública.

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

Dra. Dña. Juana Fernández López
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A mi familia, por ayudarme a cumplir mis objetivos como persona y estudiante

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QUALITY INDEX OF PUBLICATIONS

Publication 1

Andreu, L., Nuncio-Jáuregui, N., Carbonell-Barrachina, Á. A., Legua, P., & Hernández, F. (2018). Antioxidant properties and chemical characterization of Spanish *Opuntia ficus-indica* Mill. cladodes and fruits. ***Journal of the Science of Food and Agriculture,*** *98* (4), 1566 1573. https://doi.org/10.1002/jsfa.8628

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JCR® category		Chemistry, Applied; Agriculture, Multidisciplinary; Food Science and Technology	
Quartile 2018	Q1	**Rank**	8/58
Impact factor	2. 422 (2018)	**5-year impact factor**	3.802

Publication 2

Mena, P., Tassotti, M., **Andreu, L.**, Nuncio-Jáuregui, N., Legua, P., Del Rio, D., & Hernández, F. (2018). Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis. ***Food Research International*****,** *108*, 301-308. https://doi.org/10.1016/j.foodres.2018.03.062

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Research domain		Food Chemistry	
JCR® category		Food Science and Technology	
Quartile 2018	Q1	**Rank**	9/144
Impact factor	3.579 (2018)	**5-year impact factor**	6.508

Publication 3

Andreu-Coll, L., Cano-Lamadrid, M., Sendra, E., Carbonell-Barrachina, Á., Legua, P., & Hernández, F. (2019). Fatty acid profile of fruits (pulp and peel) and cladodes (young and old) of prickly pear [*Opuntia ficus-indica* (L.) Mill.] from six Spanish cultivars. ***Journal of Food Composition and Analysis***, *84*, 103294. https://doi.org/10.1016/j.jfca.2019.103294

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Quartile 2019	Q1	**Rank**	34/163
Impact factor	3.721 (2019)	**5-year impact factor**	4.890

Publication 4

Kolniak-Ostek, J., Kita, A., Miedzianka, J., **Andreu-Coll, L.**, Legua, P., & Hernandez, F. (2020). Characterization of Bioactive Compounds of *Opuntia ficus-indica* (L.) Mill. Seeds from Spanish Cultivars. ***Molecules*****,** *25*(23), 5734. https://doi.org/10.3390/molecules25235734

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Publication 5

Andreu-Coll, L., Noguera-Artiaga, L., Carbonell-Barrachina, Á. A., Legua, P., & Hernández, F. (2020). Volatile composition of prickly pear fruit pulp from six Spanish cultivars. ***Journal of Food Science***, *85*(2), 358-363. https://doi.org/10.1111/1750-3841.15001

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Publication 6

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JCR® category		Agronomy	
Quartile 2020	Q1	**Rank**	20/91
Impact factor	2.925 (2020)	**5-year impact factor**	3.044

Publication 7

Hernández, F., **Andreu-Coll, L.**, Bento-Silva, A., Serra, A.T., Mena, P., Legua, P. & Bronze, M.R. (2022). Phytochemical Profile of *Opuntia ficus-indica* (L.) Mill Fruits (cv. 'Orito') Stored at Different Conditions. ***Foods***, *11*, 160. https://doi.org/10.3390/foods11020160

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Impact factor	4.350 (2020)	**5-year impact factor**	4.957

Publication 8

Andreu-Coll, L., Cano-Lamadrid, M., Noguera-Artiaga, L., Lipan, L., Carbonell-Barrachina, Á. A., Rocamora-Montiel, B., Legua, P., Hernandez, F. & López-Lluch, D. (2020). Economic estimation of cactus pear production and its feasibility in Spain. ***Trends in Food Science & Technology***. 103, 379-385. https://doi.org/10.1016/j.tifs.2020.07.003

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Impact factor	12.563 (2020)	**5-year impact factor**	14.466

Publication 9 (Book chapter)

Hernández, F.; **Andreu-Coll, L.**; Cano-Lamadrid, M.; López-Lluch, D.; Carbonell- Barrachina, Á.A.; Legua, P. Valorization of Prickly Pear [*Opuntia ficus-indica* (L.) Mill]: Nutritional Composition, Functional Properties and Economic Aspects. In Invasive Species-Introduction Pathways, Economic Impact, and Possible Management Options; IntechOpen: Rijeka, Croatia, 2020; pp. 1–16. http://dx.doi.org/10.5772/intechopen.92009

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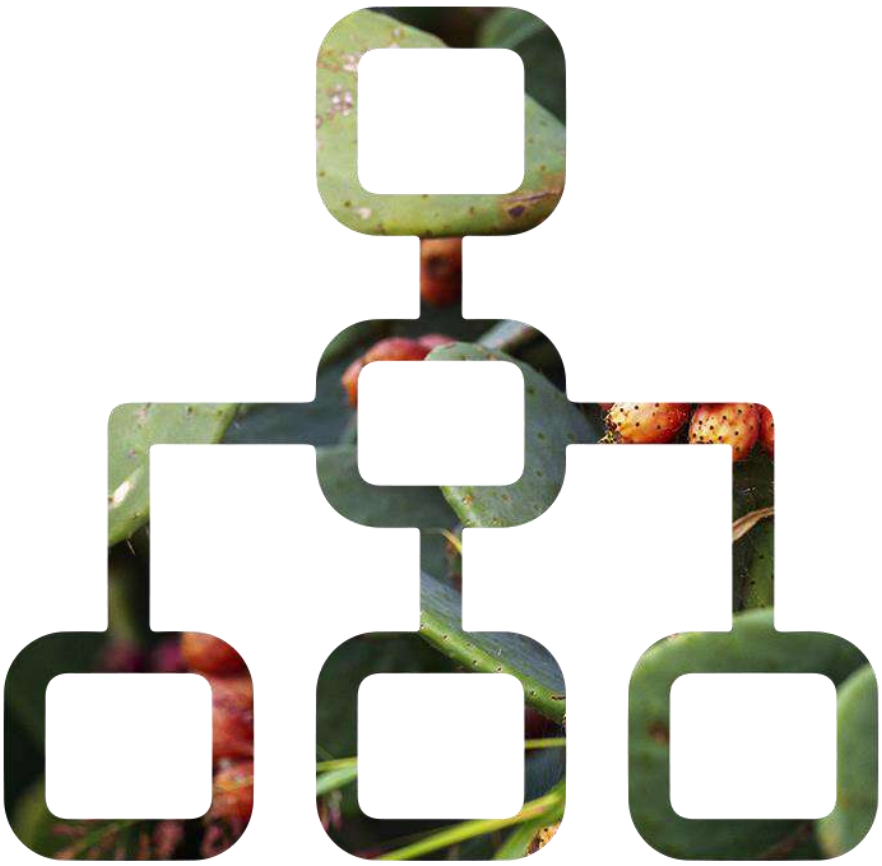


1. DOCTORAL THESIS STRUCTURE

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

- **Abstract/*Resumen*.** The most relevant result and conclusions are described in this section (English and Spanish).
- **Introduction.** This section contains a brief bibliographic review botanical parts composition, taxonomy, origin, uses, and economic importance of *Opuntia ficus-indica* (L.) Mill.
- **Objectives.** The main objective and specific goals are showed in this part.
- **Material and Methods**. This part contains a description of plant material, methodology and statistical analyses used to reach the objectives of this research.
- **Publications:** The seven publications and the book chapter used to develop this Doctoral Thesis are listed below:

Publications:

1- Antioxidant properties and chemical characterization of Spanish *Opuntia ficus-indica* Mill. cladodes and fruits. 2018. ***Journal of the Science of Food and Agriculture,*** *98* (4), 1566-1573. https://doi.org/10.1002/jsfa.8628

2- Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis. 2018. ***Food Research International*****,** *108*, 301-308 https://doi.org/10.1016/j.foodres.2018.03.062

3- Fatty acid profile of fruits (pulp and peel) and cladodes (young and old) of prickly pear [*Opuntia ficus-indica* (L.) Mill.] from six Spanish cultivars. 2019. ***Journal of Food Composition and Analysis***, *84*, 103294. https://doi.org/10.1016/j.jfca.2019.103294

4- Characterization of Bioactive Compounds of *Opuntia ficus-indica* (L.) Mill. Seeds from Spanish Cultivars. 2020. ***Molecules*****,** *25*(23), 5734. https://doi.org/10.3390/molecules25235734

5- Volatile composition of prickly pear fruit pulp from six Spanish cultivars. 2020. ***Journal of Food Science***, *85*(2), 358-363. https://doi.org/10.1111/1750-3841.15001

6- Influence of Storage on Physiological Properties, Chemical Composition, and Bioactive Compounds on Cactus Pear Fruit (*Opuntia ficus*-indica (L.) Mill.). 2021. ***Agriculture*****,** *11*(1), 62. https://doi.org/10.3390/agriculture11010062

7- Phytochemical Profile of *Opuntia ficus-indica* (L.) Mill Fruits (cv. 'Orito') Stored at Different Conditions. ***Foods***, 11, 160. https://doi.org/10.3390/foods11020160

8- Economic estimation of cactus pear production and its feasibility in Spain. 2020. ***Trends in Food Science & Technology***. 103, 379-385. https://doi.org/10.1016/j.tifs.2020.07.003

Book chapter:

9- Valorization of Prickly Pear [*Opuntia ficus-indica* (L.) Mill]: Nutritional Composition, Functional Properties and Economic Aspects. 2020. ***Cactaceae-Current Trends and Future Perspectives, Intechopen***: Rijeka, Croatia; pp.1-5. http://dx.doi.org/10.5772/intechopen.92009

- **Results and Discussion**. This section gathers the key results and a brief discussion of each publication.
- **Conclusions/*Conclusiones*.** This section includes the main conclusions reached with this doctoral thesis and the future research lines (English and Spanish).
- **References.** The present section contains all the literature used to write and justify this Doctoral Thesis following APA 7th edition.

2. ABBREVIATION

AA	Antioxidant Activity
$ABTS^{\cdot+}$	2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AI	Atherogenicity index
ANOVA	*Analysis of Variance*
CAM	Crassulacean Acid Metabolism
CM	Contribution Margin
DM	Dry Matter
$DPPH^{\cdot}$	2,2-diphenyl-1-picrylhydrazyl
dw	dry weight
ESI	Electrospray Ionization
FAO	Food and Agriculture Organization of the United Nations
FID	Flame Ionization Detector
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalents
GC	Gas Chromatography
GI	Gross Incomes
HPLC	High Performance Liquid Chromatography
HRP	Horseradish Peroxidase enzyme
H-TAA	Hidrophylic Total Antioxidant Activity
IC	Incremental Costs
L-TAA	Lipophilic Total Antioxidant Activity
masl	Metters above sea level
MS	Mass Spectrometry
MSn	Multi Stage Mass Spectrometry
MUFA	Monounsaturated Fatty Acids
PTFE	Polytetrafluoroethylene
PUFA	Polyunsaturated Fatty Acids
ROAECS	Results Orientated Agro-Environment Climate Scheme
SE	Standard Error
SFA	Saturated Fatty Acids
TA	Titratable Acidity
TAA	Total Antioxidant Activity
TI	Thrombogenicity index
TPC	Total Phenolic Content
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TSS	Total Soluble Solids
U/S	Unsaturated fatty acids /Saturated faty acids
UPLC / UHPLC	Ultra-Performance Liquid Chromatography

3. ABSTRACT/RESUMEN

Abstract

Opuntia ficus-indica (L.) Mill.), known as prickly pear or cactus pear, is a tropical or subtropical part of the Cactaceae family. This plant, which can grow in arid and semiarid climates, is native of Mexico and nowadays is naturalized in all continents, mainly in America, the southeast of Spain and the Mediterranean basin. Prickly pear is the Cactaceae plant with the greatest economic importance, due to their fruits and cladodes are consumed, the last mainly in Mexico. Prickly pear is mainly known for the consumption of their fruits in fresh, which can also be consumed in different processed forms, such as juices, jams, and syrups. Besides, the cladodes are very popular in Mexico and can be consumed in fresh, cooked, in juices or dehydrated, among other preparations. In addition to their use for human consumption, the prickly pear botanical parts (fruits, cladodes, and seeds) can be used for other purposes, such as in the pharmaceutical and cosmetic industries, animal feeding, biofuel production and phytoremediation of soils, among other uses.

In addition to the optimal nutritional properties of the prickly pear fruits, cladodes and seeds, there is ample evidence of the health benefits that its consumption entails. Both the fruits (peel and pulp) as well as the cladodes and the seeds show a high quantity of bioactive compounds, among which the polyphenolic compounds stand out, which showed antioxidant activity. Besides, prickly pear presents other interesting health-promoting compounds, such as monounsaturated and polyunsaturated fatty acids (MUFA and PUFA, respectively), mainly in its seeds, which are also related to health benefits. This crop also stands out for showed lower management and economic cost in comparison with other crops and, due to its adaptability and resistance to high environmental stress conditions, FAO promotes the cultivation of the prickly pear in arid and semi-arid areas, which could provide use to territories where is difficult to develop other crops.

Therefore, the main objective of this doctoral thesis was to determine the phytochemical, nutraceutical, and functional properties of different cultivars of prickly pear which grow in the experimental field station of Miguel Hernández University, and commercial cultivars from Alicante and Murcia, as well as their economic evaluation based on production costs, its content in bioactive compounds and carbon sequestration.

The antioxidant activity, polyphenolic compounds and the fatty acid profile were determined in the fruits (peel and pulp), cladodes and seeds. The results showed that the young cladodes (less than one year old) and the peel of the fruits presented a higher antioxidant activity than the cladodes of more than one year, the pulp of the fruits and the seeds. The seeds of 'NE' cultivar and the young cladodes and the peel of the fruits of the 'FR' cultivar stood out for their antioxidant activity by the three methods ($DPPH^{\cdot}$, $ABTS^{\cdot+}$ and FRAP) and for their concentration of polyphenolic compounds, presented correlation between both parameters.

Linoleic acid, a polyunsaturated essential fatty acid, was the most abundant fatty acid in all cultivars and parts of the prickly pear, except young cladodes, which showed palmitic acid as the predominant fatty acid. The cladodes of the 'FR' cultivar showed the highest values of MUFA and PUFA, what made it the most interesting cultivar to use its cladodes in animal feed, elaboration of processed products and human consumption. Regarding the fruits, the cultivar 'NJ' stood out for its high PUFA content both in the pulp and in the peel of the fruits. The seeds stood out for their high PUFA content in comparison to the fruits and cladodes, highlighting the cultivar 'NE', although the cultivar 'NO' showed a higher fat content. The cultivars 'FR' and 'NO' stood out for their protein content, although the cultivar 'NA' showed

higher values of IAAs (indispensable amino acids) and DAAs (total dispensable amino acids).

Regarding the sensory analysis, the volatile compounds were determined in the pulp of the fruits, determining 35 different compounds. 'FR' and 'NT' cultivars showed the higher concentration of these compounds, which are related to the acceptance by consumers.

The fruits of the cultivar 'Orito' were preserved for 28 days under cold conditions (2ºC and 85-90% relative humidity). After cold conservation, the fruits were placed three days at room temperature (20ºC) to study shelf life. The results showed that the fruits of this cultivar presented a suppressed-climacteric pattern in ethylene production and respiration rate, and their conservation were optimal under both conditions during this time, showing optimal values both in the physical parameters, such as color and firmness, as well as in the chemicals, like ºBrix, antioxidant activity, phytochemical profile and concentration of phenolic compounds.

To elaborate the economic analysis of the prickly pear crop, the production in Spain was compared with that of two major producing countries, Mexico, which is the main producer and the country with the largest area under cultivation, and Italy, which showed the most developed productive sector. In addition, due to the high content of bioactive compounds in the prickly pear, its extraction could increase the profitability of the prickly pear production, although the costs of extracting these compounds would have to be analyzed. Likewise, prickly pear cultivation proved to be an effective tool to mitigate climate change in arid and semi-arid regions, with cultivation practices being a key aspect in effectively contributing to improving carbon sequestration.

These results of this work showed that the different parts of the prickly pear studied (fruits, cladodes, and seeds) presented a nutritional profile and a quantity of bioactive compounds interesting both for human consumption, for consumption in fresh or processed foods, as well as for the pharmaceutical and cosmetic industries. In addition, the peel and the seeds of the fruit are obtained as waste when the pulp is extracted to elaborate processed foods, so their use both in the industries mentioned above and in animal feed would help to reduce the waste generated in this process. For all that, added to its low production cost and its optimal conservation up to one month, make the prickly pear a very interesting crop in terms of the parameters studied in this work, which could also be an effective tool for rural development in arid and semi-arid areas in production, job creation and environment issues.

Resumen

Opuntia ficus-indica (L.) Mill.), conocida como chumbera o nopal, es una planta tropical o subtropical perteneciente a la familia de las Cactáceas. Esta planta, que puede crecer en climas áridos o semiáridos, es originaria de México y hoy en día se encuentra naturalizada en todos los continentes, principalmente en el continente americano, el sureste de España y toda la cuenca Mediterránea. La chumbera es la cactácea de mayor importancia económica, ya que tanto sus frutos como sus cladodios son consumidos, estos últimos principalmente en México, el cual es el país con la mayor área de cultivo y el mayor productor de esta planta. Aunque la chumbera es principalmente conocida por el consumo de sus frutos en fresco, los higos chumbos, estos también pueden consumirse de distintas formas procesadas, como zumos, mermeladas y siropes. Además, sus cladodios son muy populares en la cocina mexicana, pudiendo consumirse en fresco, cocinados, en zumos o deshidratados. Además de para consumo humano, las distintas partes de la chumbera (frutos, cladodios y semillas) pueden utilizarse con otros fines, como en las industrias farmacéutica y cosmética, alimentación animal, producción de biocombustibles y fitorremediación de suelos, entre otros usos.

Además de las excelentes propiedades nutricionales de los frutos, cladodios y semillas de la chumbera, existe amplia evidencia de los beneficios para la salud que conlleva su consumo. Tanto los frutos (piel y pulpa) como los cladodios y las semillas muestran una alta cantidad de compuestos bioactivos, entre los que destacan los compuestos polifenólicos, los cuales muestran actividad antioxidante. Además, presenta otros compuestos interesantes para la salud como ácidos grasos monoinsaturados y poliinsaturados, principalmente en sus semillas, los cuales también están relacionados con beneficios para la salud. También cabe destacar que es un cultivo que presenta un manejo y coste económico menor que otros cultivos y, debido a su capacidad de adaptación y resistencia a condiciones de alto estrés ambiental, la FAO promueve el cultivo de la chumbera en zonas áridas y semiáridas, lo que podría dar uso a territorios donde es difícil desarrollar otros cultivos.

Por todo ello, el principal objetivo de esta tesis doctoral fue determinar las propiedades fitoquímicas, nutracéuticas y funcionales de distintos cultivares de chumbera cultivados en la finca experimental de la Escuela Politécnica Superior de Orihuela y cultivares comerciales de Alicante y Murcia, así como su evaluación económica en base a los costes de producción, su contenido en compuestos bioactivos y el secuestro de carbono.

La actividad antioxidante y compuestos polifenólicos y el perfil de ácidos grasos se determinaron en los frutos (piel y pulpa), los cladodios y las semillas. Los resultados mostraron que los cladodios jóvenes (menos de un año) y la piel de los frutos mostraron una mayor actividad antioxidante que los cladodios de más de un año, la pulpa de los frutos y las semillas. Las semillas del cultivar 'NE' y los cladodios jóvenes y la piel de los frutos del cultivar 'FR' destacaron por su actividad antioxidante mediante los tres métodos utilizados ($DPPH^{\bullet}$, $ABTS^{\bullet+}$ y FRAP) y por su concentración de compuestos polifenólicos, existiendo correlación entre ambos parámetros.

El ácido linoleico, un ácido graso esencial poliinsaturado, fue el más abundante en todos los cultivares y partes de la chumbera, excepto en los cladodios jóvenes, en los cuales predominó el ácido palmítico. Los cladodios del cultivar 'FR' mostraron los valores más elevados de MUFA y PUFA, por lo que es el cultivar más interesante para utilizar sus cladodios en alimentación animal, elaboración de productos procesados o consumo humano. En cuanto a los frutos, destacó el cultivar 'NJ' por su alto contenido en PUFA tanto en la pulpa como en la piel de los frutos. En lo referente a las semillas, destacan por su alto

contenido en PUFA en relación con los frutos y cladodios, destacando el cultivar 'NE', aunque el cultivar 'NO' mostró un contenido en grasa más elevado. Los cultivares 'FR' y 'NO' destacaron por su contenido en proteínas, aunque el cultivar 'NA' mostró valores más elevados de IAAs (indispensable amino acids) y DAAs (total dispensable amino acids).

En cuanto al análisis sensorial, los compuestos volátiles se determinaron en la pulpa de los frutos, determinando 35 compuestos distintos, siendo los cultivares 'FR' y 'NT' los que mostraron una mayor concentración de estos compuestos, los cuales están relacionados con la aceptación por parte de los consumidores.

Se realizó la conservación de los frutos del cultivar 'Orito' durante 28 días en condiciones de frío (85-90% HR) y, para estudiar su vida útil, se mantuvieron 3 días a temperatura ambiente (20ºC) después de la conservación en frío. Los resultados mostraron que los frutos de este cultivar muestran un patrón de climaterio suprimido en cuanto a la producción de etileno y CO_2 y que su conservación es óptima bajo ambas condiciones durante este tiempo, mostrando valores óptimos tanto en los parámetros físicos, como el color y la firmeza, como en los químicos, tales como los ºBrix, actividad antioxidante, perfil fitoquímico y concentración de compuestos fenólicos.

Para la realización del análisis económico del cultivo de la chumbera se comparó la producción en España con la de dos importantes países productores, México e Italia, mostrando este último el sector productivo más desarrollado. Además, debido al alto contenido de compuestos bioactivos de la chumbera, su obtención podría incrementar la rentabilidad de su producción, aunque habría que analizar los costes de extracción de estos compuestos. Asimismo, el cultivo de la chumbera mostró ser una eficaz herramienta para mitigar el cambio climático en regiones áridas y semiáridas, siendo las prácticas de cultivo un aspecto clave a la hora de contribuir de forma eficaz a mejorar el secuestro de carbono.

Estos resultados muestran que las distintas partes de la chumbera estudiadas (frutos, cladodios y semillas) muestran un perfil nutricional y una cantidad de compuestos bioactivos interesante tanto para la alimentación humana, para su consumo en fresco o alimentos procesados, como para las industrias farmacéutica y cosmética. Además, la piel y las semillas del fruto se obtienen como deshecho cuando se realiza la extracción de la pulpa para elaborar alimentos procesados, por lo que su utilización tanto en las industrias nombradas anteriormente como en alimentación animal ayudaría a reducir los residuos generados en este proceso. Todo esto, sumado a su reducido coste de producción y su fácil conservación, hacen de la chumbera un cultivo muy interesante en cuanto a los parámetros estudiados en este trabajo, que además podría ser una eficaz herramienta para el desarrollo rural en zonas áridas y semiáridas en materia de producción, creación de empleo y medio ambiente.

4. INTRODUCTION

4.1 Origen

Opuntia ficus-indica (L.) Mill., also known as prickly pear, cactus pear or nopal cactus, belongs to Cactaceae family and it is the most important plant of this family in the world, due to it is the most exploited and commercialized plant of this family since their fruits and cladodes are consumed, mainly in Mexico (Casas & Barbera, 2002; FAO, 2018).

The interest in cactus pear goes back thousands of years. According to archaeological evidence, the species of the genus *Opuntia* proceeded from Central America. The indigenous population of this semi-arid areas was who began its cultivation, specifically the Aztec empire, in the country that today is known as Mexico (Pimienta-Barrios, 1994; Kiesling, 1998).

The domestication of *O. ficus-indica* (L.) Mill. began approximately 8000 years ago and its genetic improvement dates to pre-Hispanic times. After his introduction in Spain around the year 1500, probably in one of Columbus's trips to America, this plant and other of the same genus were dispersed and naturalized throughout the Mediterranean area, becoming a characteristic element of the local landscape. In 1550, they have spread throughout Europe (FAO, 2018; Kiesling, 1998; Mottram, 2013; Reyes-Agüero et al., 2005)

Figure 1. *Opuntia* spp. worldwide distribution, provided by (Sáenz et al., 2006).

Nowadays prickly pear is naturalized in Mediterranean basin and currently being found on all continents. In the American continent it is currently found from Canada to Chile, in Argentina, Bolivia, Brazil, Colombia, Chile, the United States, Mexico, Peru, Venezuela and in other countries of Central America and the Caribbean. When it was introduced from Mexico into Spain, from here it was distributed throughout the Mediterranean basin: France, Greece, Italy, Turkey, and Israel. The Arabs introduced this genus in Africa, reaching as far as Algeria, Egypt, Eritrea, Ethiopia, Libya, Morocco and Tunisia. We can also find prickly pears in South Africa, Australia, and India (Sáenz et al., 2006).

4.2 Taxonomy and description

The scientific nomenclature and taxonomy of cactus pear is:

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Caryophyllales
- Family: Cactaceae
- Tripe: Opuntiae
- Genus: Opuntia
- Species: *Opuntia ficus-indica* (L.) Mill.

O. ficus-indica (L.) Mill. is a succulent bushy plant, branched and usually 1.5-3 m in height, although it can reach 4 m. This plant can be divided in four main parts: cladodes, flower, fruit, and seeds (FAO, 2018; Prieto-García et al., 2006).

Cladodes or pads are succulent and typically oblong or elliptical in shape, usually 30-40 cm long and 18-25 wide. The spines of the cladodes, although they are not abundant and some cultivars lack these, are in areolas and can be glochids (small and grouped) or modified leaves (large and individual). From these areolas can develop new cladodes, flowers and fruits depending on environmental conditions. (FAO, 2018; Sáenz, 2006). Due to cactus pear is a CAM-type metabolism plant, stores high amount of water in their cladodes and the morphology and anatomy of them it has evolved to serve this function (FAO, 2018; Sáenz, 2006; Melgar, 2017).

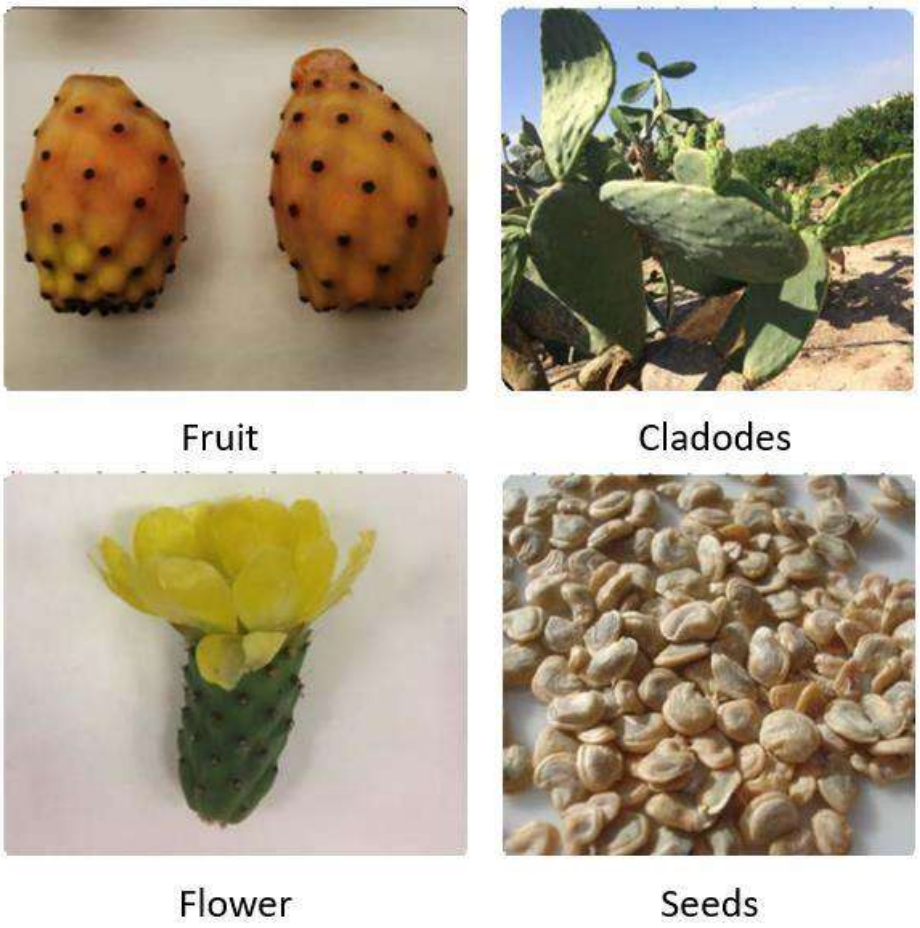


Figure 2. Botanical parts of *O. ficus-indica* (L.) Mill.

Prickly pear fruit, commonly named prickly pear, cactus pear, "tunas" (Mexico), "higo chumbo" (Spain), "fico d'India" (Italy) or "figure de Barbarie" (France), is a fleshy berry varying in shape, size and colour with a tasty pulp full of seeds. This fruit is a berry which present a semi-hard peel (pericarp) with many prickles and a very tasty pulp full of seeds, regularly arranged throughout the pulp. The varieties differ mainly in four groups by peel and pulp fruit colour: yellow-green peel and white pulp, yellow-orange peel and orange pulp, green-red peel and red pulp, and purple peel and pulp (Melgar, 2019; Sáenz, 2006).

Fruit of commercial cactus pear typically range from 120 to 200 g, with 45-60% of the fruit being edible (Stinzting et al., 2005). Peel of fruit, same as cladodes, showed areoles, glochids and spines in their surface, although the presence or absence of spies also differs between the cultivars. The pulp, which is tasty and highly flavored, contains many seeds, about 0.24 g/g, constituting about 10-15% of the edible pulp

and 30-40% on a dry weight basis, and usually discared after the extraction of the pulp (Chougui et al., 2013; FAO, 2008; Feugang, 2006; Ramadan & Mörsel, 2003).

Flowers are hermaphroditic, solitary, and sessile, and sprout from the areoles, mainly in the apical part of the cladode margin. They are usually 7 cm of length, and their color is normally yellow, orange, red, pink, or white. These flowers sprout in the cladodes after six months and their bloom is between 35-45 days after this moment, requiring a minimum temperature of 15ºC for its optimal development. Normally prickly pear flowers only sprout once a year, but under certain conditions this plan can show a second flowering (Alvarez, 2007; Melgar, 2019; Reyes-Agüero et al., 2005).

4.3 Nutritional value and health benefits

Fruits and vegetables are universally promoted as healthy and various organizations recommend the consumption of several servings per day, because its content of energy, nutrients, dietary fiber, vitamins, and minerals (Slavin & Lloyd, 2012). Nowadays, consumers look for healthy foods and avoid the consumption of others with harmful preservatives, which is why the consumption of fresh fruit has currently increased worldwide, increasing the commercialization of fruits worldwide more than five times in the last fifteen years (FAOSTAT, 2017; Vázquez-Briones et al., 2019).

Table 1 showed the chemical composition of the botanical parts of *O. ficus-indica* (L.) Mill: fruit (peel and pulp), cladodes and seeds, although these values may vary according to the cultivar, growing conditions, environmental factors and genetic diversity among other factors. Like other tropical fruits, the main components of prickly pear fruit are water and carbohydrates (Vázquez-Briones et al., 2019). Fruit pulp showed the highest values in glucose and fructose, followed by the peel. Seeds stand out for its high content of lipids and protein and no content in glucose and fructose. Cladodes are the botanical part that showed the highest values in fiber and ash.

Table 1. Chemical composition of *Opuntia ficus-indica* (L.) Mill. fruit (pulp and peel), seeds and cladodes (Andreu et al., 2018; Ayadi et al., 2009; Bensadon et al., 2010; El-Beltagi et al., 2019; Medina et al., 2007; Nassar, 2008; Salim et al., 2009).

Constituents	Pulp	Peel	Seeds	Cladodes
Moisture (%)	90.7 - 94.4	88.9 - 90.3	6.1 - 18	90.7 - 94.4
Fiber (%)	4.65 - 5.65	4.88 - 5.83	9.23 - 12.47	41.83 - 51.24
Protein (%)	0.87 - 1.62	1.45 - 4.50	4.48 - 13.72	1.13 - 8.88
Lipids (%)	0.48 - 0.70	0.32 - 1.06	3.66 - 10.43	1.22 - 4.69
Ash (%)	0.37 - 2.6	2.6 - 8	1.27 - 12.66	16.3- 23.3
Glucose (g L^{-1})	14.5 - 308	10.7 - 128	0	3.2 - 70.1
Fructose (g L^{-1})	5.1 - 201.7	10.1 - 121.6	0	tr - 164

In addition to its nutritional composition, prickly pear provides health benefits because its antioxidant activity, the mechanism by which fruits and vegetables inhibit excessive oxidation for free radicals, which are in the form of reactive oxygen species. Prickly pear stands out for its polyphenols, a group or natural compounds that are characterized by the presence of more than one phenol group in their structure. These compounds present a high scientific and therapeutic interest since they are related to the prevention and improvement of various conditions and pathologies due to its antioxidant activity (Scalbert et al., 2005; Yeddes et al., 2013). Other molecules that prickly pear presents and that show antioxidant activity are betalains, vitamin C and carotenoids. The consumption of fruits and vegetables which show high levels of antioxidant activity is related to the prevention of degenerative diseases, such as diabetes, cancer, hypercholesterolemia, cardiovascular and gastric diseases, and arteriosclerosis (Galati et al., 2003; Jiménez-Aguilar et al., 2014; Yeddes et al., 2014). However, antioxidant activity in fresh fruits is dependent of the maturity, type of cultivar, environmental conditions, cultivation and harvesting practices, but during postharvest handling and processing the most significant changes can occur (Vázquez-Briones et al., 2019).

Prickly pear can also provide health benefits due to its fatty acid profile, because the high percentage of monounsaturated and polyunsaturated fatty acids, which are related to the improvement of different health conditions such as obesity, diabetes

mellitus and cardiovascular diseases. Linolenic (an essential polyunsaturated fatty acid), oleic and palmitic acid are the predominant fatty acids in fruit, cladodes, and seeds (Andreu-Coll et al., 2019; De Wit et al., 2017; El-Beltagi et al., 2019).

4.4 Economic importance and marketability

Opuntia genus has important cultural and economic importance around Americas and some arid and semiarid regions in the world, as crops for both alimentary and forage products (Rodríguez-López et al., 2020). Nowadays, cactus pear crop takes up more than 100,000 ha in arid and semiarid areas in at least 18 countries, but only Mexico, Italy, Chile, South Africa, and Argentina produce this crop in a commercial way. In many communities of Africa, Asia, Europe and America, the consumption of this crop is limited to local ethnic markets and there is little export (FAO, 2008; Inglese et al., 2002). There is little information available regarding the cactus pear crop areas and there is not statistic information available from most countries.

Figure 1 shows the cultivated area destinated to prickly pear fruit production and the yield in different countries. Mexico is the main producer of prickly pear in the world (45% of world production, followed by Italy (12.2%) and South Africa (3.7%) (FAO, 2018; Reyes-Agüero et al., 2013). Although Mexico is the country with the largest extension of prickly pear crop, Italy has a highly developed production sector, being the main exporter of this product and having the highest yield. In Spain, this crop can be found mainly in home orchards and gardens. Only Andalusia, Valencian Community, Murcia Region and Canary Islands have a little productive structure in a total of 195 Ha, and more than 130,000 scattered trees have been counted (FAO, 2018; MAPA, 2018). For some years now, FAO ("Food and Agriculture Organization") has been promoting the cultivation of prickly pear and other species of Opuntia genus in arid and semiarid zones, due to their adaptative capacity and resistance to high environmental stress conditions. In this way, it is possible to use territories where it is difficult to develop other crops (FAO, 2018; Sáenz, 2006).

The common names of cultivars varieties change according to the country. In Mexico, the most popular varieties are 'Reina', 'Rojo Pelón' and 'Esmeralda', while in Italy these cultivars are named 'Giallia', 'Rosso' and 'Bianca' respectively. In Spain,

they are called 'Verdales', 'Morados', 'Sanguinos' y 'Blancos'. The most popular varieties in the international market are those of are different from the typical green color, specially the red, yellow, pink, and purple ones, due to the attractive color of the fruit and its low content of sugars (Álvarez, 2007; Melgar, 2019).

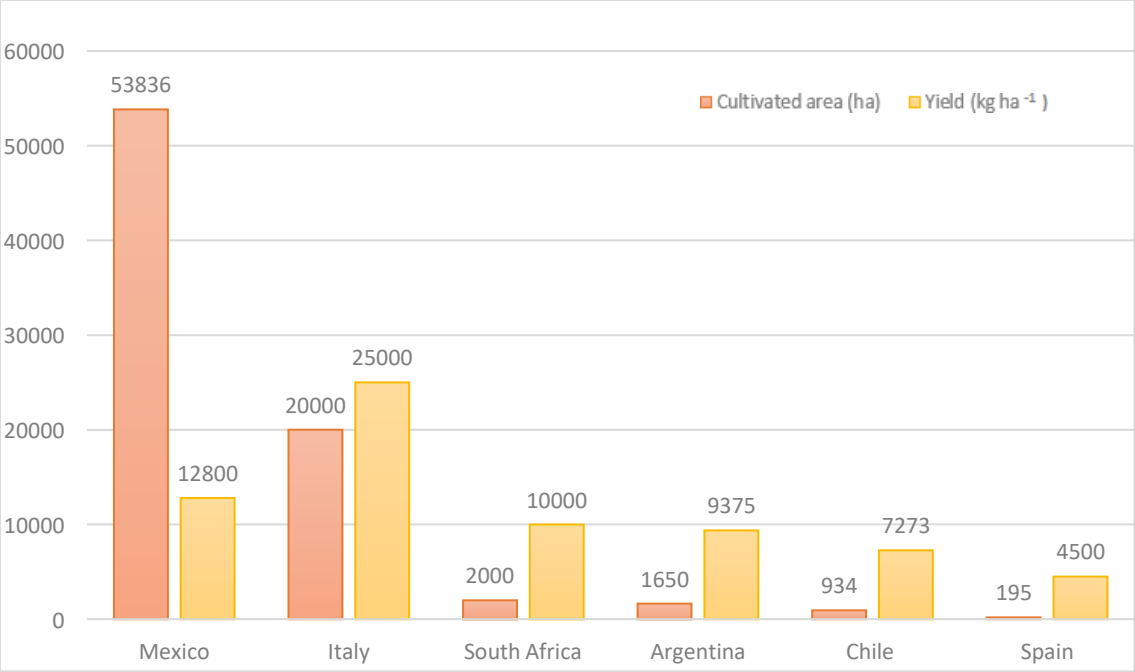


Figure 3. Cultivated area destinated to prickly pear fruit production (ha) and yield (kg ha $^{-1}$) in different countries, data provided by (FAO, 2018; Lloret, 2016; MAPA, 2018; Sáenz, 2006).

Fruit quality is a very important factor in fruit production, also in cactus pear fruit, due to consumers prefer fruit with good taste and good nutritional quality. Quality of fruit is higher at harvest time and decreases over time according to different factors: the variety or cultivar, postharvest treatments, environmental factors, and storage and distribution conditions. Thus, cactus pear fruit must be harvested when it shows the highest edible quality, although for transportation to distant markets early harvest is more appropriate to prolong the postharvest life. For the determination of the best harvest stage of cactus pear fruit, there are some signs for the determination the best harvest stage of cactus pear fruits, such as color of peel, total soluble solids (> 13%) and firmness. These parameters have based in different factors: varieties, producing country, destination of the fruit and final use (Cantwell, 1995; FAO, 2018).

Cactus pear fruit is highly perishable and physical damage during harvest and transport can affect fruit quality and shelf life. It is classified as non-climacteric fruit,

which do not show significant variations in their respiration rate and ethylene synthesis during the ripening stage, but these factors can be influenced by the cultivar, maturity stage at harvest time and environmental conditions among others (Cantwell, 1995; FAO, 2018; Lakshminarayana & Estrella, 1978). Such as other tropical fruit, cactus pear is susceptible to cold damage during storage although some factors like postharvest treatments can decrease this susceptibility. Besides, due to its composition and tissue consistency, it is also highly susceptible to spoilage by pathogenic fungi, yeast, and bacteria, mainly in areas affected by physical damage (FAO, 2018). Due to the growing demand of cactus pear fruit, postharvest treatments are required to improve cactus pear marketability. Refrigeration is the main strategy to prolong the postharvest life of this fruit, although there are limitations due to the susceptibility to cold damage. Other strategies are storage in controlled atmospheres, immersion in hot water and wrapping in films, among others (D'Aquino, 2012; FAO, 2018; Shumye et al., 2014).

5. OBJECTIVES

The overall aim of this Doctoral Thesis was to determine the phytochemical, nutraceutical, and functional properties of the botanical parts (cladodes, fruits and seeds) of different cultivars of prickly pear, with the purpose of evaluate their use either for human diet, animal feeding or industrial use.

To reach the main purpose, the following specific objectives were established (Figure 4):

- Objective 1: Phytochemical, nutraceutical, and functional characterization of the fruit, cladodes and seeds.
- Objective 2: Sensory analysis.
- Objective 3: Evaluation of the quality parameters of prickly pear fruits during their conservation under different conditions.
- Objective 4: Economic estimation of prickly pear production and its feasibility in Spain.

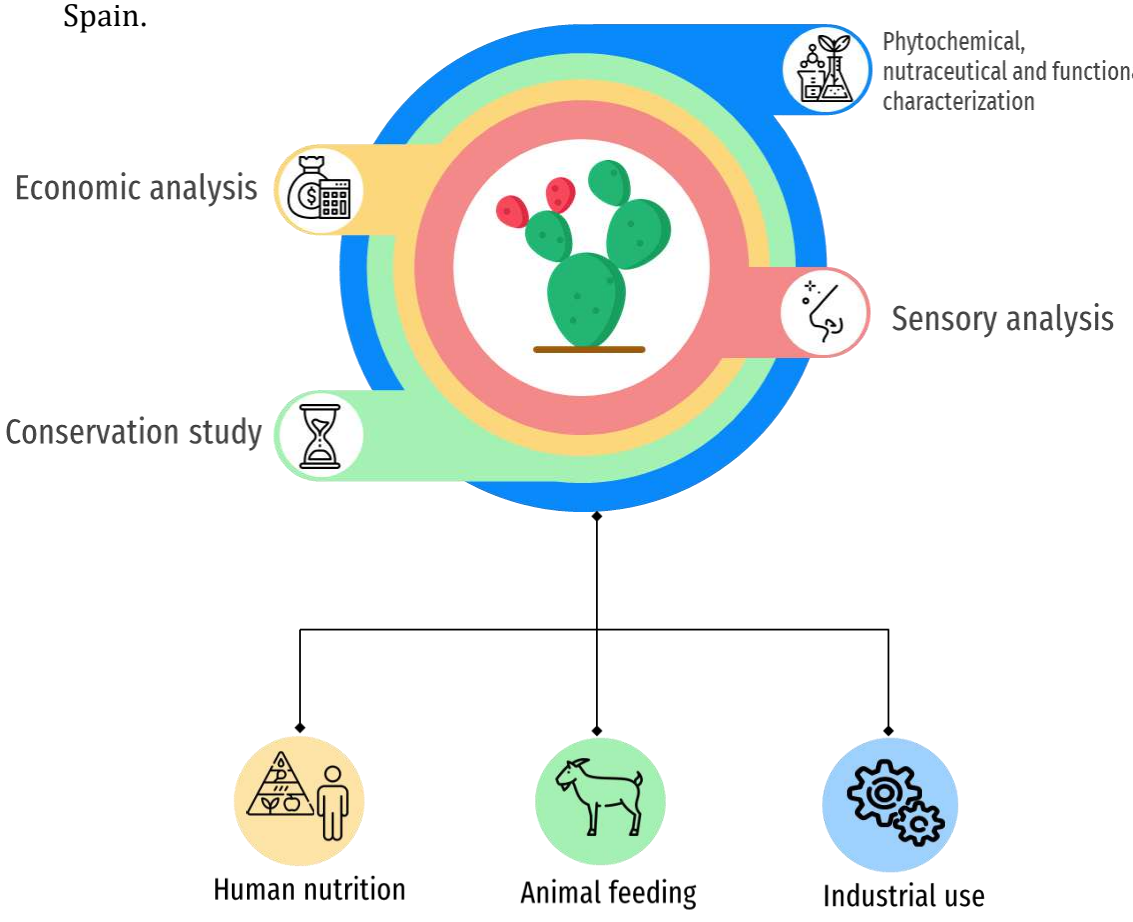


Figure 4. Graphical visualization of the Doctoral Thesis objectives

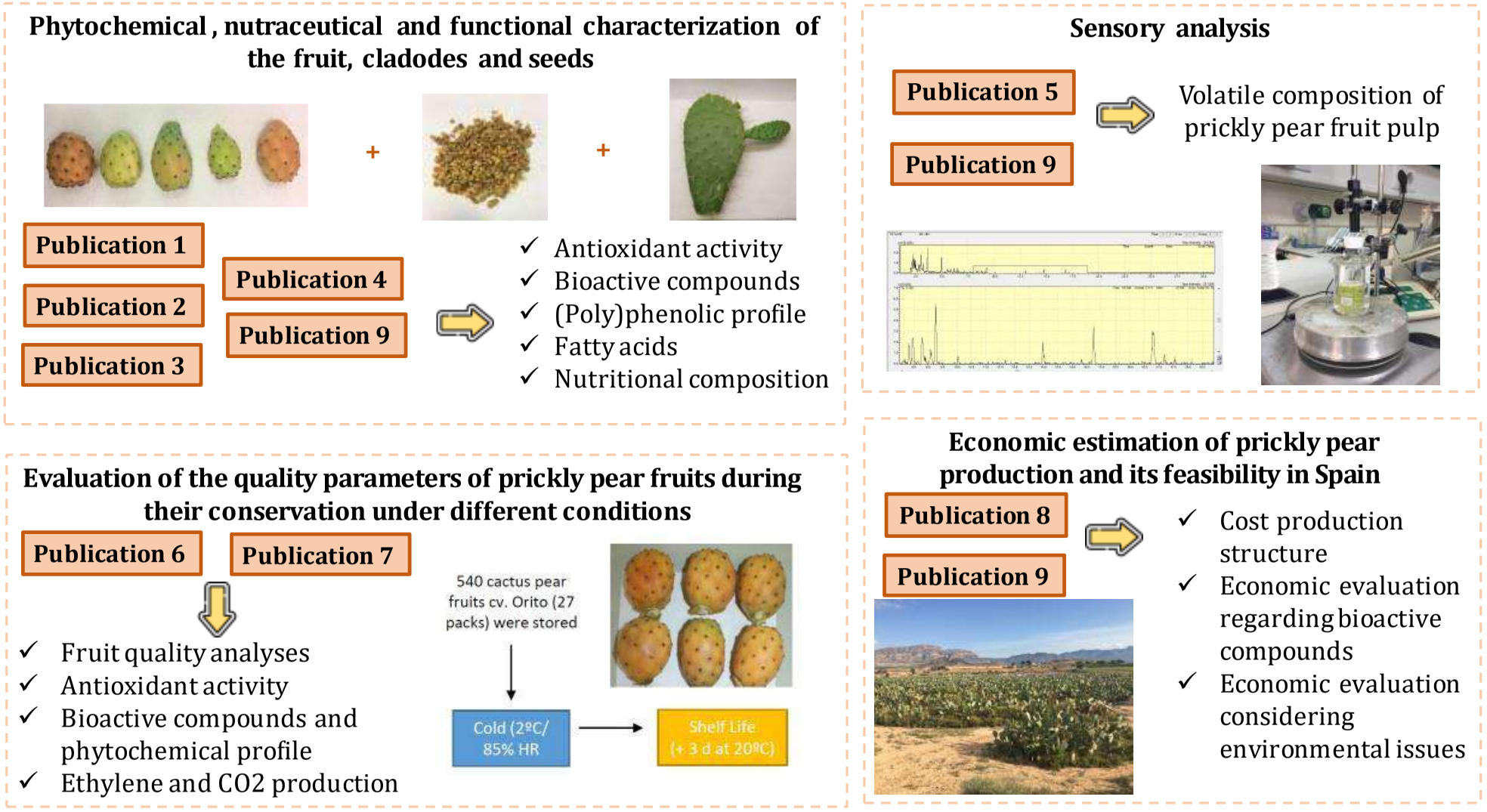
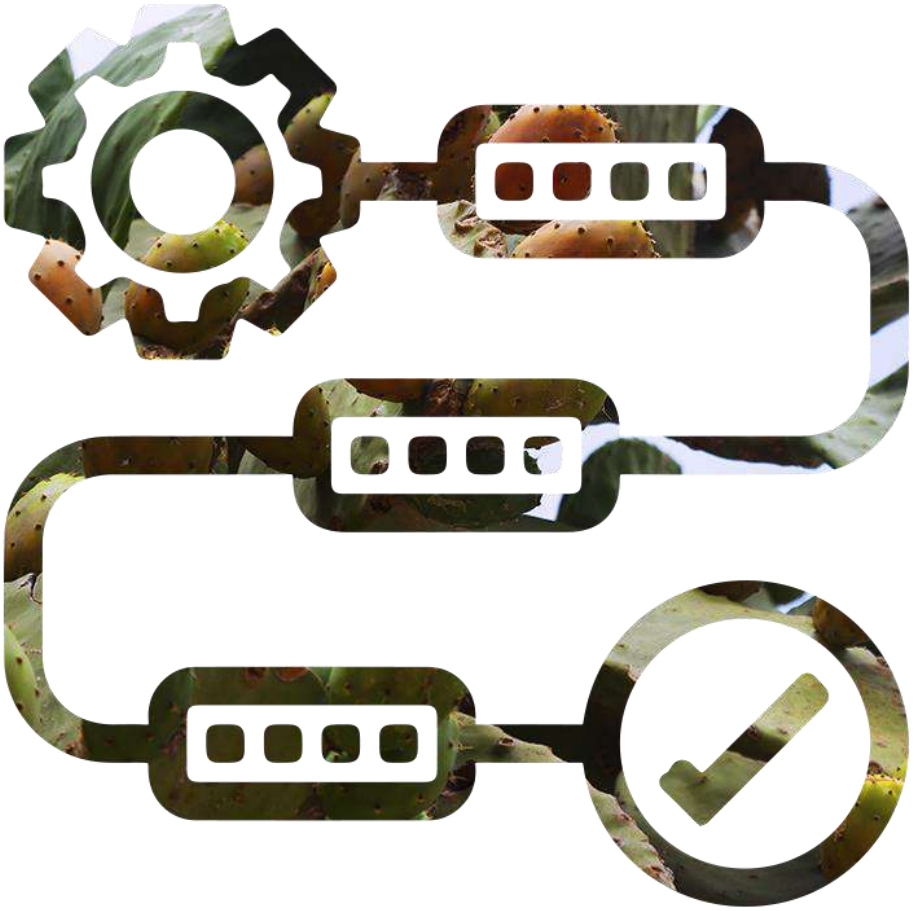


Figure 5. Graphical visualization of the doctoral thesis objectives with the publications obtained based on them.



6. MATERIALS AND METHODS

This section includes a brief description of the plant material and experimental design for the phytochemical, nutraceutical, functional, and sensory methods used for the characterization of the studied prickly pear cultivars. The tools used for the economic estimation prickly pear production cost and value are also included. Additional details on the methodology and all the materials used can be found in thepublished manuscripts that compose this thesis.

6.1 Plant material, growing conditions, and sample processing

Cladodes, fruits (peel and pulp) and seeds of seven cultivars of *Opuntia ficus-indica* (L.) Mill. were used for the different studies. Four cultivars, named 'Nopal Alargado', 'Nopal Tradicional', 'Nopal Espinoso' and 'Nopal Ovalado', were collected at the experimental field station of the Miguel Hernández University, in the province of Alicante, Spain (02°03'50''E, 38°03'50''N, and 25 masl). Cultivars called 'Fresa', 'Orito' and 'Nalle' were harvested from the private farms of Murcia ('Fresa') and Alicante ('Orito' and 'Nalle'). All these three farms are geographically close, have the same climatic conditions, similar soils and plant material was collected at the same time for each experiment. Plant species were identified by an expert botanist from de Department of Plant Sciences and Microbiology, using the protocol by García-Rollán (1981). Table 2 presents the characteristics of the analyzed prickly pear cultivars.

Table 2. Characteristics of the analyzed prickly pear cultivars.

Cultivar	Code	Characteristics
Fresa	FR	Red cultivar. Weight of the fruit: 100-140 g
Nalle	NJ/NL	Green cultivar. Average weight of the fruit: 90-100 g
Nopal alargado	NA	Green-yellow cultivar without prickles. Weight of the fruit: 120-160 g
Nopal espinoso	NE	Highly spiny green cultivar Weight of the fruit: 60-80 g
Nopal ovalado	NO	Green-yellow cultivar. Weight of the fruit: 90-120 g
Nopal tradicional	NT	Traditional cultivar (orange). Weight of the fruit: 90-120 g
Orito	ORI	Orange cultivar. Hight fruit production. Weight of the fruit: 110-140 g

The young (less than one year) and old (more than two years old) cladodes as well as the fruits were harvested during the spring and summer of 2015 - 2019, depending on the experiment. After picking, the plant material was

immediately transported to the laboratory. The spines from the cladodes were removed manually, while the fruits were washed under tap water with a brush for 2 minutes.

Quality parameters, such as weight loss, color, and fruit firmness, and in the evaluation of ethylene production and respiration rate, the measures were performed using whole fruit. In the rest of analyses, fruit peel was removed manually.

A portion of fresh plant material (pulp, peel, young and old cladodes) was squeezed to get the juice to analyse pH, total soluble solids (TSS), titratable acidity (TA), organic acids and sugar profile.

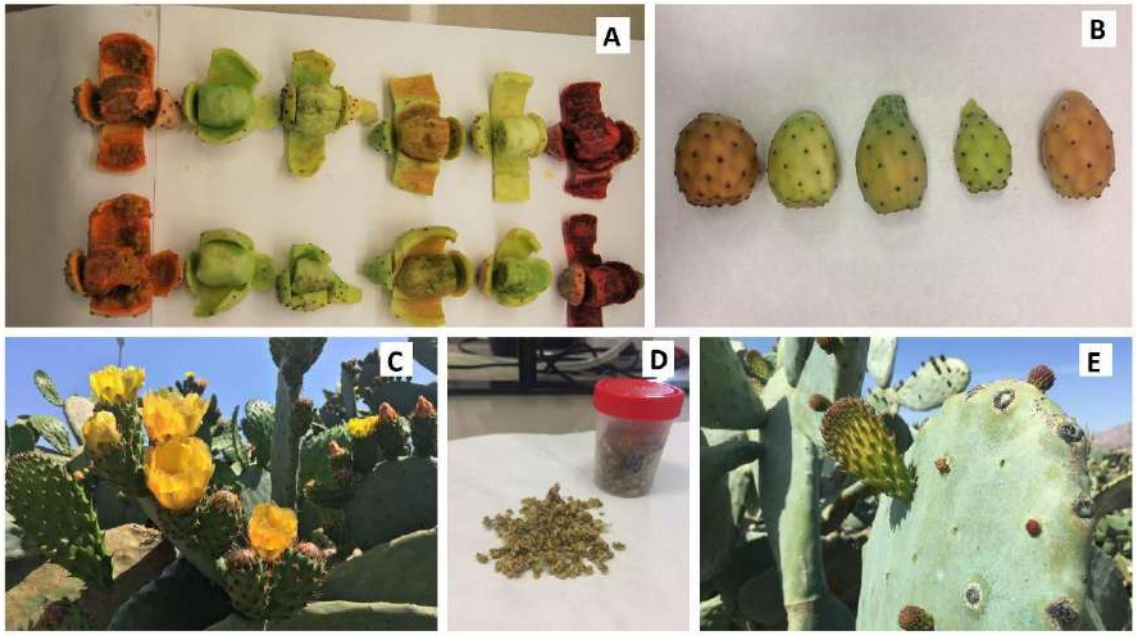


Figure 6. A: Peel and pulp of prickly pear fruits, from left to right: 'NT', 'NO', 'NE', 'NA', 'NA' and 'FR'. B: Whole fruit of prickly pear, from left to right: ORI, NO, NA, NE, NT. C: Flowers and young cladodes from 'ORI cultivar. D: Seeds from NO cultivar. E: Young and old cladodes of NO cultivar.

Some analyses were performed using freeze plant material. For the analysis of volatile compounds, fruit pulp was cut, grinding for 10 s in a grinder (Taurus Aromatic Ver II; Taurus Group, Barcelona, Spain), and frozen at -80ºC until the time of analysis. In the case of the hydrophilic and lipophilic total antioxidant activity (H- TAA and L-TAA respectively), total phenolics and total carotenoids, fruit pulp were cut in pieces and frozen at -80ºC until the time of analyses.

In the case of seeds, the fruit pulp was cut into small pieces and submerged in water for a week to make the removal of the pulp easier. After this time, the

water was removed, and the seeds were washed under tap water for two minutes to remove the pulp completely. After that, the seeds were placed on blotting paper and were left to dry at room temperature for ten days, and frozen at -80ºC until the time of analysis.

Other experiments, that will be detailed below, were performed using freeze-dried plant material. These plant material (fresh cladodes, pulp and peel) were immediately frozen in liquid nitrogen and later freeze dried in an Alpha 2-4 freeze drier (Christ Alpha 2-4; Braum Biotech) for 24 h at a pressure reduction of 0.220 mbar. The temperature in the drying chamber was −25ºC, while the heating plate reached 15ºC. At the end of freeze-drying, the samples were powdered and packedin vacuum until the time of analysis.

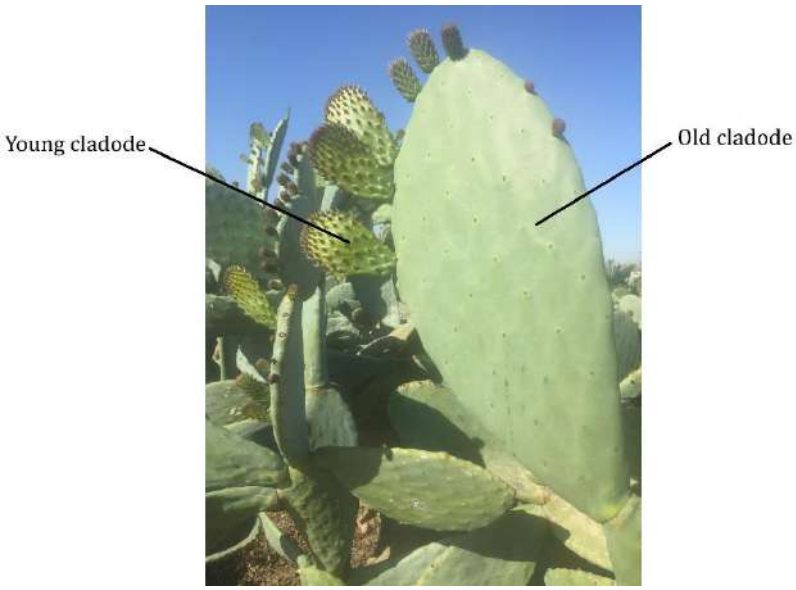


Figure 7. Young and old cladodes of 'NO' cultivar

6.2 Quality parameters

6.2.1 Weight loss, firmness, and color

To calculate weight loss, fruits (27 lots of 20 fruits) were weighed at harvest and after the storage period, using a digital balance (model BL-600; Sartorius, Madrid, Spain). Fruit firmness was determined in each whole fruit as force deformation (N mm^{-1}) by using a flat steel plate coupled with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK), which employed a force causing a 5 or 10% of deformation of the fruit diameter. Color, as L*, a*, and b* parameters, were measured with a Minolta colorimeter CR200 model/Minolta Camera Co., Osaka, Japan) by using the CIEL*a*b* System and was expressed as Hue angle ($\tan^{-1}(b^*/a^*)$). For these parameters, the results were expressed as

the mean plus/minus (±) standard error (SE) of individual determinations made in three replicates of fivefruits.

6.2.2 Total soluble solids, pH, total titratable acidity, and ripening index

TSS were measured in the juice of fruit pulp, peel,and cladodes, by using a digital refractometer (Atago refractometer model N-20; Atago, Bellevue, Wash., USA, and Atago Pocket PAL-1, Atago Co. Ltd., Tokyo, Japan) at 20 ∘C with values being expressed as degrees Brix (∘Bx) or percentage (%). TA and pH were determined by acid–base potentiometer (877 Titrino plus, Metrohm ion analyses CH9101, Herisau, Switzerland, and TitraLab AT1000 series, Hach Tokyo, Japan), using 0.1 mol L^{-1} NaOH up to pH 8.1; and values were expressed as grams of citric acid per liter and grams of malic acid equivalent per kg. These analyses were run in triplicate. Ripening index (RI) was calculated as the ratio between TSS and TA.

6.3 Ethylene production and respiration rate

Ethylene production and respiration rate were determined at harvest and after the storage period during the conservation study. Both were measured by placing each lot of fruits in a 2 L glass jar hermetically sealed with a rubber stopper for one hour. One mL of the holder atmosphere was withdrawn with a gas syringe and used to quantify ethylene concentration into a Shimadzu TM GC-2010 gas chromatograph (Kyoto, Japan), equipped with a flame ionization detector (FID) and 3, stainless steel column with an inner diameter of 3.5 mm, containing activated aluminia of 801/100 mesh. Carrier gas was helium, column temperature was 90 º C, and injector and detector temperatures were 150ºC. Another sample of 1 mL of the same atmosphere was used to quantify respiration rate by measuring CO_2 concentration into a gas chromatograph GC 14B (Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD), with the characteristics explained in Díaz-Mula (2011). Ethylene production and respiration rate was expressed as nmol kg^{-1} s^{-1}. These analyses were made in duplicate; the results were expressed as the mean ± SE of determinations made in three replicates.

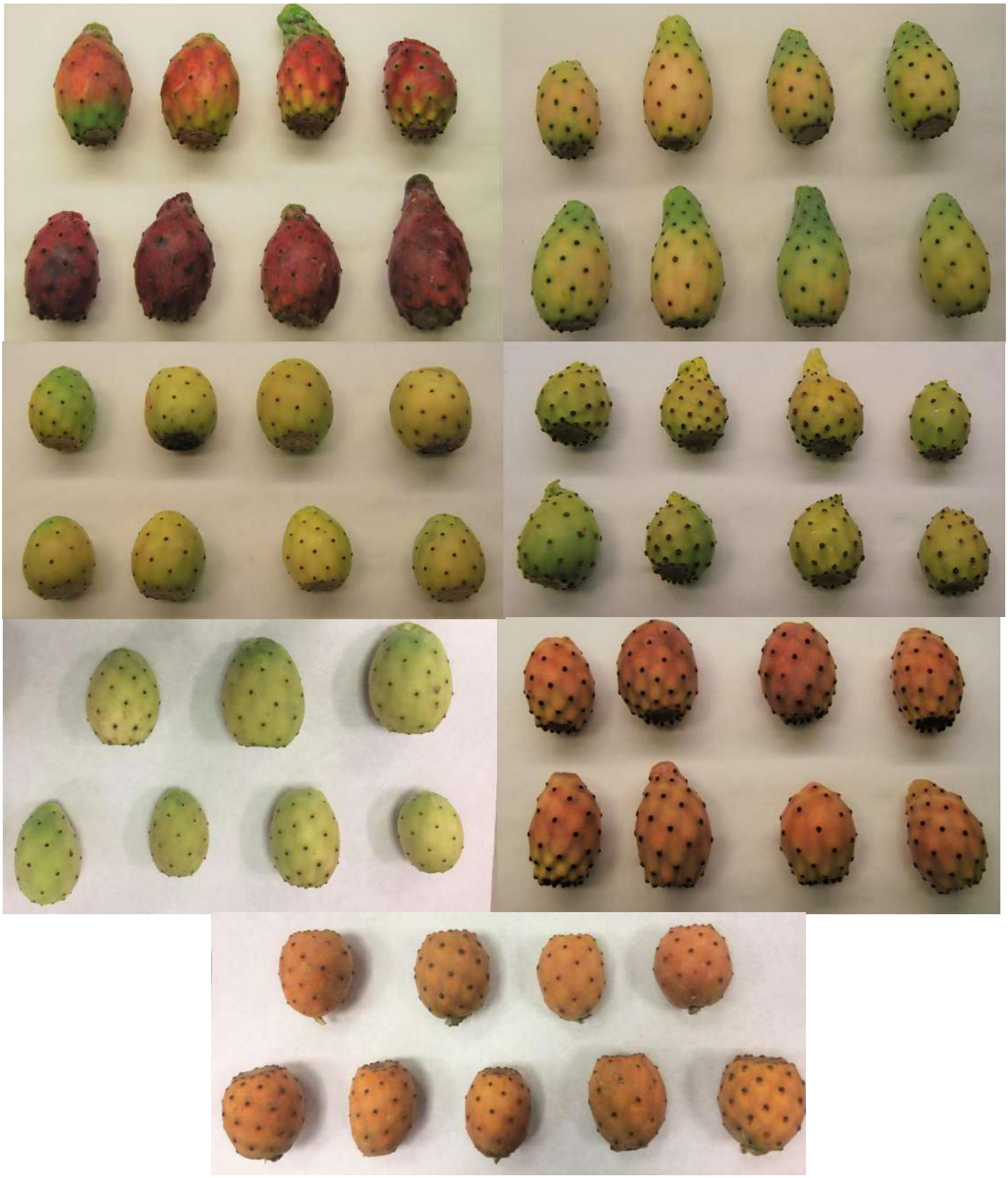


Figure 8. Fruits of the cultivars analyzed in this doctoral thesis. From left to right and from top to bottom: 'FR', 'NA', 'NJ', 'NE', 'NO', 'NT' and 'ORI'

6.4 Organic acids and sugars profile

Organic acids and sugars profile were quantified according to Hernandez *et al.* (2016) with a few modifications. The juice obtained by squeezing cladodes, the pulp and peel of fruits were homogenized with 10 mL of 50 mmol L^{-1} Tris-acetate bufferpH 6.0 and 10 mmol L^{-1} CaCl and centrifuged at 15,000 × *g* for 20 min (Sigma 3–18 K, Osterode and Harz, Germany). Then, 1 mL of supernatant was filtered through a 0.45 µm Millipore filter and 10 µL was injected into a Hewlett-Packard high- performance liquid chromatography (HPLC) series 1100 (Hewlett-Packard, Wilmington, DE, USA). A column (Supelcogel TM C-610H column 30 cm × 7.8 mm) and a pre-column (Supelguard 5 cm× 4.6 mm, Supelco, Inc., Bellefonte, PA) were used for the analyses of both organic acids and sugars. The elution buffer consisted of 0.1% phosphoric acid, and organic acid absorbance was measured at 210nm using a diode-array detector. These same HPLC conditions (elution buffer, flow rate,and column) were used for the analysis of sugars. The detection was conducted using a refractive index detector. Standards of organic acids (oxalic, citric, tartaric, malic, quinic, shikimic, and fumaric acids) and sugars (glucose, fructose, and sucrose) were obtained from Sigma (Poole, Dorset, UK). Calibration curves were used for the quantification of organic acids and sugars and showed good linearity (R^2 ≥0.999). Analyses were run in triplicate and results were expressed as the mean ± SE and units in grams per liter.

6.5 Antioxidant activity, total phenolic content and carotenoids

During the experiments carried out during the preparation of this doctoral thesis, various methodologies have been used to determine antioxidant activity and total phenol content.

6.5.1 Extraction procedure for total polyphenols content and antioxidant activity

The extraction procedure for TPC and AA quantification was prepared as described by Wojdyło et al. (2008) in the case of peel, pulp, and cladodes. Freeze-dried plant materials (0.5 g) were weighed into a test tube. After that, a total of 10 mL of 80% ofaqueous methanol with 1% of HCl was added and the suspension was slightly stirred. Tubes were sonicated for 15min and left for 24

h at 4ºC. Then the extract was again sonicated for 15 min and centrifuged for 15 min at 15,000 × *g*. The supernatants were collected to be used in subsequent analyses.

In the case of seeds, the samples were powdered, and 0.5 g were extracted with 10 mL of the same extractant described above. The extraction was performed by incubation of 20 min under sonication with occasional shaking. Next, the slurry was centrifuged at 19,000 x g for 10 min, and the supernatant was filtered through a hydrophilic PTFE 0.2 μm membrane and used for analysis.

6.5.2 Quantification of total phenolic content

The TPC was measured using the Folin–Ciocalteu colorimetric method described by Chong *et al.* (2013). Cladodes and fruit extracts (0.1 mL) were mixed with Folin– Ciocalteu reagent (0.2 mL) and of H_2O (2 mL). Then, the mixture was incubated at room temperature for 3 min. Then, 1mL of 20% sodium carbonate was added to the mixture. TPC were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue color was measured at 765nm using a UV–visible spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). Calibration curves with concentrations of gallic acid as standard were used for quantification. All determinations were performed in triplicate and results were expressed as grams of gallic acid equivalent (GAE) per kilogram of dry weight (dw).

6.5.3 Determination of antioxidant activity by three different methods (DPPH·, $ABTS^{\cdot+}$ and FRAP)

- Determination of antioxidant activity by DPPH· method

The DPPH· radical scavenging activity was determined using the method proposed by Brand-Williams *et al.* (1995) in the case of this determination in peel, pulp and cladodes, and the methodology proposed by Chen (2012) in the case of seeds. These methods are very similar, and all of these are based in the ability of DPPH· radical to react with antioxidant compounds through the transfer of a hydrogen atom provided by the oxidizing agent. Due to this reaction, the decrease in absorbance can be measured. All determinations were

performed in triplicate, and results were expressed in mmol and μmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic acid) per kilogram (dw).

- Determination of antioxidant activity by $ABTS^{\cdot+}$ method

The free-radical-scavenging activity was determined by $ABTS^{\cdot+}$ radical cation described by Re et al. (1999). The $ABTS^{\cdot+}$ solution was produced by reacting aqueous ABTS·+ solution (7 mmol L^{-1}) with potassium persulfate (2.45 mmol L^{-1}, final concentration) and kept in the dark at room temperature for 12–16 h before use. The radical was stable in this form for more than 2 days when stored in the darkat room temperature. Diluted $ABTS^{\cdot+}$ solution with an absorbance of 0.702 ± 0.1 at 734 nm was employed in the analysis. The reactions were performed by adding 990μL of $ABTS^{\cdot+}$ solution to 10 μL of each extract solution. The absorbance reading was exactly 6min after initial mixing. All determinations were performed in triplicate and the results were expressed in mmol and μmol of Trolox per kilogram (dw).

- Determination of antioxidant activity by Ferric reducing/antioxidant power (FRAP)

The antioxidant potential was determined using a FRAP assay by Benzie and Strain (1996). The assay was based on the reducing power of antioxidant compounds to reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 μmol L^{-1} pH 3.6), a solution of 10 μmolL^{-1} TPTZ in 40 μmol L^{-1} HCl, and 20 μmol L^{-1} $FeCl_3$ at 10: 1: 1 (v/v/v). The reagent (300 μL) and sample solutions (10 μL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of Trolox. All determinations were performed in triplicate and results were expressed in μmol and mmol of Trolox perkilogram(dw).

6.5.4 Determination of hydrophilic and lipophilic total antioxidant activity

Total antioxidant activity (TAA) was determined in duplicate for each lot according to the methodology of Arnao et al. (2001), which allows the determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA)

in the same extract. In summary, 5 g of the homogeneous sample of frozen pulp were homogenized in 15 mL of methanol:water (80:20, v/v) containing 1% of HCl (39%) and 2 mmol L^{-1} of NaF to inactivate polyphenol oxidase activity, and then centrifugated at 15,000 x g at 4ºC for 15 min. For the quantification of L-TAA was used the upper fraction, and thelower one was used to quantify L-TAA, both made in duplicate. The reaction medium included 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt ($ABTS^{\cdot+}$), horseradish peroxidase enzyme (HRP), and its oxidant substrate (hydrogen peroxide). Trolox ((R)-(+)-6-hydroxy 2,5,7,8-tetramethylcroman- 2- carboxylic acid) (0–20 nmol) from Sigma (Madrid, Spain) was used as a standard antioxidant to perform a calibration curve for both H-TAA and L-TAA, and results were expressed as mg Trolox equivalents kg^{-1} (fresh weight basis). Results were the mean the mean ± SE of measures made in duplicate in each of the three replicates.

6.5.5 Total phenolic content

In this experiment, total phenolics were extracted according to Tomás–Barberán et al. (2010), using the same extractant that the one used for the determination of L- TAA and H-TAA and quantified using the Folin-Ciocalteu reagent. Briefly, 200 μL of the hydrophilic extract were diluted in the extractant described above and mixed with 2.5 mL of water diluted Folin–Ciocalteau reagent. The mixture was incubated for 3 min at room temperature. Then, 2 mL of sodium carbonate (75 g L^{-1}) was added, and the mixture was shaken. At last, the mixture was incubated at 60ºC for 5 min, and absorbance was measured at 760 nm. Gallic acid was used for performing a calibration curve. Results were expressed as mg gallic acid equivalent per kg fresh weight. Results were the mean the mean ± SE of measures made in duplicate in each of the three replicates.

6.5.6 Carotenoids

Total carotenoids were quantified in the same extract that the L-TAA was determined (Arnao et al., 2001) by reading the absorbance at 450 nm in a

UNICAM Helios-α spectrophotometer (Cambridge, UK). Results were the mean ± SE and were expressed as mg of β-carotene equivalent kg^{-1} fresh weight, considering the $\varepsilon^{1\%}cm=2560$.

6.6 Extraction, determination, identification, and quantification of polyphenols by the UPLC-PDA-MS method in prickly pear seeds

For the extraction and determination of polyphenols in prickly pear seeds, a protocol described before by Kolniak-Ostek (2016) was followed.

Identification of polyphenols of prickly pear seeds extracts was carried out using an ACQUITY Ultra Performance LC system equipped with a photodiode array detectorwith a binary solvent manager (Waters Corporation, Milford, MA, USA) with a mass detector G2 Q-Tof micromass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative mode. The separation of individual polyphenols was carried out using a UPLC BEH C18 column (1.7 mm, 2.1 x 100 mm, Waters) at 30ºC.

The samples (10 μL) were injected, and the elution was completed in 15 min with a sequence of linear gradients and constant flow rates of 0.42 mL min^{-1}. The mobile phase consisted of solvent A (0.1% formic acid, v/v) and solvent B (100% acetonitrile). The linear gradient was as follows: 0.0–1.0 min, 99% A, 0.42 mL/min (isocratic), 1.0–12.0 min, 65.0% A, 0.42 mL min^{-1} (linear), 12.0–12.5 min, 99% A, 0.42 mL/min (linear), 12.5–13.5 min, 99% A, 0.42 mL min^{-1} (isocratic). The analysis was carried out using full-scan, data-dependent MS scanning from m/z 100–1500. Leucine enkephalin was used as the reference compound at a concentration of 500 pg/mL, and the $[M-H]^-$ ion at 554.2615 Da was detected. The $[M-H]^-$ ions were detected during a 15 min analysis performed within ESI–MS accurate mass experiments, which were permanently introduced via the LockSpray channel usinga Hamilton pump. The lock mass correction was ±1.000 for the mass window. The mass spectrometer was operated in negative-ion mode, set to the base peak intensity (BPI) chromatograms, and scaled to 12,400 counts per second (cps) (100%). The optimized MS conditions were as follows: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 100 C, desolvation temperature of 300ºC, and desolvation gas (nitrogen) flow rate of 300 L/h.

Collision-induced fragmentation experiments were performed using argon as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Characterization of the single components was carried out via the retention time and the accurate molecular masses. Each compound was optimized to its estimated molecular mass in the negative mode, before and after fragmentation. The data obtained from UPLC– MS were subsequently entered into the MassLynx 4.0 ChromaLynx Application Manager software (Waters).

The runs were monitored at the following wavelengths: phenolic acids at 320 nm and flavonol glycosides at 360 nm. The PDA spectra were measured over the wavelength range of 200–600 nm in steps of 2 nm. The retention times and spectrawere compared to those of the authentic standards.

The quantification of phenolic compounds was performed by external calibration curves (R2 > 0.999), using reference compounds selected based on the principle of structure-related target analyte/standard (chemical structure or functional group). Standard stock solutions were diluted to appropriate concentrations (five calibration points were used in each case) for the plotting of calibration curves. The linearity was obtained by plotting the peak areas versus the corresponding concentrations (ppm) of each analyte. The calibration curve for caffeic acid was usedto quantify caffeic acid hexosides. The calibration curve of ferulic acid was used to quantify ferulic acid derivatives. Protocatechuic acid hexoside was quantified with protocatechuic acid calibration curve.

The calibration curves of quercetin, quercetin rutinoside, and 3-O-galactoside were used to quantify quercetin derivatives. For isorhamnetin quantification, isorhamnetin 3-O-rutinoside and 3-O-glucoside were used.

All determinations were run in triplicate. The results were expressed as mg kg^{-1} perkg of dry matter (DM).

6.7 Extraction, determination, identification and quantification of polyphenols by the UHPLC-ESI-MSn method in prickly pear fruits and cladodes

The (poly)phenolic compounds in prickly pear cladodes (young and old) and fruits (pulp and skin) were extracted following a protocol previously reported (Sánchez- Salcedo et al., 2015). Briefly, 200 mg of freeze-dried powder plant

material were mixed with 1 mL of 80% aqueous methanol acidified with formic acid (1%). This mixture was then sonicated for 25 min, centrifuged at 10,480g for 5 min at room temperature, and the supernatant was collected. Two additional extractions were performed for each sample with additional 0.5 mL of the extraction solvent, as described above, after which they were centrifuged. The three supernatants were pooled before UHPLC-ESI-MSn analysis. Each sample was extracted in triplicate.

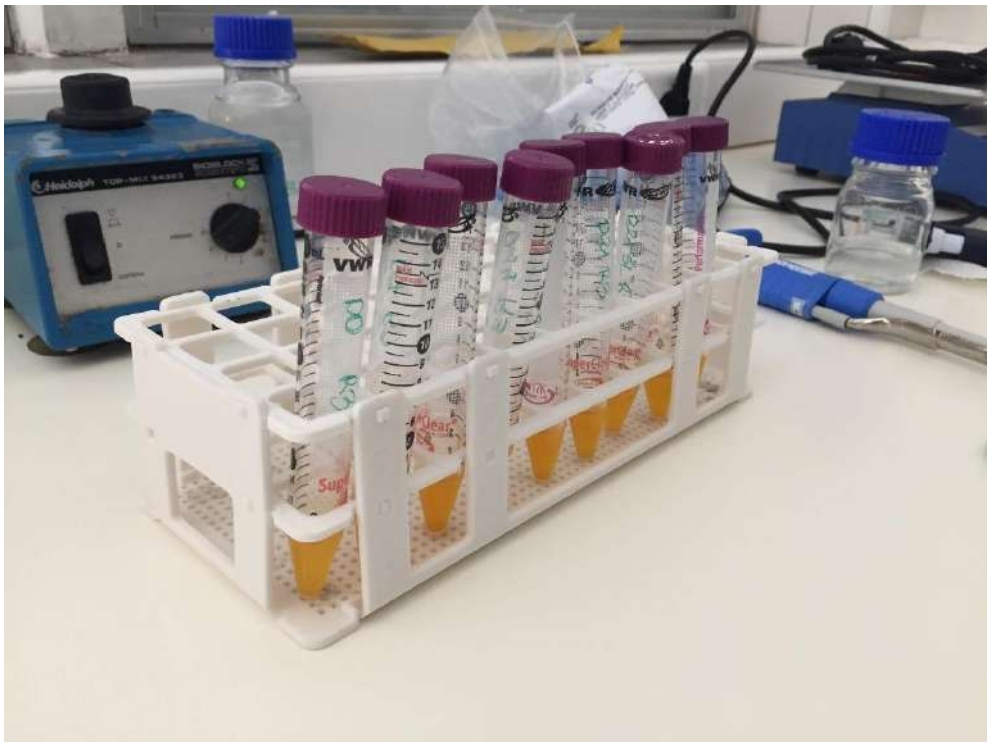


Figure 9. Extraction procedure of polyphenolic compounds by the UHPLC-ESI-MSn method

Methanolic extracts of prickly pear parts were analyzed using an Accela UHPLC 1250equipped with a linear ion trap-mass spectrometer (MS) (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization (ESI) probe (H-ESI-II; Thermo Fisher Scientific Inc., San Jose, CA, USA). Separations were performed using a XSelect HSS T3 (50×2.1 mm), 2.5 µm particle size (Waters, Ireland). Volume injected was 5 µL and column oven was set to 30ºC.

Two complementary MS experiments were performed, one in negative mode, for non-coloured phenolics, and one using positive ionization, for betalains, following an analytical approach previously developed for the comprehensive

identification of (poly)phenolic compounds (Mena et al., 2012). Each sample was analysed in duplicate for each experimental condition.

The experimental condition optimized in negative ionization mode for the analysis of non-coloured phenolics was based on the following conditions. The MS worked with a capillary temperature equal to 275ºC, while the source heater temperature was set to 250ºC. The sheath gas flow was 40 units, while both auxiliary and sweepgas were set to 5 units. The source voltage was 3 kV. The capillary and tube lens voltages were −9 and −53 V, respectively. Elution was performed at a flow rate of 0.2 mL/min. The gradient started with 90% of 0.1% aqueous formic acid and 10% of acetonitrile 0.1% formic acid, followed by a 13-min linear gradient of 10% to 70% acidified acetonitrile. From 13.5 to 14 min the acidified acetonitrile was increased to 80%, followed to 2.5 min of 80% acetonitrile and then 4 min at the start conditions to re-equilibrate the column. Analyses were carried out using full scan mode, data-dependent MS3 scanning from m/z 100 to 2000, with collision induced dissociation (CID) equal to 30 (arbitrary units). Pure helium gas was used for CID.

For the analysis of betalains, in positive ionization mode, the MS worked with a capillary temperature equal to 275ºC, while the source heather temperature was set to 200ºC. The sheath gas flow was 40 units, while auxiliary gas was set to 5 units, without sweep gas. The source voltage was 4 kV. The capillary voltage and tube lenswere 39 and 110 V, respectively. The chromatographic conditions were identical tothose used for the previous experimental condition.

Data processing was performed using Xcalibur software from Thermo Scientific. All compounds were identified by comparing with standards, when available, and mass spectral and chromatographic data reported in literature. For quantification purposes, area calculation was performed in selected ion monitoring mode byselecting the relative base peak at the corresponding mass to charge ratio (m/z). The quantification of (poly) phenolics was carried out by comparison with commercial standards, when available. For those compounds that could not be quantified with their corresponding standards, a reference compound was selected based on structural similarity and considering the functional groups that may affect the ionization properties (i.e., flavonols were quantified as rutin equivalents, lignans as secosiolariceresinol, etc.). Finally, the molecules responding to the ESI source in a unique way with respect to the

reference compound of choice, or not reaching the limit of quantification of the corresponding reference compound, were not quantified.

6.8 Extraction, determination, and identification of phytochemical profile by the HPLC-DAD and HPLC-DAD-MS/MS analyses in prickly pear fruit pulp.

Phytochemicals were extracted by the protocol described by Mena et al. (2018) described above. The extracts were analysed in a HPLC Dionex Ultimate 3000 equipped with a C-18 LiChrospher® 100 RP-18 (5 μm) column (250 × 4.0 mm) (Sigma-Aldrich, San Luis, MO, USA) operating at 35º C, coupled to a DAD-3000 detector (Thermo Scientific, MA, USA). The mobile phase consisted of water-formic acid (0.5% v/v) (eluent A) and acetonitrile (90%) + formic acid (0.5%) + water (eluent B) at a flow rate of 0.3 mL/min with an injection volume of 20 μL. Samples were also analysed by an HPLC-DAD-MS/MS system: a Waters Alliance 2695 (Waters®, Dublin, Ireland) separation module with an autosampler (20 μL injection volume), a quaternary pump and a solvent degasser, coupled to a Photodiode Array Detector Waters 996 PDA (Waters, Dublin, Ireland) scanning wavelength absorption between 210 and 600 nm. A LiChrospher® 100 RP-18 5 μm column at 35 ◦ C (stabilized by a column oven) was used. Tandem mass spectrometry (MS/MS) detection was carried out with a Micromass® Quattro Micro triple quadrupole (Waters, Dublin, Ireland), using an electrospray ionization source in both positive (ESI+) and negative (ESI-) modes. A full scan mode (m/z: 60–1100) record was applied for the mass spectra of the compounds separated by HPLC, using a collision energy of 20 eV. For data acquisition and processing, MassLynx® 4.1 software (Waters, Dublin, Ireland) was used. Results were the mean ± SE and were expressed as average of area (mAU*min).

6.9 Fatty acids determination in fruits (peel and pulp) and cladodes

In this case, fatty acid extraction-methylation were performed directly on freeze- dried pulp, peel, and cladodes. First, fatty acid methyl esters (FAMEs) were prepared by transmethylation using boron trifluoride (BF_3) catalyst according to ISO 12966- 2:2011(ISO, 2011). Then, FAMEs were analyzed in a gas chromatogram (GC17A) coupled to a mass spectrometry detector GC–MS QP5050, Shimadzu (Kyoto, Japan) with a SupraWax-280 column, 100%

polyethylene glycol (Teknokroma S. Co. Ltd., 165 Barcelona, Spain; 30m length ×0.25mm internal diameter ×0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1.1 mL min^{-1}. The temperature program for the oven was as follows: (i) an initial temperature of 80ºC was held for 2 min, (ii) then, increased at a rate of 8.0ºC min^{-1} to 160ºC; (iii) and increased at a rate of 4ºC min^{-1} from 160 to 220ºC and held for 13 min, and (iv) and further increased at a rate of 10ºC min^{-1} from 220 to 260ºC and held for 6 min. Injector and detector temperatures were held at 230 and 260ºC, respectively. Injection volume was 0.5 μL injected at a split radio of 1:10. Identification was made by comparison with the retention time of standards. Analyses were run in triplicate. The ratio S/N for each peak of the chromatogram was calculated and the lowest S/N ratio for a peak was 4, which ensured that peaks were quantified above the LOQ of the equipment (0.01%). Results were the mean ± SE and were expressed as % of total fatty acid profile.

Besides, the indexes of atherogenicity (AI) and thrombogenicity (TI) were also calculate. They were defined by Ulbricht and Southgate (1991) and the higher they are, the higher the risk of atherogenicity and thrombogenicity of the dietary fat, so they are valuable indicators of the potential effects of fats on the prevention of atherosclerosis, thrombosis, and cardiovascular health. These indexes were calculated according to the following formulas:

$$AI = (C12:0+4 \times C14:0 + C16:0) / [\Sigma MUFA + \Sigma PUFA\ (n-6)\ and (n-3)]$$

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

6.10 Fatty acid determination in seeds

Fatty acid composition of seeds was determined by GC, according to the American Oil Chemists' Society Official Method Ce 1-62 (2005). Boron trifluoride (BF_3) in methanol was used as methylating agent. Fatty acid methyl esters (FAMEs) were analyzed by an Agilent 7820A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA), equipped with a capillary column RTX-2330, 105 m length, 0.25 mm i.d., 0.2 microm film thickness (Restek, Bellefonte, PA, USA). Injector and detector (FID) temperatures were 260 º C and 280 º C, respectively. Column temperature was set to 200 º C for 21 min, then

increased to 250ºC at a rate of 10ºC min^{-1}; the final temperature was held for 6 min. Helium was used as a carrier gas, at a linear flow rate of 35 cm sec^{-1}. Individual FAMEs were identified using the Certified Reference Material (CRM) 47885 (Supelco, Bellefonte, PA, USA). Results were the mean ± SE and were expressed as % of total fatty acid profile. Analyses were run in six samples.

6.11 Determination of volatile compounds

These analyses were performed in grinded frozen (-80ºC) samples. Headspace solid-phase microextraction (HS-SPME) was the method selected to study the volatile composition of the samples under analysis. After several preliminary test to optimize the extraction system, each sample (10 g of each batch, which were prepared 10 uniform fruits of each cultivar) was placed together with 10 mL of water, 1.5 g of salt, and *β*-ionone as internal standard (10 µL of 1,000 mg/L) into 50 mL vials with polypropylene caps and a polytetrafluoroethylene/ silicone septum. Then, a magnetic stirring bar was added, and the vial was placed in a water bath with controlled temperature and automatic stirring. The vials were equilibrated during 5 min at 40ºC in the bath and after that a 50/30 µm divinylbenzene/ carboxen/polydimethylsiloxane fiber was exposed to the sample headspace for 30 min at 40ºC. Later, desorption of the volatile compounds from the fiber coating was performed in the injection port of the CG-MS during 3 min. Extraction experiments were run in triplicate.

After this procedure, the isolation and identification of volatile compounds were carried out on a gas chromatograph (GC), Shimadzu GC- 17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (MS) QP- 5050A. The GCMS system was equipped with a SLB-5 ms capillary column, 95% dimethylpolysiloxane, and 5% diphenylpolysiloxane (Sigma- Aldrich, Spain; 30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 13 mL/min, in a split ratio of 1:20, and the following temperature program: (a) initial temperature 80ºC; (b) rate of 3.0ºC/min to 170ºC and hold for 1 min; (c) rate of 25ºC/min from 170 to 300ºC and hold for 1.8 min. Injector and detector temperatures were held at 230 and 300ºC, respectively.

Figure 10. Volatile compounds extraction procedure

For the identification of volatile compounds were used three analytical methods: retention indices of each problem compound (retention indices), GC-MS retention times (authentic standard), and mass spectra (authentic chemicals and NIST05 spectral library collection; NIST, 2011). Tentatively identified compounds, based on only mass spectral data, were also included in this study.

The semiquantification of the volatile compounds was performed on a GC, Shimadzu GC-17A, with a fame ionization detector (FID). The column and chromatographic conditions were the same that those reported for the GC-MS analysis. The injector temperature was 300ºC and nitrogen was used as carrier gas (1 mL/min). The relative abundance was obtained from electronic integration measurements using FID. As internal standard, *β*-ionone was added (10 μL of 1,000 mg/L) and the areasfrom all compounds were normalized using its area; this compound was chosen after checking that it was not present in the prickly pear cultivars under study. Due to no standard curves were performed for each one of the quantified volatile compounds, data included in this study should be considered as semiquantitative. However, relative values are suitable for comparing differences between prickly pear cultivars.

All the analyses described in this section were run in triplicate. Results were the mean ± SE and were expressed in μg 100 g^{-1} and mg 100 g^{-1}.

6.12 Total protein content and fat content

These analyses were performed in seeds. The total protein content was evaluated according to the Kjeldahl method of the Association of Analytical Chemists (1996). A sample of 1 g of powdered seeds was hydrolyzed with 25 mL concentrated sulfuricacid (H_2SO_4) containing one catalyst tablet in a heat block (Büchi Digestion Unit K- 424, Labortechnik AG, Flawil, Switzerland) at 370º C for 2 h. After cooling, H_2O wasadded to the hydrolysates before neutralization, using a Büchi Distillation Unit K- 355 (Athens, Greece) and titration. A nitrogen to protein conversion factor of 6.25 was used to calculate total protein. Fat content was determined according to the standard method of the Association of Official Analytical Chemists International. (1995). A sample of 2 g of ground seeds was hydrolyzed using 4N HCl. Fat extraction and solvent (diethyl ether) removal were performed in an automated Soxhlet apparatus B-811 (Büchi Labortechnik AG, Flawil, Switzerland); the extraction time was 180 min. Results were the mean ± SE and were expressed as g 100 g^{-1} of dry matter (dw). Analyses were run in six samples of each cultivar.

6.13 Amino acid analysis

The amino acid composition analysis was carried out in prickly pear seeds by ion- exchange chromatography after 23 h hydrolysis with 6 N HCl at 110ºC. After cooling, filtering and washing, the hydrolyzed sample was evaporated in a vacuum evaporator at a temperature below 50ºC. The dry residue was dissolved in a buffer of pH 2.2. The prepared sample was analyzed using the ninhydrin method (Simpson et al., 1976; Moore et al., 1958). The pH 2.6, 3.0, 4.25, and 7.9 buffers were applied. The ninhydrin solution was buffered at pH 5.5. The hydrolyzed amino acids were determined using an AAA-400 analyzer (INGOS, Prague, Czech Republic). A photometric detector was used, working at

two wavelengths, 440 nm and 570 nm. A column of 350 x 3.7 mm, packed with ion exchanger Ostion LG ANB (INGOS) was utilized. Column temperature was kept at 60-74ºC and the detector at 121ºC. The calculations were carried out relative to an external standard. No analysis of tryptophan was carried out. Results were the mean ± SE and were expressed as g 100 g^{-1} of protein. Analyses were run in four samples of each cultivar.

6.14 Quantitative evaluation of protein quality

The amino acid content in prickly pear seeds was expressed on the nitrogen basis (g per 16 g N) and it was compared to a reference protein. The amino acid pattern for high-quality protein established by the Joint Food and Agriculture Organisation/World Health Organisation (FAO/WHO) Committee in 1991. Levels were calculated based on the essential amino acid composition of the chemical scores (CS), according to the Mitchell and Block method (Osborne, 1978) and the integrated EAA index (Oser, 1951).

6.15 Tools used for calculating the economic estimation of prickly pear production cost and value

6.15.1 Economic evaluation of cactus pear production structure

In this point, production environment for Mexico, Italy and Spain was compared. Then, economic evaluation of cactus pear production structure was done through cost accounting (Romero et al., 2006). All operations are considered self-financing to avoid introducing financial variables. Economic assessment does not include fixed costs because these costs can introduce bias that do not affect the production process. Data from other countries were obtained through published research (Basile et al., 2002; Losada et al., 2017).

Figure 11. Prickly pear fruit collecting and transporting processes

Average value of 1.0 € equal to 1.129 US$ is considered during 2017 (European Central Bank, 2018) for comparisons with Losada et al. (2017) and 1.259 for comparisons with Timpanaro and Foti (2014). Information was updated using inflation information from European Central Bank (2018). Spanish production information was obtained through *in situ* interviews in three steps: (i) open interviews with farmers; (ii) questionnaires sent by post; and, (iii) audits and information validations with specific questions directed to interviewees. This data collection covered 3 full seasons in Spain. The total variable production cost was established and was included in working assets costs. Opportunity costs were calculated as the next-best alternative use of working capital in risk-free financial assets; 2.0% interest rate was assumed, depending on money current cost and inflation adjustment. Production variables obtained from secondary data and interviews were used to calculate costs and incomes. Differences in categories are due to the different processes undertaken for getting information and to country cultivation techniques differences. Gross income and total variable costs can be calculated by using contribution margin (CM), which is the margin used before considering depreciation and fixed costs. CM is calculated by taking the difference between gross incomes (GI) and incremental costs or variable costs (IC).

6.15.2 Economic analysis of cactus pears bio-functional, medicinal, nutraceutical and cosmetic properties

Data about the contents of components with bio-functional, medicinal, nutraceutical and cosmetic properties found in cactus pears has been reviewed and will be presented in tables together with economic data regarding their cost and estimated prices. Market prices of these compounds were obtained through a questionnaire among main producers. Then, an estimation of the quantities that could be obtained from 1 ha of cactus pear in Spain was calculated considering production data obtained in questionnaires carried out to producers.

Figure 12. Prickly pear commercial crop in Orito (Alicante, Spain)

6.15.3 Economic estimation of cactus pear production value considering environmental issues

An estimate of cactus plant CO_2 accumulation will be presented based on scientific literature together with the price to be paid for carbon sequestration, which was calculated considering not only carbon sequestration but also the benefits on the environment generated by its cultivation. To estimate the exchange surface of each plant, 50 of them were measured in width, height, and length. Then, number of cladodes *per* plant was counted and 20 of each were

measured in height and length to estimate their surface area. Plant average area and average cladode surface were calculated to estimate exchange surface and CO2 daily net intake *per* m2 and day. Cactus plant weight was calculated counting cladodes per plant and weighting 25 of them; roots were not considered.

7. PUBLICATIONS

PUBLICATION 1 (Literal transcription):

Antioxidant properties and chemical characterization of Spanish *Opuntia ficus-indica* Mill. cladodes and fruits

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Antioxidant properties and chemical characterization of Spanish *Opuntia ficus-indica* Mill. cladodes and fruits

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Abstract

BACKGROUND: Recent studies have demonstrated that consumption of *Opuntia ficus-indica* Mill. has an important positive health benefit, mainly due to antioxidant properties, which justifies this research. This study examined antioxidant activity, organic acid and sugar profile, total phenolic, and physicochemical characteristics of six *O. ficus-indica* cultivars growing in the Spanish Mediterranean. It should be noted that, in this study, both cladodes (young and adult) and fruits (peel and pulp) wer e analyzed.

RESULTS: The antioxidant activity (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl methods) was higher in fruit peel than in cladodes. The young cladodes presented an important antioxidant activity by the ferric-reducing ability of plasma method as well as a higher total phenolic content (18.90 g gallic acid equivalent per kilogram). High-performance liquid chromatography (HPLC) with diode-array detector analysis revealed the absence of sucrose and the presence of glucose and fructose, which the values were higher in pulp fruits. HPLC with refractive index detector analysis showed that citric, malic, and succinic acids were the main organic acids in all cultivars, with a significant higher content in old cladodes.

CONCLUSION: These investigations valorize *O. ficus-indica* fruits in comparison with cladodes. In general, this plant can be considered as an ingredient for the production of health-promoting food, highlighting mainly in the antioxidant activity and total polyphenols content found in young cladodes and peel fruits.

Keywords: acids; antioxidant activity; cladodes; *Opuntia*; total phenols

INTRODUCTION

Opuntia ficus-indica, commonly called prickly pear or cactus pear, belongs to the Cactaceae family. Its geographic distribution covers mainly Mexico and Latin America; however, this plant can grow in arid and semiarid climates, allowing it to develop in South Africa and in Mediterranean countries.[1] In Spain, the prickly pear is distributed especially along the Mediterranean coast, Andalucía, Murcia, and the Balearic and Canary Islands.[2] It is normally found on sunny slopes, roadsides, abandoned fields, degraded scrub, and so on.[3]

This plant can be divided into four main parts: cladodes, flower, fruit, and seeds.[4,5] Cladodes contain bioactive compounds such as fiber, minerals,flavonoids, phenolics, and other nutrients.[6,7] The fruit consists of pulp, peel, and seeds, with weight proportions of 28 – 58%, 37 – 67%, and 2 – 10% respectively.[8] The pulp is rich in glucose, fructose, and pectin.[9] Among other nutrients, the fruits contain ascorbic acid, flavonoids, betalains, and phenols in which some studies have indicated that the concentration of these phenolic compounds is related to the color and cultivar of the fruit.[8,10] *O. ficus-indica* (especially cladodes and fruits) is used commonly as a fresh edible food ingredient and in products like jams, alcoholic beverages, natural liquid sweeteners, or animal feed.[6,7,11] In addition, Mexicans use the cladodes and fruits for their medicinal benefits, such as treating arteriosclerosis, diabetes, gastritis, and hyperglycemia.[11]

Recent studies have demonstrated that consumption of *O. ficus-indica* has important positive health benefits, mainly due to antioxidant properties.[8,12] There are a few reasons to research this

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plant: (i) the nutritional and potential uses of the different parts of the prickly pear; (ii) it is not a common crop in Spain; (iii) most of the research focuses on the study of only one part of the plant. These reasons have motivated our investigation about the chemical and antioxidants characterization of cladodes (young and old) and fruit (pulp and peel) to evaluate the bioactive compounds and antioxidant properties possessed in order to encourage cultivation and potential uses.

MATERIALS AND METHODS

Plant material and sample processing

Cladodes and fruits of six different cultivars of *O. ficus-indica* were used for this study. Four cultivars were collected at the experimental field station of the Miguel Hernandez University in the province of Alicante, Spain (02°03′50″E, 38°03′50″N, and 25 masl); these cultivars were termed NA, NT, NE and NO. Another two cultivars were collected from the private farms of Murcia (FR) and Alicante (NJ).

The young (less than a year) and old (2-year-old) cladodes and the fruits were harvested during spring and summer of 2015. After picking (10 cladodes and 10 fruits per cultivar from three *O. ficus-indica* plants), the plant materials were transported into the laboratory. The spines were removed from the cladodes and the fruits were washed for 2 min under tap water with a brush, and the peel was removed from the fruit manually. A portion of the plant material was squeezed to get the juice to analyze pH, total soluble solids (TSS), titratable acidity (TA), organic acids, and sugar profile. The other fresh cladodes, pulp, and peel were immediately frozen in liquid nitrogen and later freeze-dried in an Alpha 2-4 freeze drier (Christ Alpha 2-4; Braum Biotech) for 24 h at a pressure reduction of 0.220 mbar. The temperature in the drying chamber was −25 °C, while the heating plate reached 15 °C. At the end of freeze-drying, the samples were powdered and packed in vacuum, then total polyphenols content (TPC) and antioxidant activity (AA) – 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric-reducing ability of plasma (FRAP) methods – were analyzed.

Total soluble solids, pH, and total titratable acidity

The TSS were measured with a digital Atago refractometer (model N-20; Atago, Bellevue, Wash., USA) at 20 °C with values being expressed as degrees Brix (°Bx). TA and pH were determined by acid – base potentiometer (877 Titrino plus, Metrohm ion analy- ses CH9101, Herisau, Switzerland), using 0.1 mol L^{1} NaOH up to pH 8.1; the analyses were run in three replications and values were expressed as grams of citric acid per liter.

Organic acids and sugars

Organic acids and sugars profile were quantified according to Hernández *et al.*[13] with a few modifications. The juices were obtained by squeezing the cladodes; the pulp and peel were homogenized with 10 mL of 50 mmol L^{-1} Tris-acetate buffer pH 6.0 and 10 mmol L^{-1} CaCl and centrifuged at 15 000 × *g* for 20 min (Sigma 3 – 18 K, Osterode and Harz, Germany). Then 1 mL of supernatant was filtered through a 0.45 μm Millipore filter and 10 μL was injected into a Hewlett-Packard high-performance liq- uid chromatography (HPLC) series 1100 (Hewlett-Packard, Wilm-ington, DE, USA). A column (Supelcogel TM C-610H column 30 cm × 7.8 mm) and a pre-column (Supelguard 5 cm × 4.6 mm, Supelco, Inc., Bellefonte, PA) were used for the analyses of both organic acids and sugars. The elution buffer consisted of 0.1% phosphoric acid, and organic acid absorbance was measured at 210 nm using a diode-array detector. These same HPLC conditions (elution buffer, flow rate, and column) were used for the analysis of sugars. The detection was conducted using a refractive index detector. Stan- dards of organic acids (oxalic, citric, tartaric, malic, quinic, shikimic, and fumaric acids) and sugars (glucose, fructose, and sucrose) were obtained from Sigma (Poole, Dorset, UK). Calibration curves were used for the quantification of organic acids and sugars, and showed good linearity ($R^2 \geq 0.999$). Analyses were run in three replications and results were expressed as mean plus/minus stan- dard error and units in grams per liter.

Extraction procedure for total polyphenols content and antioxidant activity

The extraction procedure for TPC and AA quantification was pre-pared as described by Wojdyło *et al.*[14] Plant materials (0.5 g) were weighed into a test tube. A total of 10 mL of 80% of aqueous methanol with 1% of HCl was added and the suspension was slightly stirred. Tubes were sonicated for 15 min and left for 24 h at 4 °C. Then the extract was again sonicated for 15 min and centrifuged for 15 min at 15 000 × *g*. The supernatants were collected to be used in subsequent analyses.

Quantification of total phenolic content

The TPC was measured using the Folin– Ciocalteu colorimetric method described by Chong *et al.*[15] Cladodes and fruit extracts (0.1 mL) were mixed with 0.2 mL of Folin – Ciocalteu reagent and 2 mL of H_2O. Then, the mixture was incubated at room temperature for 3 min and 1 mL of 20% sodium carbonate was added to the mixture. TPC were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue color was measured at 765 nm using a UV – visible spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). Calibration curves with concentrations of gallic acid as standard were used for quantification. All determinations were performed in triplicate and results were expressed as grams of gallic acid equivalent (GAE) per kilogram of dry weight (dw).

Determination of antioxidant activity by three different methods

DPPH method

The DPPH radical scavenging activity was determined using the method proposed by Brand-Williams *et al.*[16] Briefly, 10 μL of the supernatant were mixed with 40 μL of MeOH and added to 950 μL of DPPH solution. The mixture was shaken vigorously and placed in a dark room for 10 min. The decrease in absorbance was measured at 515 nm in a UV– visible spectrophotometer (Termo- spectromic Helios Gamma UVG 1002 E). All determinations were performed in triplicate, and results were expressed in millimoles of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per kilogram (dw).

ABTS method

The free-radical-scavenging activity was determined by ABTS radical cation described by Re *et al.*[17] The $ABTS^{\cdot+}$ solution was produced by reacting aqueous ABTS solution (7 mmol L^{-1}) with potassium persulfate (2.45 mmol L^{-1}, final concentration) and kept in the dark at room temperature for 12 – 16 h before use. The radical was stable in this form for more than 2 days when stored

in the dark at room temperature. Diluted $ABTS^{\cdot+}$ solution with an absorbance of 0.70 at 734 nm was employed in the analysis. The reactions were performed by adding 990 µL of $ABTS^{\cdot+}$ solution to 10 µL of each extract solution. The absorbance reading was exactly 6 min after initial mixing. All determinations were performed in triplicate and the results were expressed in millimoles of Trolox per kilogram (dw).

Ferric reducing/antioxidant power

The antioxidant potential was determined using a FRAP assay by Benzie and Strain.[18] The assay was based on the reducing power of antioxidant compounds to reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 µmol L,$^{-1}$ pH 3.6), a solution of 10 µmol L^{-1} TPTZ in 40 µmol L^{-1} HCl, and 20 µmol L^{-1} $FeCl_3$ at 10: 1: 1 (v/v/v). The reagent (300 µL) and sample solutions (10 µL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of Trolox. All determinations were performed in triplicate and results were expressed in millimoles of Trolox per kilogram (dw).

Statistical analysis

All experiments and analyses were carried out in triplicate, with mean values and standard deviations calculated accordingly. The differences between them and values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey's procedure. Significance was defined at $P \leq 0.05$. Statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD).

RESULTS AND DISCUSSION

Total soluble solids, pH, total titratable acidity, and moisture

Table 1 shows the significant differences in the results obtained from the main quality parameters of cladode and fruit. TSS is an index related to the sweetness and acidic balance; these two parameters are related to consumer preference.

Table 1. Quality physicochemical parameters in *O. ficus-indica* cladodes and fruits

Part	Cultivar	Part of the fruit and ripeness	pH	TA (g citric acid L^{-1})	TSS (°Bx)	Moisture (%)
Cladode	NJ	Young	6.75 ± 0.11†	2.22 ± 0.14	4.97 ± 0.03	91.4 ± 0.2
		Old	6.40 ± 0.07	1.30 ± 0.01	4.2 ± 0.01	92.2 ± 0.3
	FR	Young	6.10 ± 0.10	2.10 ± 0.09	3.93 ± 0.22	91.6 ± 0.2
		Old	5.61 ± 0.01	3.04 ± 0.01	4.23 ± 0.07	94.0 ± 0.1
	NE	Young	5.67 ± 0.03	3.49 ± 0.12	4.03 ± 0.17	92.8 ± 0.3
		Old	5.56 ± 0.01	2.80 ± 0.06	3.63 ± 0.03	94.9 ± 0.2
	NO	Young	5.79 ± 0.14	3.40 ± 0.23	4.3 ± 0.01	92.5 ± 0.1
		Old	5.73 ± 0.02	2.46 ± 0.04	3.56 ± 0.03	94.4 ± 0.2
	NA	Young	6.08 ± 0.09	2.49 ± 0.12	4.60 ± 0.06	92.2 ± 0.2
		Old	5.65 ± 0.06	2.71 ± 0.11	4.30 ± 0.06	91.7 ± 0.2
	NT	Young	5.76 ± 0.01	2.56 ± 0.09	4.40 ± 0.23	92.9 ± 0.2
		Old	5.27 ± 0.01	5.05 ± 0.11	4.93 ± 0.03	92.5 ± 0.2
Fruit	NJ	Peel	5.11 ± 0.19	2.65 ± 0.13	10.4 ± 0.5	82.9 ± 1.3
		Pulp	5.41 ± 0.09	1.63 ± 0.60	11.7 ± 0.3	83.2 ± 0.7
	FR	Peel	5.57 ± 0.14	2.16 ± 0.09	12.5 ± 0.3	83.3 ± 0.7
		Pulp	6.15 ± 0.03	0.23 ± 0.12	13.7 ± 0.2	80.4 ± 0.9
	NE	Peel	5.27 ± 0.19	1.26 ± 0.27	15.4 ± 0.2	78.6 ± 0.5
		Pulp	5.81 ± 0.09	0.53 ± 0.09	15.7 ± 0.3	79.0 ± 0.5
	NO	Peel	4.99 ± 0.01	3.40 ± 0.61	12.8 ± 0.4	83.0 ± 0.7
		Pulp	5.51 ± 0.15	1.26 ± 0.47	13.9 ± 0.5	80.1 ± 0.1
	NA	Peel	4.83 ± 0.10	2.30 ± 0.31	8.03 ± 0.48	88.6 ± 0.2
		Pulp	6.01 ± 0.03	1.43 ± 0.16	10.7 ± 0.1	84.4 ± 0.4
	NT	Peel	5.59 ± 0.03	0.61 ± 0.06	11.7 ± 0.1	82.6 ± 0.4
		Pulp	5.54 ± 0.02	1.60 ± 0.49	12.7 ± 0.2	80.1 ± 0.5
	Part	Cladode	5.48‡b	2.80 a	4.25 b	92.8 a
		Fruit	5.86 a	2.17 b	12.3 a	82.2 b
	Cladode	Young	6.02 a	2.71 b	4.37 a	92.2 b
		Old	5.71 b	2.89 a	4.14 b	93.3 a
	Fruit	Peel	5.22 b	2.06 a	11.8 b	83.2 a
		Pulp	5.74 a	1.11b	13.1 a	81.2 b
ANOVA		Cultivar	**	*	***	***
		Part	***	*	***	***
		Cultivar/part	***	***	***	***

*, **, and ***, significant at $P < 0.05$, 0.01 and 0.001 respectively.
†Values are the mean of three replications (plus/minus standard error).
‡Values followed by the different letters (a, b) within the same column are statistically different according to Tukey's test.

Table 2. Organic acids and sugar profile in *O. ficus-indica* cladodes and fruits

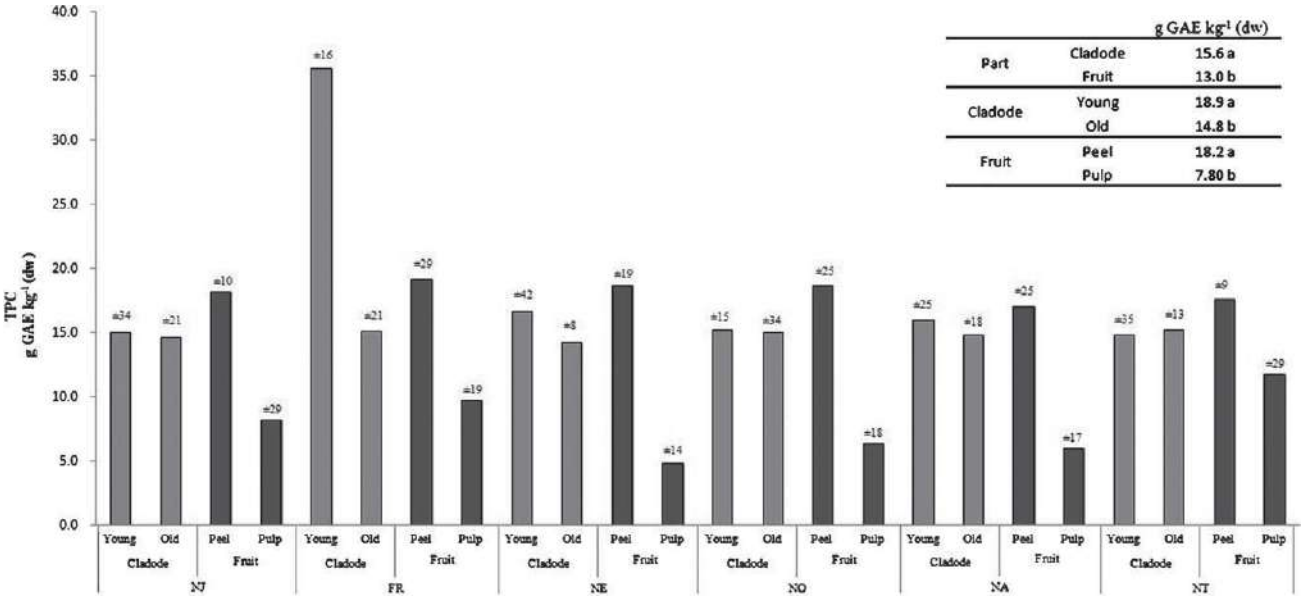
Part	Cultivar	Part of the fruit and ripeness	Concentration (g L^{-1})				
			Glucose	Fructose	Citric	Malic	Succinic
Cladode	NJ	Young	48.7 ± 0.14†	tr	13.2 ± 0.14	60.0 ± 0.14	49.6 ± 0.14
		Old	70.1 ± 0.58	130 ± 0.9	45.9 ± 0.06	74.3 ± 0.30	7.8 ± 0.01
	FR	Young	tr	tr	15.8 ± 0.37	39.9 ± 0.15	60.0 ± 0.12
		Old	26.3 ± 0.11	164 ± 0.1	76.8 ± 0.09	99.5 ± 0.11	8.9 ± 0.01
	NE	Young	23.1 ± 0.09	tr	13.8 ± 0.09	64.5 ± 0.07	79.0 ± 0.76
		Old	3.2 ± 0.01	163 ± 0.3	59.9 ± 0.01	119 ± 0.1	12.4 ± 0.21
	NO	Young	55.3 ± 0.19	tr	12.9 ± 0.03	68.8 ± 0.14	69.1 ± 0.32
		Old	tr	75.2 ± 0.15	49.2 ± 0.01	76.5 ± 0.08	42.0 ± 0.13
	NA	Young	46.9 ± 0.15	tr	14.5 ± 0.05	50.8 ± 0.07	82.5 ± 0.21
		Old	30.3 ± 0.53	112 ± 1.3	58.5 ± 0.01	74.0 ± 0.63	30.5 ± 0.21
	NT	Young	7.10 ± 0.10	29.3 ± 0.29	13.0 ± 0.45	36.4 ± 0.14	60.0 ± 0.30
		Old	tr	112 ± 0.1	78.9 ± 0.04	97.9 ± 0.07	19.2 ± 0.11
Fruit	NJ	Peel	90.2 ± 0.52	27.9 ± 0.26	3.20 ± 0.07	1.04 ± 0.01	nd
		Pulp	131 ± 0.2	57.8 ± 0.30	1.61 ± 0.01	1.20 ± 0.01	nd
	FR	Peel	92.5 ± 0.20	69.1 ± 0.35	3.0 ± 0.02	2.81 ± 0.03	nd
		Pulp	114 ± 0.3	88.0 ± 0.21	0.80 ± 0.01	2.03 ± 0.01	nd
	NE	Peel	117 ± 0.3	81.8 ± 0.18	2.31 ± 0.03	2.20 ± 0.01	nd
		Pulp	141 ± 0.1	79.5 ± 0.24	1.08 ± 0.01	2.10 ± 0.01	nd
	NO	Peel	128 ± 0.2	34.8 ± 0.13	3.41 ± 0.02	1.41 ± 0.03	nd
		Pulp	144 ± 0.2	66.1 ± 0.10	1.22 ± 0.01	1.60 ± 0.01	nd
	NA	Peel	57.0 ± 0.84	46.7 ± 0.49	1.60 ± 0.01	1.52 ± 0.01	nd
		Pulp	103 ± 0.1	77.2 ± 0.17	0.30 ± 0.01	1.51 ± 0.01	nd
	NT	Peel	61.1 ± 0.26	51.7 ± 0.06	3.00 ± 0.01	2.01 ± 0.01	nd
		Pulp	106 ± 0.2	61.5 ± 0.19	0.71 ± 0.02	1.71 ± 0.01	nd
	Part	Cladode	25.9‡b	60.9	37.7	71.8 a	43.4
		Fruit	107 a	61.8	1.80	1.70 b	nd
	Cladode	Young	30.2 a	4.91 b	13.9 b	53.4 b	66.7 a
		Old	21.6 b	117 a	61.5 a	90.2 a	20.1 b
	Fruit	Peel	91.0 b	52.0 b	2.80 a	1.80	nd
		Pulp	123 a	71.7 a	0.91 b	1.70	nd
ANOVA		Cultivar	***	NS	NS	NS	NS
		Part	***	**	***	***	***
		Cultivar/part	***	NS	NS	NS	NS

NS: not significant *F* ratio ($P < 0.05$). *, **, and ***, significant at $P < 0.05$, 0.01 and 0.001 respectively.
nd: not detected; tr: traces.
†Values are the mean of three replications (plus/minus standard error).
‡Values followed by the different letters (a, b) within the same column are statistically different according to Tukey's test.

The maturity stage of the cladodes has interest from an industrial point of view. The tender sprouts are used for 'nopalitos' production and are consumed fresh; however, when the cladodes are partially ripe they are used for the production of flour and other products.[19] Owing to the cladode nature, the content of soluble solids was lower than the fruit values (mean value 4.25 °Bx in cladode and 12.3 °Bx in fruit). The soluble solids and fruit size are used as a reference for the harvest time and fruit quality; to ensure that the fruit has good quality with established values of soluble solids, values of >12–13 °Bx are required.[20,21] Therefore, the values obtained in this study for fruit pulp are near or within the stated values, except for the NA cultivar, which presented the lowest values (10.7 °Bx).

The values obtained for TA were in the range 1.3 – 5.05 g citric acid L^{-1} for cladode and 0.2 – 3.4 g citric acid L^{-1} for fruits (Table 1). These values show the significant difference for TA among the cladode (young and old) and fruit (pulp and peel) of the *O. ficus-indica* plant. Owing to the cladode nature, TA values are higher in cladodes than in fruit, especially in old cladodes (mean value of 2.89 g citric acid L^{-1}); in the morning, because of their malic acid content, the cladodes have considerable acidity and are not desired by cattle. However, at noon and in the evening, when the sugars have been produced, cladodes lose acidity and the cattle consume them.[22]

Less variation was found in the pH values, ranging from 5.2 to 6.02, values similar to those reported by Celis-Fabian[23] (5.0 – 6.6). Regarding the moisture, significant differences were found among cladodes and fruit. The cladode is characterized by its high moisture content, so that the average value was higher in the cladodes (92.8%) than in the fruit (82.2%). The prickly pear has a photosynthetic metabolism of the crassulacean type called *crassulacean acid metabolism* (CAM), which allows the production of biomass in arid and drought conditions of their characteristic habitat.[24,25] To avoid water loss through stomata during the photosynthetic process, CAM plants have developed a specific mechanism that prevents stomata opening through the hottest daylight hours. During



		g GAE kg^{-1} (dw)
Part	Cladode	15.6 a
	Fruit	13.0 b
Cladode	Young	18.9 a
	Old	14.8 b
Fruit	Peel	18.2 a
	Pulp	7.80 b

Figure 1. TPC (g GAE kg^{-1} (dw)) in *O. ficus-indica* cladodes and fruits. The values represented in the bars are the mean of three replications. Values followed by the different letters (a, b) within the same table column are statistically different according to Tukey's test.

the winter, the *O. ficus-indica* plant loses moisture due to transpiration and is used as fodder. In addition, cladodes could be left with the plant and directly consumed by animals or harvested and stored for later use.

Organic acids and sugars

Table 2 shows the values obtained for sugars and organic acids. Despite analyzing different organic acids, only relevant results were obtained for citric and malic acids; of the rest (oxalic, tartaric, quinic, shikimic, and fumaric acids), only traces were obtained. Regarding citric and malic acids, it is seen that the majority acid is malic with a value of 71.8 g L^{-1} in cladodes, followed by succinic and citric acids (43 g L^{-1} and 37.7 g L^{-1} respectively). A significant difference in these values is noted compared with those obtained in the fruit, where the values are lower. This is due to the CAM metabolism of *O. ficus-indica*, especially in the cladodes, in which organic acids accumulate in the vacuole during the night phase, predominantly as malic acid, and also suffer a reciprocal reserve carbohydrates accumulation, such as starch, soluble glucans, or hexoses, during the daytime phase.[10] Likewise, the values of citric and malic acids in cladode were higher in old than in young cladodes, possibly due to the accumulation that has been generated during cladode ripening. The composition of cladodes changes with age, so their use and industrial interest are different. The TA values obtained can be correlated with the content of citric and malic acids, since in both cases the old cladodes had the highest content in these three parameters (TA 2.89 g citric acid L^{-1}; citric acid 61.5 g L,$^{-1}$ and malic acid 90.2 g L^{-1}). Corrales-Garcia[26] indicated that, according to popular medicine in countries such as Mexico, consumption of cladodes can reduce sudden changes in pH in the digestive tract, partly because of the therapeutic effect it has on gastrointestinal disorders (gastritis). The NE and FR cultivars were those that showed the highest values in the contents of citric and malic acids, especially in old cladodes. The presence of organic acids in the fruit is lower in comparison with cladodes; however, it is characterized by high sugar content.

Sucrose, glucose, and fructose sugars were determined as components in juice samples. Table 2 shows that glucose was detected in all sample juices. Fructose was detected in fruit juices, old cladodes, and only in the NT cultivar of young cladodes. However, sucrose was not detected. The amount of glucose and fructose in pulp juices was greater than in the peel juices of the six cultivars. Our results were in agreement with previously reported data by Zenteno-Ramirez *et al.*[9] in the juice of prickly pear cultivars, with glucose predominating (96 g L^{-1}), followed by fructose (64 g L^{-1}). The results obtained in total sugars are greater than those reported for glucose and fructose in mature wolfberry (2 and 40 g kg^{-1}),[27] grapefruit pulp (22 and 25 g kg^{-1}),[28] and in passion fruit (96 and 97.6 g kg^{-1} dw).[29] The NE and NO cultivars were those that showed the highest values in the glucose content; likewise, the FR and NE cultivars were those that showed the highest values in the fructose content, especially in pulp fruit.

Since cactus pear juice is rich in both glucose and fructose, it makes it a good source of energy and as a natural source of sweetness for food preparations. For its part, the fructose contributes to a sweet taste, which is typical of this fruit, due to its high sweetness compared with glucose and sucrose.[26] Furthermore, glucose is an energy metabolite of the brain and nerve cells and in the fruit analyzed (mainly in pulp with values of 123 g kg^{-1}) is present as free sugar, which is directly absorbed by the human body. Among the potential uses presented by The National Institute of Ecology in Mexico on the chemical composition of an antidiabetic *O. ficus-indica* extract is the reduction of sugar content, mostly glucose, which is interesting since glucose is associated with the disease. Likewise, studies by the Mexican Social Security Institute have shown that administration fasting on cactus pads in diabetic individuals has resulted in decreased glucose levels. It is believed that *O. ficus-indica* function on glucose is due to the presence of a substance, which is identified as isolated polysaccharides, that sequesters glucose molecules, so that if insulin is low it is sufficient to regulate blood sugar. According to these results in the content of glucose, fructose, and citric and malic acids, cladodes and fruit can be used as a good food supplement and could be an important additive for functional foods.

Table 3. AA in *O. ficus-indica* cladodes and fruits

Part	Cultivar	Part of the fruit and ripeness	AA (mmol Trolox kg^{-1} (dw)) ABTS	DPPH	FRAP
Cladode	NJ	Young	20.0 ± 0.5†	18.8 ± 0.6	99.0 ± 0.7
		Old	11.8 ± 0.9	10.6 ± 0.7	77.8 ± 0.3
	FR	Young	28.4 ± 0.2	55.2 ± 0.2	106 ± 2
		Old	13.3 ± 0.6	11.3 ± 1.2	74.4 ± 0.6
	NE	Young	25.7 ± 0.2	18.8 ± 2.0	114 ± 4
		Old	12.4 ± 0.2	7.56 ± 0.70	60.8 ± 0.5
	NO	Young	20.3 ± 0.3	12.8 ± 0.7	81.8 ± 0.8
		Old	16.0 ± 1.3	11.6 ± 0.2	78.2 ± 1.3
	NA	Young	22.4 ± 0.5	18.3 ± 0.6	98.7 ± 6.5
		Old	16.1 ± 0.8	14.5 ± 0.3	74.6 ± 0.7
	NT	Young	15.8 ± 0.6	18.4 ± 0.1	73.8 ± 3.3
		Old	16.2 ± 1.0	10.4 ± 0.3	83.5 ± 2.8
Fruit	NJ	Peel	33.3 ± 0.2	56.6 ± 1.3	50.1 ± 3.5
		Pulp	6.40 ± 0.3	59.7 ± 0.7	17.9 ± 1.3
	FR	Peel	36.0 ± 0.8	59.6 ± 2.0	55.1 ± 0.4
		Pulp	10.2 ± 1.0	60.1 ± 1.0	32.3 ± 0.1
	NE	Peel	36.2 ± 0.8	54.8 ± 0.7	40.2 ± 2.2
		Pulp	29.0 ± 0.3	60.0 ± 0.9	17.3 ± 1.1
	NO	Peel	36.9 ± 0.5	57.4 ± 2.0	42.2 ± 0.9
		Pulp	21.2 ± 0.6	58.9 ± 0.5	21.4 ± 1.5
	NA	Peel	14.7 ± 1.4	56.0 ± 0.8	116 ± 5
		Pulp	29.2 ± 0.8	58.4 ± 1.9	15.0 ± 0.5
	NT	Peel	37.3 ± 0.9	55.1 ± 1.5	46.6 ± 4.4
		Pulp	30.6 ± 0.8	59.0 ± 1.5	28.1 ± 2.4
	Part	Cladode	18.8‡b	17.4 b	85.3 a
		Fruit	26.8 a	58.0 a	40.2 b
	Cladode	Young	22.1 a	23.7 a	95.7 a
		Old	14.3 b	11.0 b	74.9 b
	Fruit	Peel	32.4 a	56.6	58.4 a
		Pulp	21.1 b	59.4	22.0 b
ANOVA		Cultivar	NS	**	NS
		Part	***	***	***
		Cultivar/part	NS	*	NS

NS: not significant *F* ratio ($P < 0.05$). *, **, and ***, significant at $P < 0.05$, 0.01 and 0.001 respectively.
†Values are the mean of three replications (plus/minus standard error).
‡Values followed by the different letters (a, b) within the same column are statistically different according to Tukey's test.

Total phenolic content

Fruit and vegetables, besides being composed of essential nutrients for human metabolic processes, also have other substances that may serve as protectors against certain diseases; these phenolic compounds are known as bioactive or functional compounds,[30] which mostly are characterized by their AA. In this context, Fig. 1 shows the TPC values for cladodes, with average values of 18.9 g GAE kg^{-1} and 14.8 g GAE kg^{-1} (dw) in young and old cladodes respectively, and for fruit, with average values of 18.2 g GAE kg^{-1} and 7.80 g GAE kg^{-1} (dw) in peel and pulp respectively. The TPC values have been analyzed mainly in the fruit juice: Abdel-Hameed *et al.*[11] reported values of 11.5 g GAE L^{-1} and 10.6 g GAE L^{-1} respectively in peel and pulp of red cactus fruit. TPC values obtained in the cladodes and fruits of *O. ficus-indica* were higher than those found in other *Opuntia* species. Morales-Montelongo[31] reported values of 8.59 g GAE kg^{-1} and 9.18 g GAE kg^{-1} (dw) respectively in peel and pulp of xoconostle (*O. matudae*). The values reported in this study are higher than those reported for cranberries, which are in the range 4.95–9.80 g GAE kg,$^{-1}$ [32] and garambullo fruit, with 12.3 g GAE kg^{-1} in ripe fruits.[33]

The FR cultivar showed significantly higher values of TPC in young cladodes (35.6 g GAE kg^{-1} (dw)); FR and NE cultivars presented the highest values in the peel fruit (19.2 g GAE kg^{-1} and 18.2 g GAE kg^{-1} (dw) respectively). The presence of TPC was detected in the majority of young cladodes and in peel fruit and it corresponds with the antioxidant effect (Table 3). These results are clear evidence that both cladodes and fruit can be used in areas such as nutrition, traditional medicine, and other industrial applications.

Antioxidant activity

AA is one of the major mechanisms by which fruits and vegetables provide health benefits, in addition to having the ability to inhibit excessive oxidation due to free radicals which are in the form of reactive oxygen species. Polyphenols exhibit antioxidant properties and are attached to a benzene ring; the hydroxyl groups give

the polyphenol the ability to act either as a donor of a hydrogen atom or as a donor of an electron to a free radical (or other reactive species).[34]

The AAs of *O. ficus-indica* extracts were conducted by three complementary methods to take into account the various mechanisms of antioxidant action. DPPH radical is scavenged by antioxidant compounds present in the extracts, which determines its ability to capture radicals, the ABTS method captures the cationic $ABTS^{\cdot+}$ radical, and finally the FRAP method measures the ability to reduce Fe^{3+} in the sample.

Table 3 shows significant differences in the AA of *O. ficus-indica* depending on the part analyzed (cladodes or fruits). The scavenging activity of DPPH and ABTS methods was higher in fruits than in cladodes, especially in the peel. The AA mean values of fruit by DPPH and ABTS methods were 26.8 mmol Trolox kg^{-1} and 58.0 mmol Trolox kg^{-1} (dw) respectively. Regarding these two methods, the FR cultivar presented the highest value in peel fruit (60.1 mmol Trolox kg^{-1} (dw)). Several authors have reported a higher AA in the peel than in the pulp fruits; for example, Calín-Sánchez *et al.*[35] in pomegranate fruit, Marquina *et al.*[36] in guava fruit, and Oszmiański *et al.*[37] in berries. Also, the results obtained in this study are comparable to those reported by Teleszko and Wojdyło[38] in different fruits using ABTS and FRAP: apple (87.2 mmol L^{-1} Trolox equivalents (TE) kg^{-1} dw and 34.4 mmol L^{-1} TE kg^{-1} dm respectively) and quince (78.5 mmol L^{-1} TE kg^{-1} dw and 54.3 mmol L^{-1} TE kg^{-1} dw respectively).

Regarding the FRAP method, the results showed that cladode presented the highest values, even more than the fruit (85.3 mmol L^{-1} TE kg^{-1} dw), mainly in young cladodes for FR cultivar (106 mmol L^{-1} TE kg^{-1} dw) (Table 3). Comparing the AA results by the three methods, together with the values obtained in the TPC, it is concluded that the young cladodes and peel fruit have a higher AA than old cladodes and pulp fruit. In fact, positive correlations were observed among the TPC and the AA measured by the ABTS assay for young cladodes ($r = 0.76$, $P \leq 0.05$) and for peel fruit ($r = 0.51$, $P \leq 0.05$).

CONCLUSIONS

This study provides information about antioxidant properties and chemical characterization in both *O. ficus-indica* fruits and cladodes from cultivars grown in Spain. Results show that the young cladodes, especially FR cultivar, present significant levels of phenolic compounds that play an important role against oxidation, as well as the AA by the FRAP method. Using the DPPH and ABTS methods the fruits presented higher content in AA, especially in the peel of FR, NE, and NT cultivars. The old cladodes presented higher contents of citric and malic acids. Glucose and fructose were detected in all *O. ficus-indica* parts, especially in pulp fruits.

Their easy adaptation to arid conditions, as well as their rapid spread and economic maintenance, makes the cladodes and the fruits a valuable resource for ruminant feeding in arid and semiarid areas of the country. In addition, their total phenolic content makes this material interesting for food in both fresh consumption and for the production of food products. However, further investigations are needed to analyze other important components in these cultivars, such as minerals and fatty acids.

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Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-MS^n metabolomic profiles and their chemometric analysis

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Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MS^n metabolomics profiles and their chemometric analysis

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ABSTRACT

Prickly pear is an important source of bioactive compounds. However, a comprehensive characterization of the phytochemical profile of its aerial botanical parts, considering genotypic differences, has not been conducted. This study evaluated the phytochemical composition of four botanical parts (fruit pulp and skin, and young and adult cladodes) of six cultivars. Analysis was carried out by using two non-targeted UHPLC-ESI-MS^n experimental conditions and assisted with multivariate analysis to facilitate data interpretation. Up to 41 compounds, mainly (poly)phenolic molecules, were identified and quantified, 23 compounds being reported for the first time in *Opuntia ficus-indica*. Phenolic composition varied significantly depending on the part of the plant. Betalains were detected only in the fruit of a red cultivar. This study provided novel insights in terms of identification of bioactives and thorough characterization of botanical parts of prickly pears. This information may be used for the development of prickly pear-derived products with high levels of bioactive compounds.

1. Introduction

Cactus prickly pear (*Opuntia ficus-indica* (L.) Mill.) is a plant that could be easily cultivated in arid and semiarid climates (Russell & Felker, 1987). It produces edible fruits (called "tuna") and cladodes (fleshy flattened stems, commonly called "nopal"), both used as food and as feed. Prickly pear is employed for nutrition, cosmetic, and ethnopharmacological purposes in the forms of tea, jam, juice, and oil -extracted from the seeds- (Stintzing et al., 2005). Recently, some authors have highlighted the prospects of different prickly pear aerial parts as good sources of phytochemicals with proven biological activities and high-added value for the food/nutraceutical industry (Barba et al., 2017; Msaddak et al., 2017; Sánchez-Tapia et al., 2017). This interest in *Opuntia* bioactives becomes even more relevant when considering the need to cope with climate change challenges. Taking into account the tolerance of cactus species to extreme climatic/soil conditions (Russell & Felker, 1987), the exploitation of its phytochemical content may contribute to its sustainable production.

The main phytochemical compounds in prickly pear fruits and cladodes are vitamins, carotenoids, betalains, and (poly)phenolic compounds (Barba et al., 2017; Fernández-López, Almela, Obón, & Castellar, 2010; Stintzing et al., 2005). Fruits are good sources of betalains, but the real physiological relevance of these compounds has not been fully unraveled (Moreno, García-Viguera, Gil, & Gil-Izquierdo, 2008). Among the different prickly pear phytochemicals, (poly)phenolic compounds are likely those attracting more attention due to their health-related effects (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014; Zanotti et al., 2015). The (poly)phenolic fingerprint of prickly pear products is characterized mainly by flavonols and phenolic acids (Fernández-López et al., 2010; Kuti, 2004; Mata et al., 2016; Moussa-Ayoub et al., 2014; Serra, Poejo, Matias, Bronze, & Duarte, 2013; Stintzing et al., 2005; Yeddes, Cherif, & Trabelsi Ayadi, 2014). However, despite considerable characterizations have been reported (Guevara-Figueroa et al., 2010; Mata et al., 2016; Moussa-Ayoub et al., 2014; Serra et al., 2013; Yeddes et al., 2014), a detailed profiling of the bioactive compounds of the aerial parts of prickly pear is lacking.

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The accurate characterization of the phytochemical fingerprinting of any vegetal matrix is key to better understand its biological, technological, and nutritional properties (Mena et al., 2012). The use of mass spectrometric (MS) metabolomics techniques, assisted by chemometric analysis, has been identified as a valuable technique in the evaluation of the phytochemical profile of different plant materials rich in bioactive compounds (Calani et al., 2013; Sánchez-Salcedo et al., 2016). Analytical approaches allowing easy sample handling and quick, high-throughput chromatographic screening are encouraged to accomplish this task (Filigenzi, Ehrke, Aston, & Poppenga, 2011). Nevertheless, the comprehensive study of bioactive compounds may pose some analytical constraints due to the varying capability of diverse chemical scaffolds to respond to the MS ionization settings. Thus, versatile experimental conditions leading to the identification of different phytochemical classes are required (Mena et al., 2016).

The present work aimed at investigating the phytochemical composition of four different botanical parts (young and adult cladodes, fruit pulp, and skin) of six prickly pear cultivars grown in Spain, extending a preliminary characterization of this plant material (Andreu, Nuncio-Jáuregui, Carbonell-Barrachina, Legua, & Hernández, 2018). The study was performed by using two complementary non-targeted UHPLC-ESI-MS^n experimental conditions and paired with multivariate analysis to facilitate a comprehensive screening. The high number of samples and the presence of different matrices and classes of phytochemicals represented a major analytical challenge; however, the insights provided in terms of both identification of bioactive compounds and thorough characterization are of interest.

2. Materials and methods

2.1. Chemicals

Protocatechuic acid, ferulic acid, quercetin-3-*O*-rutinoside (rutin), naringenin-7-*O*-rutinoside (narirutin), secoisolariceresinol, and betanin were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade solvents were also purchased from Sigma-Aldrich. Water for HPLC analysis was purchased from VWR Chemicals (Fontenay-sous-bois, France).

2.2. Plant material

Cladodes and fruits of six different cultivars of *Opuntia ficus-indica* were used for this study. Four cultivars, named "NA", "NT", "NE", and "NO", were collected at the experimental field station of the Miguel Hernandez University in the province of Alicante, Spain (02°03'50"E, 38°03'50"N, and 25 m above sea level). The other two cultivars were collected from private farms in Murcia ("Fresa" cultivar) and Alicante ("Nalle" cultivar) (SE Spain) (< 50 km far from the experimental station).

Young (less than a year) and old cladodes (2 years old), as well as the fruits, were manually harvested during spring and summer of 2015. Ten young cladodes, 10 adult cladodes, and 10 fruits from three *Opuntia ficus-indica* plants per cultivar were harvested. After picking, the plant material was immediately transported to the lab. The spines from the cladodes were removed manually, while the fruits were washed under tap water with a brush for 2 min. The peels from the fruits were removed manually. The fresh cladodes (young and old), the pulp plus seeds, and the peel were immediately frozen in liquid nitrogen, to be later freeze-dried in an Alpha 2–4 freeze drier (Christ Alpha 2–4; Braum Biotech, Osterode am Harz, Germany) for 24 h at a pressure reduction of 0.220 mbar. The temperature in the drying chamber was −25 °C, while the heating plate reached 15 °C. Thereafter, seeds were removed from the pulp, and all the samples were powdered (particle size < 0.4 mm) and packed under vacuum.

2.3. Extraction of (poly)phenolic compounds

The (poly)phenolic compounds in prickly pear cladodes (young and old) and fruits (pulp and skin) were extracted following a protocol previously reported (Sánchez-Salcedo, Mena, García-Viguera, Martínez, & Hernández, 2015). Briefly, 200 mg of freeze-dried powder were mixed with 1 mL of 80% aqueous methanol acidified with formic acid (1%). This mixture was then sonicated for 25 min, centrifuged at 10,480*g* for 5 min at room temperature, and the supernatant was collected. Two additional extractions were performed for each sample with additional 0.5 mL of the extraction solvent, as described above, after which they were centrifuged. The three supernatants were pooled before UHPLC-ESI-MS^n analysis. Each sample was extracted in triplicate.

2.4. Liquid chromatography-mass spectrometry (UHPLC-ESI-MS^n) analysis

Methanolic extracts of prickly pear parts were analysed using an Accela UHPLC 1250 equipped with a linear ion trap-mass spectrometer (MS) (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization (ESI) probe (H-ESI-II; Thermo Fisher Scientific Inc., San Jose, CA, USA). Separations were performed using a XSelect HSS T3 (50 × 2.1 mm), 2.5 μm particle size (Waters, Ireland). Volume injected was 5 μL and column oven was set to 30 °C. Two complementary MS experiments were performed, one in negative mode, for non-coloured phenolics, and one using positive ionization, for betalains, following an analytical approach previously developed for the comprehensive identification of (poly)phenolic compounds (Mena et al., 2012). Each sample was analysed in duplicate for each experimental condition.

The experimental condition optimized in negative ionization mode for the analysis of non-coloured phenolics was based on the following conditions. The MS worked with a capillary temperature equal to 275 °C, while the source heater temperature was set to 250 °C. The sheath gas flow was 40 units, while both auxiliary and sweep gas were set to 5 units. The source voltage was 3 kV. The capillary and tube lens voltages were −9 and −53 V, respectively. Elution was performed at a flow rate of 0.2 mL/min. The gradient started with 90% of 0.1% aqu- eous formic acid and 10% of acetonitrile 0.1% formic acid, followed by a 13-min linear gradient of 10% to 70% acidified acetonitrile. From 13.5 to 14 min the acidified acetonitrile was increased to 80%, followed to 2.5 min of 80% acetonitrile and then 4 min at the start conditions to re-equilibrate the column. Analyses were carried out using full scan mode, data-dependent MS^3 scanning from *m/z* 100 to 2000, with collision induced dissociation (CID) equal to 30 (arbitrary units). Pure helium gas was used for CID.

For the analysis of betalains, in positive ionization mode, the MS worked with a capillary temperature equal to 275 °C, while the source heather temperature was set to 200 °C. The sheath gas flow was 40 units, while auxiliary gas was set to 5 units, without sweep gas. The source voltage was 4 kV. The capillary voltage and tube lens were 39 and 110 V, respectively. The chromatographic conditions were identical to those used for the previous experimental condition.

Data processing was performed using Xcalibur software from Thermo Scientific. All compounds were identified by comparing with standards, when available, and mass spectral and chromatographic data reported in literature. For quantification purposes, area calculation was performed in selected ion monitoring mode by selecting the relative base peak at the corresponding mass to charge ratio (*m/z*). The quantification of (poly) phenolics was carried out by comparison with commercial standards, when available. For those compounds that could not be quantified with their corresponding standards, a reference compound was selected based on structural similarity and considering the functional groups that may affect the ionisation properties (i.e., flavonols were quantified as rutin equivalents, lignans as secoisolariciresinol, etc.). Finally, the molecules responding to the ESI source in a unique way with respect to the reference compound of choice, or not

reaching the limit of quantification of the corresponding reference compound, were not quantified. Details on the identification and quantification of the phytochemicals are presented in the Supplementary Table S1.

2.5. Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics 23 software package (SPSS Inc., Chicago, IL, USA) and performed at $p < 0.05$ of significance level. Data are presented as mean ± standard deviation (SD) since the distribution of these variables was normal. A one-way ANOVA with post hoc Tukey HSD test was employed for mean comparisons among cultivars for each botanical part. The assessment of the main effects (botanical part, cultivar, and the interaction of botanical part × cultivar) was also carried out with Bonferroni *post-hoc* tests for multiple comparisons. Principal component analysis (PCA) with varimax was performed to explore the differences in the phytochemical profile of the different cultivars and prickly pear parts.

3. Results

3.1. Identification of phytochemicals in Opuntia ficus-indica cladodes and fruits

The phytochemical screening of prickly pear cladodes (young and old) and fruits (pulp and skin) belonging to six different cultivars was carried out by using two complementary MS experimental conditions. About 120 mass spectra were evaluated for each botanical part, cultivar, experimental condition, and analytical replicate. This exhaustive analysis of the *Opuntia ficus-indica* phytochemical composition allowed the tentative identification of up to 41 compounds (Table 1). Taking into account the number of compounds identified in prickly pear parts, flavonoids were the most relevant class of phytochemicals (16 flavonols, compounds 6, 13, 15, 16, 18, 20–22, 24, 26–28, 31, 32, 37, and 38, and 2 flavanones, 30 and 33). Phenolic acids (6 hydroxycinnamic acids, 4, 7, 9, 12, 14, and 36, 2 phenylpyruvic acids, 8 and 35, 2 hydroxyphenylpropionic acids, 19 and 23, and 2 hydroxybenzoic acids, 3 and 11) and lignans (6 compounds, 5, 10, 17, 25, 29, and 34) were also present. In addition, some other compounds such as betalains (compounds 39–41) and organic acids (compounds 1 and 2) were detected. Two compounds (24 and 39) were identified by comparison with their respective analytical standards. Thirty-nine compounds were identified based on their retention time, fragmentation patterns obtained from mass spectra (MS^2 and MS^3 experiments) (Table 1), and by comparing their mass spectral characteristic with the available literature (see Supplementary material, Table S1). The interpretation of the mass spectra fragmentation patterns reported in the literature was not discussed unless of special interest. In this sense, compounds 19, 22, and 26 were tentatively identified according to their characteristic aglycone fragment ions. Compounds 22 and 26 presented a major MS^2 fragment ion at *m/z* 315 and showed MS^3 fragments matching those of other isorhamnetin derivatives (compounds 20, 31, 32, and 37). Compounds 22 and 26 (*m/z* 755 and 609) also had losses of *m/z* 440 and 294, respectively, which might correspond to sambubioside-rhamnoside and sambubioside moieties; however, the full structure could not be identified and, hence, they were classified simply as isorhamnetin derivatives. Compound 19 presented the same fragmentation pattern of compound 23 and was identified as an isomer of dihydrosinapic acid-hexoside. 23 compounds (3–6, 10–19, 21, 23, 25, 29, 30, 33, 34, 37 and 38) were tentatively identified for the first time, as far as we know, in *Opuntia ficus-indica.*

Most of the compounds were identified in all the botanical parts analysed, while some compounds were detected only in some of them (Supplementary material, Table S1). In the case of betalains, they were only detected in the pulp and skin of the "Fresa" cultivar, the only one presenting an intense red colour.

3.2. Quantification of major (poly)phenolic compounds in Opuntia ficus-indica

The total amount of (poly)phenolic compounds for each botanical part and cultivar is reported in Fig. 1. There were significant main effects of botanical part, cultivar, and the interaction of botanical part × cultivar on the content of (poly)phenolic compounds ($p < 0.001$ for all). Regarding the botanical part, the highest (poly)phenolic content was found in young cladodes > old cladodes > skin > pulp ($p < 0.05$). Comparison among cultivars for each botanical part showed statistically significant differences on the content of (poly)phenolic compounds (Fig. 1). The concentration of these compounds varied between 5.3 ("NE") and 14.3 ("Fresa") mg/g dw for young cladodes and from 4.2 ("NO") to 12.4 ("NE") mg/g dw for old cladodes. The content of (poly)phenolic compounds in fruit skin ranged from 4.3 to 7.1 mg/g dw for "NA" and "NT", respectively, while it varied from 0.7 to 5.1 mg/g dw for "NO" and "Nalle", respectively, in fruit pulp.

The profile of individual (poly)phenolic compounds for each botanical part was dependent on the cultivar (Tables 2-5, Supplementary Fig. S1). Twenty-six phenolic compounds were quantified in young cladodes, with flavonoids (in particular, flavonols) being the main (poly)phenolic compounds (Table 2). Individual phenolics in young cladodes varied greatly among prickly pear varieties. Myricetin-hexoside (6) was the predominant compound in most of the tested cultivars, except for "NE", where it was present at a very low amount. Young cladodes were also characterized by the presence of relevant amounts of some isorhamnetin derivatives (20, 22, and 31), rutin (24), and ferulic acid-hexoside (9) (Table 2). In the case of old cladodes, up to 25 compounds were quantified (Table 3). Similar to what was reported for young cladodes, flavonols were the major group of (poly)phenolic compounds, and several isorhamnetin glycosides (20, 22, 26, and 31), together with myricetin-hexoside (6) and ferulic acid-hexoside (9), were the main individual phenolics (Table 3). With respect to fruit skin and pulp, a higher prevalence of phenolic acids over flavonols was noted (Tables 4 and 5). Twenty-six (poly)phenolic compounds were quantified in prickly pear skin, with ferulic acid-hexoside (9), sinapic acid-hexoside (12), dihydrosinapic acid-hexoside (23), and isorhamnetin-rutinoside (31) present in high concentrations for most of the cultivars (Table 4). Prickly pear pulp presented a lower number of quantifiable phenolics (21 compounds), the main amount corresponding to a ferulic acid derivative (36) (Table 5).

Betalains were not quantified due to the lack of commercially available, pure reference standards (i.e, the purity of the Sigma-Aldrich's betanin and that of other chemical providers was not enough to use them as reliable analytical standards, to our concern).

3.3. Chemometric classification

Principal component analysis (PCA) was used to better understand the relationships among different botanical parts and cultivars of the species *Opuntia ficus-indica* in terms of (poly)phenolic composition. Only quantified phenolic compounds (reported in Supplementary Table S1) were taken into account for the PCA.

Two principal components (PCs) were able to explain 61.3% of the total variability. The first PC (PC1), representing 39.3% of the total variance, was positively linked to isorhamnetin derivatives (20, 22, 26, 31, 32, 37), quercetin derivatives (15, 16, 21, 24, 27), kaempferol derivatives (18, 28), and a ferulic acid derivative (7) (Fig. 2A), while negatively associated with compounds 10 and 36. PC2 accounted for 22% of the total variance and it was positively correlated with compounds 9, 12, 23, 25, 29, 30, and 38, while it was inversely correlated to compounds 17 and 34 (Fig. 2A).

Sample scores for each PC accounted mostly for the similarities among cultivars and the differences among botanical parts (Fig. 2B). All cultivars presented a similar negative PC1 value for the pulp (low

Table 1
Retention time (RT) and characteristic MS ions of phytochemical compounds identified in different *Opuntia ficus-indica* cultivars and botanical parts.

Id.	Compounds	RT (min)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)[b]	MS^3 (*m/z*)[b]
1	L-Malic acid	1.32	133[a]	115 (100), 87 (10)	71 (100), 115 (20)
2	Citric acid	1.51	191	111 (100), 173 (40)	111 (100), 67 (25)
3	Protocatechuic acid-hexoside[c]	1.92	315	153 (100)	109 (100)
4	Caffeic acid-hexoside[c]	2.69	341	179 (100), 161 (20), 135 (5)	135 (100)
5	Guaiacyl(8-*O*-4)ferulic acid[c]	2.80	389	343 (100)	139 (100), 283 (50), 223 (45)
6	Myricetin-hexoside[c]	3.97	479	317 (100)	179 (100), 151 (45)
7	Ferulic acid derivative	4.10	517	193 (100), 337 (60), 175 (50)	149 (100), 134 (55), 178 (40)
8	Piscidic acid	4.18	255	165 (100), 193 (30), 221 (20)	135 (100), 107 (60), 147 (40)
9	Ferulic acid-hexoside	4.26	355	193 (100), 217 (30), 175 (20)	134 (100), 149 (90), 178 (40)
10	Guaiacyl(t8-*O*-4)guaiacyl-hexoside[c]	4.38	537	375 (100)	327 (100), 195 (50), 179 (20)
11	Salicylic acid-hexoside[c]	4.42	299	137 (100)	93 (100), 137 (50)
12	Sinapic acid-hexoside[c]	4.47	385	223 (100)	179 (100), 153 (75), 205 (70), 161 (30)
13	Quercetin-malonyl-hexoside[c]	4.51	549	505 (100), 356 (40), 461 (20)	356 (100), 461 (20)
14	Ferulic acid-*C*-hexoside[c]	4.78	355	265 (100), 235 (90), 295 (70), 193 (50)	193 (100), 149 (10)
15	Quercetin-rhamnose-hexoside-rhamnose[c]	4.84	755	300 (100), 591 (60), 489 (40)	271 (100), 255 (40), 179 (20), 151 (15)
16	Rutin-pentoside[c]	4.90	741	300 (100), 591 (80), 609 (50), 475 (45)	271 (100), 255 (60), 179 (25), 151 (20)
17	Syrinigyl(t8-*O*-4)guaiacyl[c]	5.03	613	405 (100), 567 (20)	357 (100), 195 (70), 209 (60)
18	Kaempferol-di-rhamnose-hexoside[c]	5.18	739	575 (100), 285(60), 393 (20)	339 (100)
19	Dihydrosinapic acid-hexoside isomer[c]	5.20	387	225 (100)	151 (100)
20	Isorhamnetin- rhamnose-rutinoside	5.25	769	315 (100), 605 (80)	300 (100)
21	Quercetin-hexoside-pentoside[c]	5.30	595	300 (100), 445 (20), 475 (15)	271 (100), 255 (70), 179 (30), 151 (20)
22	Isorhamnetin derivative	5.35	755	315 (100), 605 (90), 300 (35), 623 (25)	300 (100)
23	Dihydrosinapic acid hexoside[c]	5.68	387	255 (100)	
24	Quercetin-3-*O*-rutinoside (rutin)	5.70	609	301 (100)	179 (100), 151 (60)
25	Secoisolariciresinol-hexoside[c]	5.71	523	388 (100), 243 (15)	361 (100)
26	Isorhamnetin derivative	5.75	609	315 (100), 459 (20), 300 (15)	300 (100)
27	Quercetin-hexoside	5.80	463	301 (100)	179 (100), 151 (60), 257 (20)
28	Kaempferol-rutinoside	5.98	593	285 (100)	257 (100), 267 (80), 229 (59), 241 (50)
29	Syringaresinol[c]	6.00	417	181 (100), 402 (40), 166 (35)	166 (100)
30	Naringenin-hexoside[c]	6.02	433	415 (100)	271 (100)
31	Isorhamnetin-rutinoside	6.09	623	315 (100), 300 (20)	300 (100)
32	Isorhamnetin-*C*-hexoside	6.31	477	314 (100), 315 (70), 357 (20), 449 (10)	300 (100), 285 (80), 271 (50)
33	Naringin[c]	6.33	579	459 (100), 271 (30)	357 (100), 235 (80), 271 (75), 441 (60)
34	Guaiacyl(8-*O*-4)syrinigyl(8–8)guaiacyl-hexoside[c]	6.38	745	583 (100)	535 (100), 369 (50), 357 (30)
35	Eucomic acid	7.09	239	179 (100), 149 (80), 221 (20)	107 (100), 151 (20)
36	Feruloyl derivative	7.15	562	337 (100), 386 (80)	193 (100), 175 (90)
37	Isorhamnetin pentoside[c]	7.47	447	315 (100)	161 (100)
38	Trihydroxy-methoxy-flavonol[c]	8.55	315	300 (100)	271 (100), 255 (50)
Id.	Compounds	RT (min)	$[M]^+$ (*m/z*)	MS^2 (*m/z*)	MS^3 (*m/z*)
39	Betanin	8.22	551	389 (100)	345 (100), 150 (50), 194 (40)
40	Proline-betaxanthin	8.37	309	265 (100), 263 (90)	221 (100), 152 (40)
41	Isobetanin	8.66	551	389 (100)	345 (100), 150 (50), 194 (40)

[a] MS ions in bold were those subjected to further MS fragmentation.
[b] Abundance relative of each fragment ions is reported in brackets. Compounds 1–38 were identified in negative ionization mode, while compounds 39–41 were detected in positive mode. RT, retention time.
[c] Compounds (tentatively) identified for the first time in *Opuntia ficus-indica*.

content in flavonoids, rich in lignans), differing only in their scores for PC2: "Fresa", "NT", "NA", "NO", and "NE" cultivars formed a sub-cluster with negative scores for PC2, while "Nalle" had positive PC2 values (higher content in phenolic acids). For the skin samples, all cultivars displayed neutral scores for PC1 and positive scores for PC2 (medium content in most of the phenolic compounds). "Nalle" cultivar was the skin sample showing a higher value for PC2, characterised by a high content of sinapic acid-hexoside (12), dihydrosinapic acid-hexoside (23) and secoisolariciresinol-hexoside (25). Most of the cladodes presented similar values for both PCs, although old cladodes had slightly lower PC1 and PC2 scores than young ones. In this sense, young cladodes exhibited a higher flavonol content than old cladodes. Nevertheless, some samples showed very high positive scores for PC1, accounting for high concentrations of quercetin and isorhamnetin derivatives, which was the case for the old cladodes of "NE" cultivar and the young cladodes of "Fresa".

4. Discussion

This work investigated the phytochemical profile of four different botanical parts of six prickly pear cultivars by using two complementary MS experimental conditions. Although some accurate works have been reported in the literature (Guevara-Figueroa et al., 2010; Mata et al., 2016; Moussa-Ayoub et al., 2014; Serra et al., 2013; Yeddes et al., 2014), this challenging study provided an exhaustive characterization of the phytochemical profile (betalains, flavonols, flavanones, phenolic acids, lignans, and organic acids) of the aerial parts of *Opuntia ficus-indica*. Obviously, the range of molecules present in prickly pear phytochemical pool comprises way > 41 structures, but these may be considered those contributing to a better extent to the definition of its phytochemical fingerprinting, regardless of genotypic differences. From a methodological point of view, this work also reinforces the need for versatile, high-throughput experimental conditions allowing the identification of several groups of bioactives (Filigenzi et al., 2011; Mena et al., 2012; Mena et al., 2016; Rak, Fodor, & Abrankó, 2010).

While the role of betalains as some of the most interesting phytochemicals in *Opuntia* genera has been widely discussed for pigmented cultivars during the latest years (Cejudo-Bastante, Chaalal, Louaileche, Parrado, & Heredia, 2014; Mata et al., 2016; Stintzing et al., 2005), the (poly)phenolic profile of prickly pear has been scarcely assessed. It is

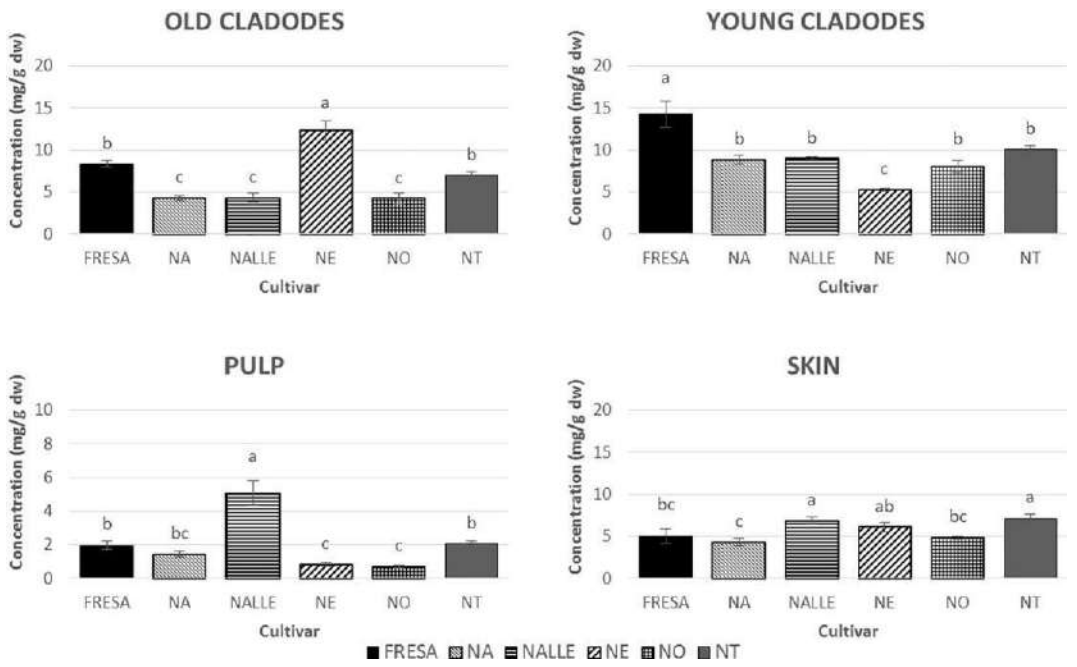

Fig. 1. Total (poly)phenolic content of the different aerial parts of prickly pear for different cultivars, obtained as the sum of individual phenolics. Letters above bars denote significant differences at $p < 0.05$.

Table 2
Concentration (mg/g dw) of (poly)phenolic compounds in young cladodes of six cultivars of *Opuntia ficus-indica*.

Id.	Compounds	FRESA	NA	NALLE	NE	NO	NT
3	Protocatechuic acid-hexoside	0.09 ± 0.03 a	0.03 ± 0.01 b	0.05 ± 0.02 ab	0.02 ± 0.00 b	0.07 ± 0.01 ab	0.03 ± 0.00 b
6	Myricetin-hexoside	4.27 ± 0.43 a	2.66 ± 0.33 b	4.71 ± 0.26 a	0.03 ± 0.00 c	3.38 ± 0.23 b	3.21 ± 0.18 b
7	Ferulic acid derivative	0.36 ± 0.04 ab	0.36 ± 0.03 ab	0.37 ± 0.01 a	0.13 ± 0.03 c	0.27 ± 0.03 b	0.29 ± 0.02 ab
9	Ferulic acid-hexoside	0.86 ± 0.10 ab	1.19 ± 0.13 a	0.65 ± 0.06 bc	0.31 ± 0.16 c	0.81 ± 0.10 b	0.96 ± 0.11 ab
12	Sinapic acid-hexoside	0.17 ± 0.01 b	0.06 ± 0.02 cd	0.02 ± 0.01 d	0.47 ± 0.03 a	0.02 ± 0.02 d	0.10 ± 0.02 c
15	Quercetin-rhamnose-hexoside-rhamnose	0.15 ± 0.01 a	0.09 ± 0.01 b	0.04 ± 0.00 c	0.05 ± 0.01 c	0.04 ± 0.01 c	0.08 ± 0.01 b
16	Rutin-pentoside	0.10 ± 0.02 a	0.06 ± 0.03 ab	0.03 ± 0.01 b	0.08 ± 0.02 ab	0.04 ± 0.01 b	0.09 ± 0.00 ab
17	Syrinigyl(t8-*O*-4)guaiacyl	0.15 ± 0.02 a	0.06 ± 0.01 cd	0.10 ± 0.03 bc	0.03 ± 0.00 d	0.12 ± 0.02 ab	0.03 ± 0.01 d
18	Kaempferol-di-rhamnose-hexoside	0.47 ± 0.13 ab	0.34 ± 0.02 ab	0.49 ± 0.08 ab	0.08 ± 0.02 c	0.53 ± 0.07 a	0.31 ± 0.05 b
20	Isorhamnetin- rhamnose-rutinoside	0.82 ± 0.06 a	0.58 ± 0.07 b	0.20 ± 0.02 c	0.58 ± 0.10 b	0.29 ± 0.06 c	1.00 ± 0.12 a
21	Quercetin-hexoside-pentoside	0.12 ± 0.02 a	0.06 ± 0.01 b	0.03 ± 0.01 b	0.05 ± 0.00 b	0.03 ± 0.01 b	0.04 ± 0.00 b
22	Isorhamnetin derivative	0.62 ± 0.04 ab	0.39 ± 0.07 bc	0.20 ± 0.02 c	0.75 ± 0.19 a	0.29 ± 0.06 c	0.84 ± 0.08 a
23	Dihydrosinapic acid hexoside	0.11 ± 0.01 b	0.06 ± 0.01 c	0.04 ± 0.00 cd	0.16 ± 0.01 a	0.02 ± 0.00 d	0.07 ± 0.01 c
24	Quercetin-3-*O*-rutinoside (rutin)	1.80 ± 0.29 a	0.61 ± 0.23 b	0.41 ± 0.09 b	0.21 ± 0.04 b	0.46 ± 0.06 b	0.40 ± 0.03 b
25	Secoisolariciresinol-hexoside	–	–	0.02 ± 0.00 a	–	–	0.01 ± 0.00 b
26	Isorhamnetin derivative	0.43 ± 0.06 b	0.31 ± 0.04 bc	0.17 ± 0.03 c	0.62 ± 0.01 a	0.23 ± 0.02 c	0.64 ± 0.09 a
27	Quercetin-hexoside	1.02 ± 0.62 a	0.57 ± 0.15 ab	0.28 ± 0.06 ab	0.06 ± 0.01 b	0.29 ± 0.03 ab	0.23 ± 0.03 b
28	Kaempferol-rutinoside	0.77 ± 0.07 a	0.23 ± 0.03 c	0.46 ± 0.01 b	0.22 ± 0.03 c	0.41 ± 0.03 b	0.43 ± 0.00 b
29	Syringaresinol	0.17 ± 0.02 a	0.03 ± 0.01 b	0.05 ± 0.01 b	0.03 ± 0.01 b	0.04 ± 0.00 b	0.04 ± 0.01 b
30	Naringenin-hexoside	0.05 ± 0.01 a	0.05 ± 0.01 ab	0.03 ± 0.01 c	0.05 ± 0.01 ab	0.03 ± 0.01 bc	0.03 ± 0.00 bc
31	Isorhamnetin-rutinoside	0.94 ± 0.05 b	0.56 ± 0.11 c	0.31 ± 0.01 d	1.22 ± 0.10 a	0.40 ± 0.08 cd	0.93 ± 0.08 b
32	Isorhamnetin-*C*-hexoside	0.61 ± 0.08 a	0.46 ± 0.06 b	0.24 ± 0.03 c	0.07 ± 0.01 d	0.25 ± 0.06 c	0.19 ± 0.02 cd
33	Naringin	0.04 ± 0.01 ab	0.03 ± 0.01 b	0.05 ± 0.00 a	0.03 ± 0.00 b	0.01 ± 0.00 c	0.03 ± 0.01 ab
34	Guaiacyl(8-*O*-4)syrinigyl(8–8)guaiacyl-hexoside	0.05 ± 0.01 a	0.03 ± 0.00 ab	0.03 ± 0.00 ab	0.02 ± 0.00 b	0.02 ± 0.01 ab	0.01 ± 0.00 b
37	Isorhamnetin pentoside	0.08 ± 0.01 a	–	–	–	–	0.05 ± 0.00 b
38	Trihydroxy-methoxy-flavonol	0.05 ± 0.01 a	0.02 ± 0.00 bcd	0.02 ± 0.01 bc	0.01 ± 0.00 cd	0.01 ± 0.00 d	0.03 ± 0.01 ab

Values are presented as means ± SD (n = 3). Different letters within a raw indicate significant differences at $p < 0.05$ according to Tukey's test.

known that the concentration of (poly)phenolic compounds in prickly pear depends on genetic and environmental conditions, as well as the part of the cactus plant taken into consideration (Khatabi, Hanine, Elothmani, & Hasib, 2016;Moussa-Ayoub et al., 2014; Stintzing et al., 2005). The study of the (poly)phenolic composition of different parts of *Opuntia ficus-indica* had been previously addressed (Moussa-Ayoub et al., 2014; Yeddes et al., 2014). The effect of genotypic differences on the (poly)phenolic profile of prickly pear fruits had also been investigated (Moussa-Ayoub et al., 2014; Stintzing et al., 2005). However, there is a limited knowledge on the (poly)phenolic composition of both edible and residual parts of *Opuntia* taking into account genotypic characteristics (Moussa-Ayoub et al., 2014). This work provides novel insights in this regard, with data for individual phenolics on the basis of different botanical parts and genotypes grown under the same environmental conditions. This information may be used as starting point for the development of prickly pear-derived products with high levels of (poly)phenolic compounds, as well as for botanical purposes. In addition, the understanding of the phytochemistry of the aerial parts of prickly pear may favour an integrated exploitation of cactus orchards.

The importance of assessing the (poly)phenolic content of prickly

Table 3
Concentration (mg/g dw) of (poly)phenolic compounds in old cladodes of six cultivars of *Opuntia ficus-indica*.

Id.	Compounds	FRESA	NA	NALLE	NE	NO	NT
3	Protocatechuic acid-hexoside	0.02 ± 0.01 b	0.02 ± 0.00 b	0.06 ± 0.01 a	0.03 ± 0.01 b	0.03 ± 0.00 b	0.01 ± 0.02 b
6	Myricetin-hexoside	0.76 ± 0.13 b	0.03 ± 0.00 d	0.61 ± 0.12 bc	2.43 ± 0.06 a	0.39 ± 0.09 c	0.79 ± 0.10 b
7	Ferulic acid derivative	0.33 ± 0.01 a	0.20 ± 0.09 b	0.37 ± 0.04 a	0.33 ± 0.03 a	0.28 ± 0.04 b	0.37 ± 0.02 a
9	Ferulic acid-hexoside	1.82 ± 0.16 a	1.13 ± 0.21 bc	1.27 ± 0.16 b	0.81 ± 0.12 cd	0.41 ± 0.05 e	0.50 ± 0.04 de
12	Sinapic acid-hexoside	0.30 ± 0.05 ab	0.32 ± 0.07 a	0.11 ± 0.02 cd	0.06 ± 0.01 d	0.30 ± 0.04 ab	0.19 ± 0.02 bc
15	Quercetin-rhamnose-hexoside-rhamnose	0.07 ± 0.01 ab	0.04 ± 0.01 bc	0.02 ± 0.01 c	0.07 ± 0.01 a	0.03 ± 0.01 c	0.07 ± 0.01 ab
16	Rutin-pentoside	0.07 ± 0.01 a	0.03 ± 0.01 b	0.02 ± 0.01 b	0.06 ± 0.01 a	0.03 ± 0.00 b	0.08 ± 0.01 a
17	Syrinigyl(t8-*O*-4)guaiacyl	0.03 ± 0.00 b	0.02 ± 0.01 b	0.02 ± 0.00 b	0.21 ± 0.06 a	0.02 ± 0.00 b	0.02 ± 0.00 b
18	Kaempferol-di-rhamnose-hexoside	0.10 ± 0.02 bc	0.05 ± 0.01 bc	0.04 ± 0.02 c	0.38 ± 0.08 a	0.15 ± 0.02 b	0.10 ± 0.01 bc
20	Isorhamnetin- rhamnose-rutinoside	1.08 ± 0.18 a	0.54 ± 0.10 b	0.38 ± 0.07 b	0.35 ± 0.04 b	0.48 ± 0.06 b	1.16 ± 0.10 a
21	Quercetin-hexoside-pentoside	0.04 ± 0.01 bc	0.03 ± 0.00 bc	0.02 ± 0.01 c	0.06 ± 0.00 a	0.02 ± 0.00 c	0.04 ± 0.00 ab
22	Isorhamnetin derivative	0.76 ± 0.12 b	0.43 ± 0.07 c	0.40 ± 0.26 c	0.38 ± 0.08 a	0.40 ± 0.09 c	0.89 ± 0.06 bc
23	Dihydrosinapic acid hexoside	0.16 ± 0.03 bc	0.11 ± 0.01 b	0.28 ± 0.07 a	–	0.21 ± 0.04 ab	0.11 ± 0.01 b
24	Quercetin-3-*O*-rutinoside (rutin)	0.34 ± 0.05 b	0.09 ± 0.02 c	0.05 ± 0.05 c	1.66 ± 0.16 a	0.10 ± 0.01 c	0.15 ± 0.00 bc
25	Secoisolariciresinol-hexoside	–	–	0.01 ± 0.00 a	–	0.01 ± 0.00 a	0.01 ± 0.00 a
26	Isorhamnetin derivative	0.62 ± 0.07 b	0.42 ± 0.02 b	0.30 ± 0.04 b	1.88 ± 0.45 a	0.29 ± 0.04 b	0.74 ± 0.09 b
27	Quercetin-hexoside	0.22 ± 0.04 b	0.04 ± 0.02 b	0.01 ± 0.00 b	1.61 ± 0.29 a	0.04 ± 0.00 b	0.05 ± 0.00 b
28	Kaempferol-rutinoside	0.15 ± 0.04 bc	0.05 ± 0.01 c	0.07 ± 0.01 c	0.75 ± 0.08 a	0.20 ± 0.02 b	0.23 ± 0.01 b
29	Syringaresinol	0.06 ± 0.02 a	–	0.04 ± 0.01 ab	–	0.03 ± 0.01 b	–
30	Naringenin-hexoside	0.06 ± 0.02 a	0.02 ± 0.03 abc	–	0.01 ± 0.00 c	0.01 ± 0.00 bc	0.04 ± 0.01 ab
31	Isorhamnetin-rutinoside	1.19 ± 0.13 a	0.66 ± 0.08 c	0.16 ± 0.03 c	0.73 ± 0.06 b	0.72 ± 0.13 b	1.27 ± 0.12 a
32	Isorhamnetin-*C*-hexoside	0.09 ± 0.03 b	0.03 ± 0.01 bc	0.01 ± 0.00 c	0.50 ± 0.07 a	0.01 ± 0.00 bc	0.08 ± 0.02 bc
33	Naringin	0.03 ± 0.01 a	0.03 ± 0.00 ab	0.02 ± 0.01 ab	0.01 ± 0.00 ab	0.02 ± 0.00 ab	0.03 ± 0.00 a
34	Guaiacyl(8-*O*-4)syrinigyl(8–8)guaiacyl-hexoside	0.03 ± 0.00 a	0.02 ± 0.01 a	0.04 ± 0.02 a	0.03 ± 0.01 a	0.03 ± 0.01 a	0.02 ± 0.00 a
38	Trihydroxy-methoxy-flavonol	0.02 ± 0.00 a	0.01 ± 0.00 a	0.02 ± 0.02 a	0.03 ± 0.01 a	0.02 ± 0.01 a	0.03 ± 0.01 a

Values are presented as means ± SD (n = 3). Different letters within a raw indicate significant differences at $p < 0.05$ according to Tukey's test.

Table 4
Concentration (mg/g dw) of (poly)phenolic compounds in fruit skin of six cultivars of *Opuntia ficus-indica*.

Id.	Compounds	FRESA	NA	NALLE	NE	NO	NT
3	Protocatechuic acid-hexoside	0.01 ± 0.00 b	0.03 ± 0.01 ab	0.08 ± 0.04 a	0.02 ± 0.00 ab	0.07 ± 0.02 a	0.02 ± 0.001 ab
6	Myricetin-hexoside	0.02 ± 0.00 c	0.01 ± 0.00 c	0.03 ± 0.01 c	0.01 ± 0.00 c	0.08 ± 0.02 b	0.56 ± 0.04 a
7	Ferulic acid derivative	0.23 ± 0.06 b	0.15 ± 0.03 b	0.37 ± 0.07 a	0.23 ± 0.02 b	0.23 ± 0.03 b	0.39 ± 0.02 a
9	Ferulic acid-hexoside	1.55 ± 0.22 ab	1.03 ± 0.15 bc	1.03 ± 0.32 bc	0.82 ± 0.20 c	1.16 ± 0.15 bc	1.81 ± 0.28 a
10	Guaiacyl(t8-*O*-4)guaiacyl-hexoside	–	–	–	–	–	0.02 ± 0.00 a
12	Sinapic acid-hexoside	0.47 ± 0.08 b	0.62 ± 0.13 b	1.72 ± 0.41 a	0.81 ± 0.11 b	0.64 ± 0.08 b	0.47 ± 0.09 b
15	Quercetin-rhamnose-hexoside-rhamnose	0.03 ± 0.01 ab	0.02 ± 0.00 ab	0.01 ± 0.00 b	0.02 ± 0.00 ab	0.01 ± 0.00 b	0.03 ± 0.01 a
16	Rutin-pentoside	0.04 ± 0.02 abc	0.02 ± 0.01 c	0.03 ± 0.00 bc	0.06 ± 0.01 a	0.02 ± 0.00 c	0.05 ± 0.01 ab
17	Syrinigyl(t8-*O*-4)guaiacyl	0.03 ± 0.01 a	0.01 ± 0.00 bc	0.03 ± 0.00 ab	0.03 ± 0.00 a	–	0.03 ± 0.00 a
18	Kaempferol-di-rhamnose-hexoside	0.01 ± 0.00 ab	0.01 ± 0.00 b	0.02 ± 0.00 a	0.02 ± 0.00 ab	0.03 ± 0.00 a	0.02 ± 0.00 ab
20	Isorhamnetin- rhamnose-rutinoside	0.45 ± 0.08 ab	0.28 ± 0.05 bc	0.26 ± 0.04 bc	0.34 ± 0.03 bc	0.23 ± 0.01 c	0.61 ± 0.15 a
21	Quercetin-hexoside-pentoside	0.02 ± 0.01 b	0.02 ± 0.00 ab	0.02 ± 0.00 b	0.04 ± 0.01 a	0.01 ± 0.00 b	0.02 ± 0.01 b
22	Isorhamnetin derivative	0.42 ± 0.07 bc	0.31 ± 0.08 b	0.44 ± 0.03 bc	0.72 ± 0.07 a	0.38 ± 0.02 b	0.65 ± 0.15 ab
23	Dihydrosinapic acid hexoside	0.35 ± 0.08 c	0.55 ± 0.09 cd	1.16 ± 0.16 a	0.93 ± 0.11 ab	0.66 ± 0.08 bc	0.54 ± 0.13 cd
24	Quercetin-3-*O*-rutinoside (rutin)	0.10 ± 0.01 bc	0.10 ± 0.02 bc	0.06 ± 0.01 c	0.16 ± 0.04 ab	0.08 ± 0.01 c	0.18 ± 0.03 a
25	Secoisolariciresinol-hexoside	–	0.03 ± 0.00 bc	0.13 ± 0.04 a	0.02 ± 0.00 c	0.08 ± 0.01 b	–
26	Isorhamnetin derivative	0.30 ± 0.06 abc	0.27 ± 0.05 bc	0.33 ± 0.04 abc	0.49 ± 0.09 a	0.21 ± 0.03 c	0.44 ± 0.11 ab
27	Quercetin-hexoside	0.07 ± 0.02 a	0.04 ± 0.01 bc	0.02 ± 0.00 c	0.06 ± 0.00 ab	0.02 ± 0.01 c	0.08 ± 0.01 a
28	Kaempferol-rutinoside	0.04 ± 0.01 bc	0.02 ± 0.00 c	0.06 ± 0.01 ab	0.05 ± 0.00 b	0.07 ± 0.01 a	0.06 ± 0.01 ab
29	Syringaresinol	0.20 ± 0.03 a	0.11 ± 0.02 b	0.13 ± 0.04 b	0.13 ± 0.00 b	0.12 ± 0.01 b	0.24 ± 0.02 a
30	Naringenin-hexoside	0.06 ± 0.02 ab	0.02 ± 0.01 b	0.18 ± 0.05 a	0.12 ± 0.01 ab	0.07 ± 0.01 ab	0.07 ± 0.01 ab
31	Isorhamnetin-rutinoside	0.53 ± 0.12 b	0.53 ± 0.10 b	0.61 ± 0.04 ab	0.85 ± 0.19 a	0.58 ± 0.03 ab	0.75 ± 0.11 ab
32	Isorhamnetin-*C*-hexoside	0.03 ± 0.01 a	0.01 ± 0.00 b	–	0.04 ± 0.01 a	0.02 ± 0.00 b	0.01 ± 0.00 bc
33	Naringin	0.02 ± 0.00 c	0.03 ± 0.01 abc	0.04 ± 0.01 a	0.03 ± 0.00 abc	0.01 ± 0.00 bc	0.03 ± 0.00 ab
34	Guaiacyl(8-*O*-4)syrinigyl(8–8)guaiacyl-hexoside	0.01 ± 0.00	0.03 ± 0.02 a	0.01 ± 0.00 b	0.02 ± 0.01 ab	0.03 ± 0.00 a	0.01 ± 0.01 b
38	Trihydroxy-methoxy-flavonol	0.05 ± 0.01 b	0.06 ± 0.01 b	0.11 ± 0.02 a	0.11 ± 0.01 a	0.05 ± 0.02 b	0.05 ± 0.01 b

Values are presented as means ± SD (n = 3). Different letters within a raw indicate significant differences at $p < 0.05$ according to Tukey's test.

pear fruit pulp is due to their use as edible plants for humans. Since prickly pear fruits are rich in a series of flavonoids and phenolic acids with proven bioactivities (Del Rio et al., 2013; Rodriguez-Mateos et al., 2014; Zanotti et al., 2015), data on their actual content are key to further explore the biological prospects of prickly pear fruit consumption on human health. The content in (poly)phenolic compounds of the six cultivars was similar in line with previous reports on *Opuntia* fruits (Moussa-Ayoub et al., 2014; Yeddes et al., 2014), but slightly lower than those recently reported for this same plant material by using a colorimetric method (Andreu et al., 2018). In terms of individual phenolics, the presence of phenolic acids in juice made from pulp has been confirmed (Mata et al., 2016). Regarding flavonols, while some authors have identified a few isorhamnetin derivatives in the pulp of *Opuntia ficus-indica* fruits (Kuti, 2004; Yeddes et al., 2014), others have reported a lack of flavonols in pulp (Moussa-Ayoub et al., 2014). The present characterization accounted for the presence of up to 9 flavonols, as well as several other phenolic scaffolds, in the pulp of prickly pear fruits, which represent a step forward in the definition of the bioactives contained in the main edible part of this plant. Although these inconsistencies in the flavonoid profile of prickly pear pulp might be

Table 5
Concentration (mg/g dw) of (poly)phenolic compounds in fruit pulp of six cultivars of *Opuntia ficus-indica*.

Id.	Compounds	FRESA	NA	NALLE	NE	NO	NT
3	Protocatechuic acid-hexoside	0.02 ± 0.00 bc	0.01 ± 0.00 c	0.08 ± 0.02 a	0.02 ± 0.00 bc	0.02 ± 0.01 bc	0.03 ± 0.00 b
6	Myricetin-hexoside	–	–	–	–	–	0.01 ± 0.00 a
7	Ferulic acid derivative	0.08 ± 0.02	–	–	–	–	–
9	Ferulic acid-hexoside	0.14 ± 0.03 a	0.02 ± 0.02 b	0.06 ± 0.00 b	0.02 ± 0.00 b	0.05 ± 0.01 b	0.15 ± 0.03 a
10	Guaiacyl(t8-*O*-4)guaiacyl-hexoside	0.19 ± 0.02 b	0.19 ± 0.01 b	0.10 ± 0.00 d	0.18 ± 0.02 bc	0.14 ± 0.03 cd	0.33 ± 0.02 a
12	Sinapic acid-hexoside	0.10 ± 0.01 b	0.21 ± 0.05 b	1.71 ± 0.36 a	0.06 ± 0.01 b	0.06 ± 0.01 b	0.10 ± 0.02 b
17	Syrinigyl(t8-*O*-4)guaiacyl	0.13 ± 0.04 ab	0.12 ± 0.01 b	0.08 ± 0.01 c	0.07 ± 0.02 c	0.06 ± 0.01 c	0.17 ± 0.01 a
20	Isorhamnetin- rhamnose-rutinoside	0.01 ± 0.00 a	–	–	–	–	0.01 ± 0.00 a
21	Quercetin-hexoside-pentoside	0.01 ± 0.00 a	–	–	0.01 ± 0.00 a	–	–
22	Isorhamnetin derivative	–	–	0.01 ± 0.00 a	–	–	0.01 ± 0.00 b
23	Dihydrosinapic acid hexoside	–	–	2.39 ± 0.28 a	–	0.12 ± 0.01 b	–
25	Secoisolariciresinol-hexoside	–	–	0.10 ± 0.02	–	–	–
26	Isorhamnetin derivative	0.02 ± 0.00 a	0.01 ± 0.00 a	0.02 ± 0.00 a	0.02 ± 0.00	0.01 ± 0.00 a	0.02 ± 0.00 a
27	Quercetin-hexoside	0.01 ± 0.01 a	–	–	0.01 ± 0.00 a	–	–
29	Syringaresinol	0.07 ± 0.01 b	0.02 ± 0.00 cd	0.13 ± 0.03 a	0.02 ± 0.01 d	0.06 ± 0.01 bc	0.06 ± 0.01 bcd
30	Naringenin-hexoside	–	–	0.21 ± 0.04	–	–	–
31	Isorhamnetin-rutinoside	0.02 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.01 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a
33	Naringin	0.04 ± 0.01 ab	0.03 ± 0.00 bc	0.05 ± 0.01 a	0.01 ± 0.00 c	0.02 ± 0.00 bc	0.03 ± 0.00 ab
34	Guaiacyl(8-*O*-4)syrinigyl(8–8)guaiacyl-hexoside	0.16 ± 0.03 a	0.12 ± 0.01 ab	0.05 ± 0.01 b	0.13 ± 0.03 ab	0.04 ± 0.01 b	0.08 ± 0.08 ab
36	Feruloyl derivative	0.96 ± 0.07 a	0.7 ± 0.14 b	0.08 ± 0.01 c	0.28 ± 0.03 c	0.11 ± 0.01 c	1.06 ± 0.19 a
38	Trihydroxy-methoxy-flavonol	–	–	0.01 ± 0.00	–	–	–

Values are presented as means ± SD (n = 3). Different letters within a raw indicate significant differences at $p < 0.05$ according to Tukey's test.

attributed to geographic and genotypic differences, they could likely be due to the sensitivity and accuracy of the methodological approaches used.

A higher amount of (poly)phenolic compounds has been reported for fruit skin than for fruit pulp (Moussa-Ayoub et al., 2014; Yeddes et al., 2014), in agreement with our results. Important quantitative differences among cultivars were not found. This similarity among cultivars has also been shown for cultivars grown in different countries in terms of flavonol content (Moussa-Ayoub et al., 2014). So far, the (poly)phenolic profile of fruit skins was restricted mainly to flavonols and some phenolic acids (Fernández-López et al., 2010; Kuti, 2004; Mata et al., 2016; Moussa-Ayoub et al., 2014; Serra et al., 2013; Stintzing et al., 2005; Yeddes et al., 2014), while the present work extends the number of molecules present in this fruit part. Skins, which are usually a waste product, represent a potential source of bioactive compounds that may increase the amounts of (poly)phenolic compounds if used for juice elaboration together with the pulp (Fernández-López et al., 2010; Serra et al., 2013). Considering its phytochemical content not only in phenolics but also in betalains (Stintzing et al., 2005), prickly pear fruit skin may also be industrialized for the development of sustainable alternatives allowing the exploitation of their bioactives as nutraceuticals (Matias et al., 2014; Serra et al., 2013). This would minimize production by-products and might generate profits from a by-product generally lacking economic value.

Cladodes were rich in (poly)phenolic compounds. The (poly)phenolic profile of cladodes had been previously reported to comprise flavonols and phenolic acids (Guevara-Figueroa et al., 2010; Msaddak et al., 2017). The newly-described presence of flavanones and lignans increases the number of bioactive compounds in cladodes and, thus, its interest for human health. Young cladodes exhibited a higher content in (poly)phenolic compounds when compared to their older counterparts, which may be explained by changes in the physiology of the cladode as a consequence of the age and maturation stage (El-Mostafa et al., 2014; Rodríguez-Garcia et al., 2007). Young cladodes are frequently consumed as a green vegetable in salads, sauces, soups, stews, snacks, beverages and desserts in Mexico and Southern US (Stintzing et al.,

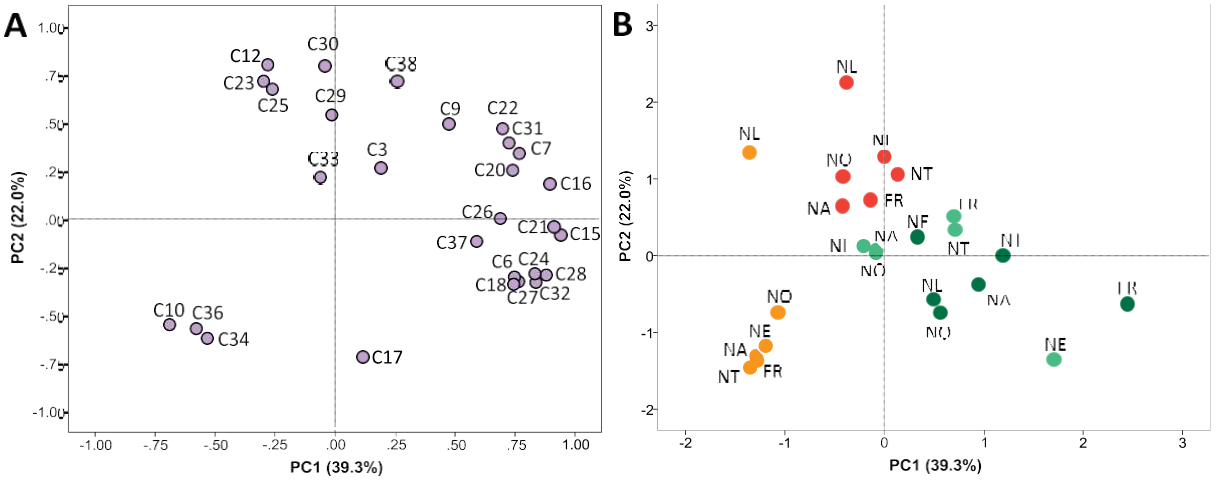

Fig. 2. Principal component analysis of different prickly pear aerial parts for six different Spanish cultivars. A) loading plot of PC1 versus PC2; B) score plot and distribution of the samples in the consensus space. In the loading plot, C# indicates the compound code, as reported in Table 1. Non-quantified compounds (1, 2, 4, 5, 8, 11, 13, 14, 19, 35, and 34–41) were excluded from the analysis. In the score plot, dark green circles correspond to old cladodes, light green ones to young cladodes, red to fruit skin, and orange to fruit pulp. "Fresa" cultivar has been abbreviated as "FR", while "Nalle" as "NL". (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2005). Therefore, considering their (poly)phenolic content, they may contribute to the total intake of (poly)phenolic compounds with the diet. With respect to old cladodes, their use as a valuable source of bioactives compounds or to produce functional products rich in bioactives should be further explored (Msaddak et al., 2017).

From a botanical/evolutionary point of view, the assessment of the (poly)phenolic profile of all the aerial parts of different cultivars of prickly pear represents an important advance in the understanding of *Opuntia* plant biology and defence. Multivariate analysis on prickly pear (poly)phenolic composition accounted for the similarity among cultivars instead of among botanical parts, which may indicate the selective synthesis of phenolic scaffolds in each plant part. Among other ecological roles, this fact could be linked to plant defence mechanisms, where (poly)phenolic compounds play a key role as antibacterial agents and reducing the palatability and nutrient digestibility for herbivores (Salminen & Karonen, 2011).

Even though this work contributes significantly to the identification of bioactive compounds in alternative plant sources, a couple of analytical constraints should be acknowledged. The first one is related to betalains. Although the most representative *Opuntia* betalains were identified only in the red coloured cultivar (Cejudo-Bastante et al., 2013), they were not quantified because of the low purity of the commercially available standard (circa 40%, as stated by the provider). Secondly, an accurate quantification of all the phenolic compounds was not possible due to the unavailability of all their respective reference standards. This led to the semi-quantification of most of the phenolics, which, however, did not impair the conclusions drawn from this study.

5. Conclusions

In summary, this analytical work allowed the characterization of the phytochemical profiles of four botanical parts from six different prickly pear cultivars. Up to 41 compounds, mainly (poly)phenolics, were identified, with 23 of them being reported in *Opuntia ficus-indica* for the first time. Moreover, some insights on plant biology with respect to phenolic distribution were provided. This information may also be used as starting point for the development of prickly pear-derived products with high levels of (poly)phenolic compounds. Lastly, this analytical approach could also be used in other plant products, supposedly rich in phytochemicals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.03.062.

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Original Research Article

Fatty acid profile of fruits (pulp and peel) and cladodes (young and old) of prickly pear [*Opuntia ficus-indica* (L.) Mill.] from six Spanish cultivars

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ABSTRACT

The aim of this research was to determine the fatty acid profile of the botanical parts (young and old cladodes, fruit pulp and peel) of six *Opuntia ficus-indica* (L.) Mill. cultivars grown in Spain to evaluate their potential uses for human nutrition, animal feeding and/or industrial use. Nine fatty acids were identified in fruit peel and young cladodes, eight in fruit pulp, and seven in old cladodes. Linoleic acid (C18:2), which is an essential fatty acid, was the main compound in the profile of the old cladodes, fruit peel and pulp. However, young cladodes had palmitic acid (C16:0) as the major compound. Old cladodes showed higher percentages of monounsaturated and polyunsaturated fatty acids than young cladodes. Polyunsaturated fatty acids were the prevailing group of fatty acids in fruits. These results suggested that prickly pear fruits have a good nutritional profile of fatty acids, rich in monounsaturated and polyunsaturated compounds.

1. Introduction

Opuntia ficus-indica (L.) Mill, usually named prickly pear or nopal cactus, is the Cactateae plant with the greatest economic relevance in the world (Kiesling, 1998). It is a tropical or subtropical plant, original from arid and semiarid regions of America, including Mexico (Pimienta-Barrios, 1994) and cultivated as a significant nutrient and food source (Matthäus and Özcan, 2011). Nowadays, it is grown throughout the American continent, in southern Spain and all over the Mediterranean basin (Sáenz, 2006). Prickly pear is mostly known for its fruits, but its cladodes are consumed as well, mainly in Mexico. They are commonly consumed fresh, but additionally, prickly pear fruits can be consumed dehydrated, as juice concentrates, jams and syrups and as fruit gummies, among others (FAO, Food and Agricultural Organization, 2018). Besides, cladodes can be stored canned and consumed as juices, or stored as dehydrated powder, which has a high content of dietary fiber (FAO, Food and Agricultural Organization, 2018). Furthermore, prickly pear also presents other uses: treatment of hyperglycemia (Basurto et al., 2006; Ramirez, 2015); production of biofuels, specifically bioethanol and biogas (Sánchez-Godoy, 2012; Santos et al., 2016), animal nutrition (Atti et al., 2006; Costa et al., 2010; Urrutia-Morales et al., 2014) and phytoremediation (Bañuelos and Lin, 2010; Escobar-Alvarado et al., 2018; Shedbalkar et al., 2010), among others. Nowadays, prickly pear is generally known due to its antioxidant properties; there are several studies about its phenolic content and antioxidant activity, which may provide potential health benefits (Ammar et al., 2015; Andreu et al., 2018; Butera et al., 2002).

Fatty acids are very important molecules in living organisms because they play different roles: source of energy, structural function, and modulators of physiological functions. They are organic compounds formed by a hydrocarbonated chain and a carboxylic group, which is usually bound to glycerol forming acylglycerides (mono-, di- or triglycerides). Besides, fatty acids can be saturated or unsaturated according to the nature of the hydrocarbonated chain (Mornar et al., 2014). The consumption of monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, respectively) has been reported to provide health benefits at all stages of life and to contribute to ameliorate various health conditions such as obesity, cardiovascular diseases, diabetes mellitus and even some types of cancer (Rodríguez-Cruz et al., 2005; Serra et al., 2013).

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Nevertheless, there are only few published studies on the fatty acid profile of prickly pear (El-Said et al., 2011; Ramadan and Mörsel, 2003a), and basically they studied the FAMEs profile in seed oil (De Wit et al., 2017). The aim of the present study was to determine the fatty acid profile of fruits (pulp and peel) and cladodes (young and old) of six cultivars of prickly pear, all grown in Spain. This information will be used to provide a basis for the selection of the most suitable cultivars for the elaboration of functional products and by-products derived from prickly pear. This is the first study including different parts (fruit peel, fruit pulp, old cladodes and young cladodes) of Spanish prickly pears.

2. Material and methods

2.1. Chemicals and reagents

The reagents used in the laboratory procedures were all HPLC grade: Sigma-Aldrich Chemie GmbH (Steinheim, Germany) provided *n*-hexane, methanol, methylene chloride, and boron trifluoride. Sodium hydroxide and anhydrous sodium sulfate were obtained from Panreac (Castellar de Vallès, Barcelona, Spain).

Peak identification was made by comparing with FAMEs (fatty acids methyl esters) standards from Sigma-Aldrich Chemie GmbH (Steinheim, Germany): lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidic acid (C20:0).

2.2. Plant material and sample processing

Six different cultivars of prickly pear and their respective cladodes and fruits were used for this study. Four of the six cultivars ("NA, "NT", "NE" and "NO") were collected at the experimental field station of Miguel Hernandez University in the province of Alicante, Spain (02°03'50''E, 38°03'50''N, and 25 masl), while the other two cultivars ("FR" and "NJ") were harvested from private farms of Murcia and Alicante, respectively. All these three farms are geographically close, have the same climatic conditions, similar soils and plant material was collected at the same time.

Young (less than one year) and old (more than 2 years old) cladodes, as well as the fruits were harvested during the spring and summer of two consecutive seasons (2015 and 2016). After picking 10 cladodes and 10 fruits per cultivar from 3 different prickly pear plants, the plant materials were transported to the laboratory. Then, the spines were removed by washing cladodes and fruits under running tap water for 2 min while gently brushing them; then, fruits were manually peeled.

Fresh fruits (peel and pulp) and cladodes (young and old) were immediately frozen in liquid nitrogen and freeze-dried in an Alpha 2–4 freeze drier (Christ Alpha 2–4; Braum Biotech) for 24 h under reduced pressure, 0.220 mbar. The temperature in the drying chamber was -25 °C, while the heating plate reached 15 °C. Later, samples were milled until reaching a fine powder and vacuum-packed. Fatty acid extraction-methylation was performed directly on freeze-dried plant material.

2.3. Fatty acids extraction

2.3.1. Methylation procedure

FAMEs where prepared by transmethylation using boron trifluoride (BF_3) catalyst according to ISO 12966-2:2011(ISO, 2011)

2.3.2. Gas chromatography (GC) analysis

Fatty acids methyl esters (FAMEs) were analyzed in a gas chromatogram (GC17A) coupled to a mass spectrometry detector GC–MS QP5050, Shimadzu (Kyoto, Japan) with a SupraWax-280 column, 100% polyethylene glycol (Teknokroma S. Co. Ltd., 165 Barcelona, Spain; 30 m length ×0.25 mm internal diameter ×0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1.1 mL min^{-1}. The temperature program for the oven was as follows: (i) an initial temperature of 80 °C was held for 2 min, (ii) then, increased at a rate of 8.0 °C min^{-1} to 160 °C; (iii) and increased at a rate of 4 °C min^{-1} from 160 to 220 °C and held for 13 min, and (iv) and further increased at a rate of 10 °C min^{-1} from 220 to 260 °C and held for 6 min. Injector and detector temperatures were held at 230 and 260 °C, respectively. Injection volume was 0.5 μL injected at a split radio of 1:10. Identification was made by comparison with the retention time of standards. Analyses were run in triplicate. The ratio S/N for each peak of the chromatogram was calculated and the lowest S/N ratio for a peak was 4, which ensured that peaks were quantified above the LOQ of the equipment (0.01%).

2.4. Indexes calculations

The atherogenic index (AI) and thrombogenic index (TI) were calculated according to the formulas described by Ulbricht and Southgate (1991):

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / [\Sigma MUFA + \Sigma PUFA\ (n\text{-}6)\ and\ (n\text{-}3)]$$

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

2.5. Statistical analysis

One-way analysis of variance (ANOVA) and multiple-range tests were used for samples comparison. The method used to discriminate among the means (multiple range test) was the Fisher's least significant difference procedure. Significance was defined at $p \leq 0.05$. Statistical analysis was performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD).

3. Results

The fatty acids profiles of each cultivar and botanical part of Spanish prickly pear plants are shown in Tables 1–4 and results have been expressed as percentage of the total fatty acid profile. In addition, unsaturation ratio (U/S), index of atherogenicity (AI) and index of thrombogenicity (TI) are also presented. Unsaturation ratio shows the proportion of unsaturated fatty acids in relation to saturated ones. The indexes of atherogenicity and thrombogenicity were defined by Ulbricht and Southgate (1991) and the higher they are, the higher the risk of atherogenicity and thrombogenicity of the dietary fat. The AI index relates the content of fatty acids that increase serum lipids (lauric, myristic and palmitic acids) to the compounds with protective action (MUFAs and PUFAs). Additionally, the TI index relates the content of myristic, palmitic and stearic acids, which have a thrombogenic effect, to that of compounds with protective action (MUFAs and PUFAs). Myristic acid is the most thrombogenic fatty acid, and n-3 PUFAs are the most antithrombogenic compounds, while n-6 PUFAs are the most antiatherogenic acids. Due to this fact, AI and TI are valuable indicators of the potential effect of fats on the prevention of atherosclerosis, thrombosis and cardiovascular health.

3.1. Fatty acids composition of fruit pulp

The pulp of prickly pear is the edible and most valued part of the fruit by humans; eight fatty acids were identified in this matrix (Table 1). The most abundant compounds were linoleic, oleic and palmitic acids. In the case of linoleic acid, its content ranged from 20.19% (NA) to 53.85% (NJ) of the total profile of fatty acids. Concerning oleic acid, its content ranged from 16.93% (FR) to 40.19% (NE), and palmitic acid ranged from 16.41% (NJ) to 29.01% (NA). Myristic and lauric acids were below the detection threshold of the analytical technique used in NJ cultivar, while arachidic acid was only found in fruit pulp of the NT cultivar.

Table 1
Fatty acid composition (% of total fatty acid profile) of *O. ficus-indica* fruit pulp as affected by cultivar.

Fatty acids (%)	Cultivar					
	NT	NO	NE	NA	FR	NJ
Lauric acid (C12:0)	3.92 ± 0.02[1] d	1.13 ± 0.21 b	0.46 ± 0.11 c	2.72 ± 0.11 c	1.06 ± 0.11 b	nd
Myristic acid (C14:0)	1.80 ± 0.06 ab	1.44 ± 0.01 a	1.46 ± 0.08 a	2.55 ± 0.11 c	1.86 ± 0.23 b	nd
Palmitic acid (C16:0)	23.5 ± 0.2 b	22.7 ± 0.7 b	18.2 ± 0.4 a	29.0 ± 0.7 c	27.3 ± 0.3 c	16.4 ± 0.1 a
Palmitoleic acid (C16:1)	nd	nd	nd	nd	nd	nd
Stearic acid (C18:0)	5.68 ± 0.10 c	4.71 ± 0.03 b	3.77 ± 0.14 a	5.80 ± 0.06 c	6.90 ± 0.43 d	4.33 ± 0.09 ab
Oleic acid (C18:1)	21.2 ± 0.3 ab	34.8 ± 3.6 c	40.2 ± 2.4 c	22.0 ± 2.4 ab	16.9 ± 0.2 a	25.4 ± 3.2 b
Linoleic acid (C18:2)	27.0 ± 0.4 b	24.0 ± 0.3 ab	25.2 ± 1.4 b	20.2 ± 1.1 a	25.1 ± 0.6 b	53.9 ± 3.1 c
Linolenic acid (C18:3)	12.34 ± 0.24 a	11.2 ± 2.0 a	10.8 ± 0.4 a	17.8 ± 0.9 b	20.8 ± 0.1 b	nd
Arachidic acid (C20:0)	4.55 ± 0.03	nd	nd	nd	nd	nd
Total MUFA	21.2 ± 0.3 ab	34.8 ± 3.7 c	40.2 ± 2.4 c	22.0 ± 2.4 ab	16.9 ± 0.2 a	25.4 ± 3.2 b
Total PUFA	39.3 ± 0.1 a	35.2 ± 1.8 a	36.0 ± 1.8 a	38.0 ± 2.0 a	46.0 ± 0.5 b	53.9 ± 3.1 c
Total SFA	39.4 ± 0.2 de	30.0 ± 1.9 c	23.9 ± 0.6 b	40.1 ± 0.4 e	37.1 ± 0.2 d	20.7 ± 0.1 a
AI[2]	0.57 ± 0.01 d	0.42 ± 0.03 c	0.32 ± 0.01 b	0.70 ± 0.01 e	0.57 ± 0.02 d	0.21 ± 0.01 a
IT[3]	1.02 ± 0.01 c	0.82 ± 0.07 b	0.61 ± 0.02 a	1.25 ± 0.03 d	0.93 ± 0.03 bc	0.52 ± 0.01 a
U/S[4]	1.54 ± 0.01 a	2.33 ± 0.21 b	3.19 ± 0.10 c	1.49 ± 0.03 a	1.69 ± 0.02 a	3.82 ± 0.03 d

[1] 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 5% significance level. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. nd, not detected.

[2] AI, index of atherogenicity: (C12:0 + 4 x C14:0 + C16:0) / [Σ MUFA + ΣPUFA (n-6) and (n-3)].

[3] AT, index of thrombogenicity: (C14:0 + C16:0 + C18:0)/[0.5 x Σ MUFA + 0.5 x Σ PUFA(n-6) + 3 x Σ PUFA(n-3) + (n-3)/(n-6)].

[4] U/S, unsaturated ratio: (MUFA + PUFA) / SFA.

The pulp of prickly pear fruits had important percentages of MUFAs (26.75%, mean value of all studied cultivars) and PUFAs (41.38%, mean value of all studied cultivars) (Table 5). The cultivar which presented the highest percentage of MUFAs was NE (40.19%), and NJ presented the maximum percentage of PUFAs in fruit pulp (53.85%). Regarding AI and IT, the NJ cultivar showed the lowest values for these two indexes, and also presented the highest value of the U/S ratio; thus, NJ was the cultivar having the most beneficial profile of fatty acids in fruit pulp (Table 1).

3.2. Fatty acid composition of fruit peel

Fruit peels are mainly used for animal feeding. In this case, nine fatty acids were detected (Table 2). The most abundant compounds were linoleic acid, which ranged from 39.58% (NJ) to 52.02% (NT), oleic acid from 6.83% (NA) to 30.99% (NE), and palmitic acid from 21.53% (NT) to 32.06% (NE). The next most abundant compounds were linolenic and stearic acids. Regarding linolenic acid, its content ranged from 18.70% (FR) to 21.88% (NA and NJ), but it was not detected in neither NT nor NE cultivars. The stearic acid content ranged from 1.74% (NO) to 3.96% (NT), but it was not detected in the NE cultivar. Myristic acid, ranged from 0.56% (NJ) to 0.65% (NA) and it was only detected in three cultivars (NA, NJ and FR). Arachidic acid was only detected in FR and NJ cultivars, and ranged from 1.46% (NJ) to 1.90% (FR). Finally, lauric acid was only detected in FR cultivar (0.50%).

Prickly pear fruit peel had high percentages of MUFAs (14.78%, mean value of all studied cultivars) and PUFAs (55.06%, mean value of all studied cultivars) (Table 5). The NE cultivar showed the maximum percentage of MUFAs (30.99%) while the NO cultivar presented the highest percentage of PUFAs (63.17%) in peel. However, the NT cultivar showed the lowest values for AI and TI and the highest one for U/S ratio (Table 2). Consequently, the NT can be considered as the cultivar with the most beneficial fatty acid profile in its fruit peels.

Table 2
Fatty acid composition (% of total fatty acid profile) of O. ficus-indica fruit peel as affected by cultivar.

Fatty acids (%)	Cultivar					
	NT	NO	NE	NA	FR	NJ
Lauric acid (C12:0)	nd	nd	nd	nd	0.50 ± 0.03	nd
Myristic acid (C14:0)	nd	nd	nd	0.65 ± 0.01 b	0.59 ± 0.01 a	0.56 ± 0.01 a
Palmitic acid (C16:0)	21.5 ± 1.6[1] a	28.2 ± 0.6 c	32.1 ± 0.2 d	27.7 ± 0.4 c	27.6 ± 0.4 c	24.9 ± 0.3 b
Palmitoleic acid (C16:1)	nd	nd	nd	0.92 ± 0.05 b	0.58 ± 0.02 a	0.65 ± 0.03 a
Stearic acid (C18:0)	3.96 ± 0.09 d	1.74 ± 0.34 a	nd	2.85 ± 0.15 c	2.61 ± 0.04 bc	2.21 ± 0.02 ab
Oleic acid (C18:1)	22.5 ± 0.3 d	6.90 ± 0.54 a	31.0 ± 0.4 e	6.83 ± 0.72 a	10.6 ± 0.6 c	8.72 ± 0.28 b
Linoleic acid (C18:2)	52.0 ± 13.5 d	42.4 ± 0.9 c	37.0 ± 0.2 a	39.2 ± 0.2 b	37.0 ± 0.4 a	39.6 ± 0.1 b
Linolenic acid (C18:3)	nd	20.8 ± 0.7 b	nd	21.9 ± 0.3 b	18.7 ± 0.2 a	21.9 ± 0.7 b
Arachidic acid (C20:0)	nd	nd	nd	nd	1.90 ± 0.07 b	1.46 ± 0.07 a
Total MUFA	22.5 ± 0.3 d	6.90 ± 0.54 a	31.0 ± 0.4 e	7.75 ± 0.67 a	11.2 ± 0.5 c	9.37 ± 0.25 b
Total PUFA	52.0 ± 1.4 b	63.2 ± 0.2 e	37.0 ± 0.2 a	61.1 ± 0.5 d	55.7 ± 0.3 c	61.5 ± 0.6 de
Total SFA	25.5 ± 1.7 a	29.9 ± 0.3 bc	32.1 ± 0.2 cd	31.2 ± 0.2 bcd	33.2 ± 0.3 d	29.2 ± 0.4 b
AI[2]	0.29 ± 0.03 a	0.40 ± 0.01 bc	0.47 ± 0.01 d	0.44 ± 0.01 cd	0.46 ± 0.01 d	0.38 ± 0.01 b
IT[3]	0.68 ± 0.06 a	0.85 ± 0.01 b	0.94 ± 0.01 c	0.81 ± 0.0003 b	0.84 ± 0.01 b	0.71 ± 0.01 a
U/S[4]	2.92 ± 0.26 c	2.34 ± 0.03 ab	2.12 ± 0.02 ab	2.21 ± 0.02 ab	2.01 ± 0.03 a	2.43 ± 0.05 b

[1] 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 5% significance level. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. nd, not detected.

[2] AI, index of atherogenicity: (C12:0 + 4 x C14:0 + C16:0) / [Σ MUFA + ΣPUFA (n-6) and (n-3)].

[3] AT, index of thrombogenicity: (C14:0 + C16:0 + C18:0)/[0.5 x Σ MUFA + 0.5 x Σ PUFA(n-6) + 3 x Σ PUFA(n-3) + (n-3)/(n-6)].

[4] U/S, unsaturated ratio: (MUFA + PUFA) / SFA.

Table 3
Fatty acid composition (% of total fatty acid profile) of *O. ficus-indica* young cladodes as affected by cultivar.

Fatty acids (%)	Cultivar					
	NT	NO	NE	NA	FR	NJ
Lauric acid (C12:0)	1.19 ± 0.02[1] ^{c}	1.12 ± 0.08 ^{c}	nd	1.45 ± 0.04 ^{d}	0.94 ± 0.01 ^{b}	0.75 ± 0.05 ^{a}
Myristic acid (C14:0)	3.17 ± 0.13 ^{d}	1.68 ± 0.09 ^{b}	1.38 ± 0.12 ^{a}	1.56 ± 0.01 ^{ab}	2.15 ± 0.06 ^{c}	1.33 ± 0.05 ^{a}
Palmitic acid (C16:0)	50.0 ± 1.0 ^{e}	33.8 ± 0.3 ^{b}	42.5 ± 1.8 ^{d}	39.8 ± 0.5 ^{cd}	37.5 ± 0.6 ^{c}	30.1 ± 1.2 ^{a}
Palmitoleic acid (C16:1)	1.84 ± 0.05 ^{b}	1.56 ± 0.12 ^{ab}	1.28 ± 0.10 ^{ab}	2.84 ± 0.24 ^{c}	3.45 ± 0.38 ^{d}	1.05 ± 0.08 ^{a}
Stearic acid (C18:0)	5.86 ± 0.17 ^{b}	4.36 ± 0.08 ^{a}	5.57 ± 0.01 ^{b}	5.47 ± 0.02 ^{ab}	5.71 ± 0.33 ^{b}	6.46 ± 0.86 ^{b}
Oleic acid (C18:1)	16.3 ± 0.5 ^{abc}	9.22 ± 0.66 ^{a}	23.7 ± 1.8 ^{c}	15.0 ± 1.1 ^{ab}	22.3 ± 3.4 ^{bc}	21.6 ± 5.5 ^{bc}
Linoleic acid (C18:2)	12.8 ± 0.6 ^{a}	27.9 ± 0.3 ^{d}	20.3 ± 4.2 ^{bc}	19.8 ± 0.1 ^{bc}	16.3 ± 0.5 ^{ab}	25.1 ± 2.5 ^{cd}
Linolenic acid (C18:3)	8.85 ± 0.41 ^{b}	20.4 ± 0.6 ^{d}	5.31 ± 0.55 ^{a}	11.5 ± 0.6 ^{c}	8.68 ± 1.51 ^{b}	10.5 ± 0.3 ^{bc}
Arachidic acid (C20:0)	nd	nd	nd	2.53 ± 0.38 ^{a}	2.98 ± 0.07 ^{a}	3.12 ± 0.42 ^{a}
Total MUFA	18.2 ± 0.6 ^{ab}	10.8 ± 0.5 ^{a}	24.96 ± 1.73 ^{b}	17.8 ± 0.8 ^{ab}	25.7 ± 3.0 ^{b}	22.7 ± 5.4 ^{b}
Total PUFA	21.6 ± 0.2 ^{a}	48.3 ± 0.9 ^{d}	25.6 ± 3.7 ^{ab}	31.3 ± 0.7 ^{bc}	25.0 ± 2.0 ^{ab}	35.6 ± 2.9 ^{c}
Total SFA	60.2 ± 0.7 ^{c}	40.9 ± 0.4 ^{a}	49.5 ± 1.9 ^{d}	50.9 ± 0.1 ^{d}	49.3 ± 1.0 ^{d}	41.7 ± 2. ^{a}
AI[2]	1.60 ± 0.04 ^{c}	0.70 ± 0.01 ^{a}	0.95 ± 0.08 ^{b}	0.97 ± 0.01 ^{b}	0.93 ± 0.03 ^{b}	0.62 ± 0.05 ^{a}
IT[3]	1.78 ± 0.01 ^{bc}	1.35 ± 0.02 ^{a}	1.96 ± 0.15 ^{c}	1.55 ± 0.03 ^{ab}	1.52 ± 0.03 ^{a}	1.30 ± 0.13 ^{a}
U/S[4]	0.66 ± 0.02 ^{a}	1.44 ± 0.02 ^{c}	1.02 ± 0.08 ^{b}	0.97 ± 0.001 ^{b}	1.03 ± 0.04 ^{b}	1.40 ± 0.14 ^{c}

[1] 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 5% significance level. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. nd, not detected.

[2] AI, index of atherogenicity: (C12:0 + 4 X C14:0 + C16:0) / [Σ MUFA + ΣPUFA (n-6) and (n-3)].

[3] AT, index of thrombogenicity: (C14:0 + C16:0 + C18:0)/[0.5 X Σ MUFA + 0.5 X Σ PUFA(n-6) + 3 X Σ PUFA(n-3) + (n-3)/(n-6)].

[4] U/S, unsaturated ratio: (MUFA + PUFA) / SFA.

3.3. Fatty acid composition of young cladodes

Young cladodes are mainly used for animal feed, but in some countries, they are also included in human diets. Nine fatty acids were identified in this botanical part (Table 3). Palmitic acid was the most abundant compound, and its content ranged from 30.05% to 49.98% in NJ and NT cultivars, respectively. The second most abundant fatty acid was linoleic acid, which ranged from 12.77% (NT cultivar) to 27.91% (NO cultivar). Oleic acid was the third most abundant fatty acid and ranged from 9.22% in NO cultivar to 23.68% in NE cultivar. Lauric acid ranged from 0.75% (NJ) to 1.45% (NA) but was not detected in NE cultivar. Arachidic acid was only detected in NA, FR and NJ cultivars and ranged from 2.53% (NA) to 3.12% (NJ); however, no significant differences were found among the three cultivars.

Young cladodes of prickly pear presented elevated percentages of PUFAs (31.21%, mean value of all studied cultivars) but also showed high contents of saturated fatty acids (SFAs) (48.75%, mean value of all studied cultivars) higher than those of fruits (both peel and pulp) and old cladodes (Table 5). The NO cultivar showed the highest percentage of PUFAs (48.28%), and the NT cultivar presented the maximum percentage of SFA (60.2%). Regarding MUFA, they ranged from 9.22% (NO) to 25.72% (FR). NJ and NO cultivars presented similar values for AI, TI (the lowest values) and U/S ratio (the highest values) (Table 3), so they were the cultivars with the most beneficial fatty acid profile in young cladodes.

3.4. Fatty acid composition of old cladodes

The main use of old cladodes is animal feeding. Seven different fatty acids were identified in the old cladodes (Table 4), with linoleic acid being the most abundant compound; its content ranged from 25.84% (NJ) to 53.77% (FR). After this, palmitic acid content ranged from 15.02% (FR) to 26.54% (NA), and oleic acid from 8.52% (NT) to 36.30% (NJ). Arachidic acid (C20:0) was only detected in NT, NO and

Table 4
Fatty acid composition (% of total fatty acid profile) of *O. ficus-indica* old cladodes as affected by cultivar.

Fatty acids (%)	Cultivar					
	NT	NO	NE	NA	FR	NJ
Lauric acid (C12:0)	nd	nd	nd	nd	nd	nd
Myristic acid (C14:0)	nd	nd	nd	nd	nd	nd
Palmitic acid (C16:0)	17.5 ± 0.1[1] ^{bc}	16.6 ± 0.5 ^{b}	18.5 ± 0.2 ^{c}	26.54 ± 0.1 ^{e}	15.0 ± 0.7 ^{a}	20.1 ± 0.1 ^{d}
Palmitoleic acid (C16:1)	nd	nd	nd	nd	0.36 ± 0.03	nd
Stearic acid (C18:0)	6.01 ± 0.23 ^{b}	4.56 ± 0.10 ^{a}	4.55 ± 0.28 ^{a}	5.01 ± 0.22 ^{a}	4.40 ± 0.48 ^{a}	4.36 ± 0.01 ^{a}
Oleic acid (C18:1)	8.52 ± 0.10 ^{a}	14.2 ± 0.5 ^{b}	10.7 ± 1.9 ^{a}	21.1 ± 0.4 ^{c}	24.8 ± 0.3 ^{d}	36.3 ± 0.1 ^{e}
Linoleic acid (C18:2)	34.7 ± 0.4 ^{c}	33.9 ± 0.1 ^{c}	37.6 ± 0.1 ^{d}	27.7 ± 0.6 ^{b}	53.8 ± 0.8 ^{e}	25.8 ± 0.1 ^{a}
Linolenic acid (C18:3)	16.4 ± 0.1 ^{c}	15.9 ± 0.3 ^{c}	16.3 ± 0.7 ^{c}	19.7 ± 0.1 ^{d}	1.69 ± 0.07 ^{a}	13.4 ± 0.1 ^{b}
Arachidic acid (C20:0)	16.8 ± 0.3 ^{c}	14.9 ± 0.2 ^{b}	12.4 ± 0.8 ^{a}	nd	nd	nd
Total MUFA	8.52 ± 0.10 ^{a}	14.2 ± 0.5 ^{b}	10.7 ± 1.9 ^{a}	21.1 ± 0.4 ^{c}	25.1 ± 0.3 ^{d}	36.3 ± 0.1 ^{e}
Total PUFA	51.1 ± 0.5 ^{c}	49.8 ± 0.3 ^{c}	53.9 ± 0.7 ^{d}	47.4 ± 0.6 ^{b}	55.5 ± 0.8 ^{d}	39.3 ± 0.1 ^{a}
Total SFA	40.3 ± 0.6 ^{a}	36.0 ± 0.8 ^{d}	35.5 ± 1.2 ^{d}	31.5 ± 0.1 ^{c}	19.4 ± 1.2 ^{a}	24.4 ± 0.1 ^{b}
AI[2]	0.29 ± 0.01 ^{d}	0.26 ± 0.01 ^{b}	0.29 ± 0.01 ^{cd}	0.39 ± 0.01 ^{e}	0.19 ± 0.01 ^{a}	0.37 ± 0.01 ^{bc}
IT[3]	0.67 ± 0.01 ^{cd}	0.56 ± 0.03 ^{b}	0.72 ± 0.03 ^{d}	0.92 ± 0.01 ^{e}	0.48 ± 0.04 ^{a}	0.65 ± 0.01 ^{c}
U/S[4]	1.48 ± 0.03 ^{a}	1.78 ± 0.06 ^{ab}	1.82 ± 0.10 ^{ab}	2.17 ± 0.01 ^{b}	4.15 ± 0.31 ^{d}	3.10 ± 0.01 ^{c}

[1] 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 5% significance level. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. nd, not detected.

[2] AI, index of atherogenicity: (C12:0 + 4 X C14:0 + C16:0) / [Σ MUFA + ΣPUFA (n-6) and (n-3)].

[3] AT, index of thrombogenicity: (C14:0 + C16:0 + C18:0)/[0.5 X Σ MUFA + 0.5 X Σ PUFA(n-6) + 3 X Σ PUFA(n-3) + (n-3)/(n-6)].

[4] U/S, unsaturated ratio: (MUFA + PUFA) / SFA.

Table 5
Comparison of fatty acid composition of the different botanical parts of *O. ficus-indica* (average of studied cultivars) and other plant species, as % of total fatty acid profile.

Fatty acid (%)	*Prickly pear* pulp	*Prickly pear* peel	*Prickly pear* young cladodes	*Prickly pear* old cladodes	*Prickly pear seed oil*	*White mulberry pulp*	*Black mulberry pulp*	*Pomegranate* seed oil	*Jujube* seed oil	*Pitaya (red flesh)* seed oil	*Pitaya (white flesh)* seed oil
C12:0	1.55[a]	0.08	0.91	nd	nd	nd	nd	nd	nd	nd	nd
C14:0	1.52	0.30	1.88	nd	0.01	0.26	0.38	nd	0.14	0.20	0.30
C16:0	22.9	27.0	39.0	19.1	15.8	12.3	14.0	3.76	4.75	17.9	17.1
C16:1	nd	0.36	2.00	0.06	0.81	0.13	0.15	nd	0.06	0.91	0.61
C17:0	nd	nd	nd	nd	0.01	nd	nd	nd	nd	nd	nd
C17:1	nd	nd	nd	nd	0.03	nd	nd	nd	nd	nd	nd
C18:0	5.20	2.23	5.57	4.82	2.66	3.40	3.50	2.03	2.70	5.49	4.37
C18:1	26.8	14.4	18.0	19.3	20.1	6.20	6.27	5.84	14.5	24.7	26.6
C18:2	29.2	41.2	20.4	35.6	59.9	74.9	73.0	7.21	41.6	49.6	50.1
C18:3	12.2	13.9	10.9	13.9	0.15	1.78	1.73	75.0	0.34	1.21	0.98
C19:0	nd	nd	nd	nd	nd	0.46	0.51	nd	nd	nd	nd
C20:0	0.76	0.56	1.44	7.34	0.19	0.01	0.01	nd	0.80	nd	nd
C20:1	nd	nd	nd	nd	0.11	nd	0.09	nd	2.88	nd	nd
C20:3	nd	nd	nd	nd	0.13	nd	nd	nd	nd	nd	nd
C21:0	nd	nd	nd	nd	nd	nd	0.03	nd	nd	nd	nd
C22:0	nd	nd	nd	nd	0.10	0.35	0.61	nd	0.86	nd	nd
C22:1	nd	nd	nd	nd	nd	0.14	nd	nd	nd	nd	nd
C24:0	nd	nd	nd	nd	0.03	nd	nd	nd	nd	nd	nd
MUFA	26.8	14.8	20.0	19.3	29.0	6.47	6.51	5.84	41.1	25.7	27.2
PUFA	41.4	55.1	31.2	49.5	60.2	76.7	74.7	82.3	49.7	50.8	51.1
SFA	31.9	30.2	48.8	31.2	18.8	16.8	19.1	5.79	9.21	23.6	21.8
AI[b]	0.45	0.40	0.92	0.28	0.18	0.16	0.19	0.04	0.09	0.24	0.23
IT[c]	0.86	0.81	1.58	0.67	0.41	–	–	–	–	0.29	0.23
U/S[d]	2.14	2.32	1.05	2.20	4.74	4.96	4.26	15.21	9.86	3.24	3.60
Reference[e]					1	2	2	3	4	5	5

[a] Values are the average of all cultivars. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids. nd, not detected.
[b] AI, index of atherogenicity: (C12:0 + 4 X C14:0 + C16:0) / [Σ MUFA + ΣPUFA (n-6) and (n-3)].
[c] TI, index of thrombogenicity: (C14:0 + C16:0 + C18:0)/[0.5 X Σ MUFA + 0.5 X Σ PUFA(n-6) + 3 X Σ PUFA(n-3) + (n-3)/(n-6)].
[d] U/S, unsaturated ratio: (MUFA + PUFA) / SFA.
[e] De Wit et al. (2017):1; Sánchez-Salcedo et al., (2015): 2; Hernández et al. (2011): 3; El Aloui et al. (2013): 4; Ariffin et al. (2009): 5.

NE cultivars, and its content ranged from 12.36% (NE) to 16.80% (NT).

High percentages of MUFAs and PUFAs were found in old cladodes of prickly pear (19.31% and 49.49% respectively, mean value of all studied cultivars), in fact, values were higher than those of the young cladodes (Table 5). The FR and NJ cultivars presented the highest percentages of PUFAs (55.45%) and MUFAs (36.30%), respectively; the last cultivar (NJ) also had the lowest percentage of SFAs (24.42%). The cultivar which showed the lowest values for AI and TI was FR, and simultaneously presented the highest value of the U/S ratio (Table 4). Consequently, the FR was the cultivar with the most beneficial fatty acid profile in old cladodes.

3.5. Principal components analysis

Principal component analysis (PCA) was applied to obtain an easier and complete understanding of the relationship among each of the FAMEs from different prickly pear parts and cultivars. One PCA was made for each studied part of prickly pear. The first principal component (F1) accounted for 60.9%, 46.6%, 43.0% and 33.5% of the total data variance in pulp (Fig. 1A), peel (Fig. 1B), young cladodes (Fig. 1C), and old cladodes (Fig. 1D), respectively; while, the second principal component (F2) accounted for 20.4%, 29.1%, 33.2% and 51.1% of the total variance, respectively. It is important to remember that the higher the distance between two parameters, the lower their correlation.

3.5.1. PCA in pulp

The first component, F1, was positively linked with the content of the following fatty acids: lauric, myristic, palmitic, stearic and linolenic acids, and negatively connected with linoleic acid. On the other hand, F2 was positively correlated with lauric, linoleic and arachidic acids and negatively correlated with oleic acid. Considering F1 as the dimension explaining the main differences, F1 also allowed the separation among cultivars. NT, FR and NA cultivars were positively linked with SFAs, while NJ, NE and NO cultivars were connected with PUFAs, with the exception of linolenic acid. This implied that NJ cultivar was characterized by a high PUFAs content, especially linoleic acid, and a low SFAs content, such as lauric, myristic, palmitic and arachidic acids. The same tendency of SFAs was noticed in NE and NO cultivars, but presenting a high content of MUFAs, oleic acid. Therefore, the pulp of NJ, NE and NO cultivars presented a good profile of fatty acids: rich in monounsaturated and polyunsaturated fatty acids. Thus, they were the most interesting cultivars for human diet and animal feeding.

3.5.2. PCA in peel

Considering the first component, F1 was positively linked with myristic, palmitoleic, linolenic and arachidic acids and negatively with oleic acid. F2 was positively linked with palmitic acid and inversely with stearic and linoleic acids. F1 also separated the studied cultivars, on one side NA, NJ and FR cultivars (positive axis), and on the other NE, NO, NT cultivars (negative axis).

Taking into account that in the F1 dimension the distance among samples was the lowest, the peels of NA, NJ and FR cultivars were characterized by a high content of linolenic, myristic, palmitoleic, and arachidic acids; lauric acid was also correlated with FR cultivar peel because of lauric acid was only identified in the mentioned cultivar. Regarding to NE cultivar, the peel showed the same tendency than the pulp of this same cultivar, and it was linked with oleic acid.

3.5.3. PCA in young cladodes

In the case of young cladodes, the main component F1 was positively correlated to linoleic and linolenic acids but negatively correlated to palmitic, stearic, and oleic acids. On the other side, the F2 component was positively linked with lauric, myristic, palmitic and

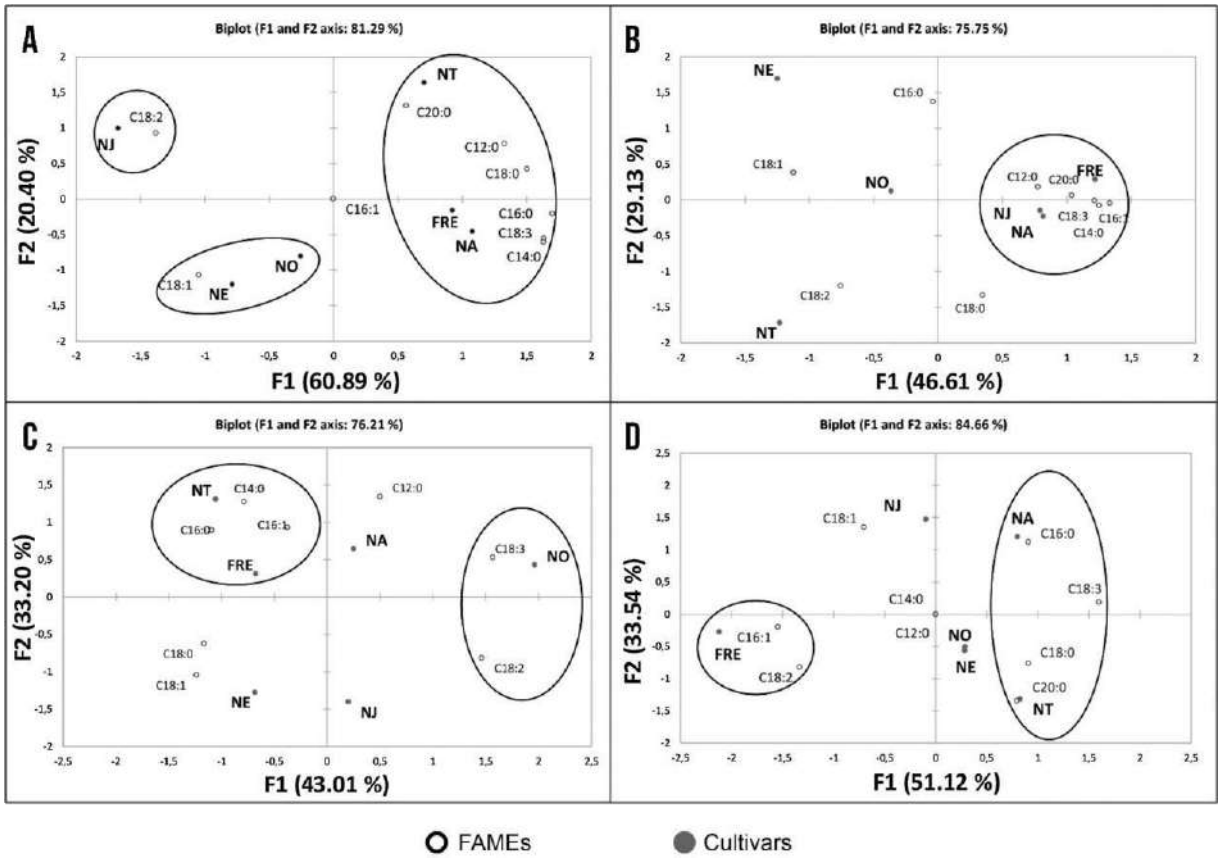

Fig. 1. Principal component analysis (F1 and F2) of different botanical parts of *O. ficus-indica*: A) fruit pulp, B) fruit peel, C) young cladodes, D) old cladodes.

palmitoleic acids and negatively with oleic and linoleic acids.

The main component F1 allowed separating NO, NA and NJ cultivars in the positive axis, in which NO cultivar was visibly differentiated by a high positive value, and FR, NT and NE cultivars in the negative axis. In this regard, NO cultivar was characterized by a high percentage in linoleic and linolenic acids, while the cultivars NT and FR were characterized with high levels of palmitic, myristic and palmitoleic acids.

3.5.4. PCA in old cladodes

The first component was positively connected to the content of linolenic acid and negatively linked to palmitoleic and linoleic acids. F2 was positively correlated with palmitic and oleic acids, and negatively correlated with stearic, linoleic, and arachidic acids. The principal component F1 also showed the differences among cultivars. For instance, old cladodes from NT and NA cultivars, which were positioned as far to the right of the graph, were correlated with saturated FAMEs (palmitic, stearic and arachidic acids, the last one was only detected in this cultivar) but with a higher amount of linoleic acid. On the contrary, old cladodes from FR cultivar were characterized by a high content of palmitoleic acid (C16:1) (this was the only cultivar which presented this fatty acid) and linoleic acid.

4. Discussion

There are no previous reports in the scientific literature dealing with the fatty acid profile of fresh fruit pulp, old cladodes and young cladodes of prickly pear, and only two articles studied fruit peel (El-Said et al., 2011; Ramadan and Mörsel, 2003a). Besides, Abidi et al. (2009) studied the fatty acid profile of cladodes but they did not differentiate between young and old cladodes. El-Beltagi et al. (2019) and Ramadan and Mörsel (2003b) studied the fatty acids in fruit oils (peel and pulp), and De Wit et al. (2017) studied the fatty acid profile of prickly pear seed oil.

Ramadan and Mörsel (2003a) identified 13 fatty acids. Regarding the content of SFAs, current results agreed with those reported by Ramadan and Mörsel (2003a). The MUFAs content of the NT and NE cultivars presented similar values to those obtained by these authors, but NO, NA, NJ and FR cultivars presented higher values than the reported previously in the literature. In the case of PUFAs, all the studied cultivars, except NE, presented higher values than those reported by Ramadan and Mörsel (2003a). El-Said et al. (2011) identified 7 fatty acids in fruit peels, with linoleic acid predominating and followed by palmitic and oleic acids; this trend agreed with current results. The values reported for SFAs also agreed with the current results, but the cultivars analyzed in the present study showed higher percentages of PUFAs and lower percentages of MUFAs, except for NE cultivar, that showed lower values of PUFAs and higher values of MUFAs.

El-Beltagi et al. (2019) studied the fatty acid composition of prickly pear pulp and peel oils. Eleven fatty acids were identified, and linoleic, palmitic and oleic acids were the predominant ones; these results agreed with the current results. The content of SFAs obtained by these authors in pulp oil was similar to those obtained in NO, NE and NJ cultivars and lower than those obtained in NT, NA and FR cultivars. PUFAs values were higher in the cultivars studied by these authors, while MUFAs percentages were lower than those obtained in this study. Regarding peel oil, El-Beltagi et al. (2019) obtained similar values in SFAs to that of the NT cultivar, but lower values than that found in the rest of the cultivars. The PUFAs percentages obtained by these authors were similar to those of NO, NA and NJ cultivars but higher than NT, FR and NE cultivars. The NT and NE cultivars showed higher values of MUFAs than those reported by these authors, but the rest of the cultivars showed lower values.

Ramadan and Mörsel (2003b) studied the fatty acid composition of

prickly pear pulp oil. They detected eight fatty acids, of which linoleic acid was the predominant one; this result agreed with the current results. Palmitic and oleic acids showed higher values in the cultivar studied by these authors. Regarding SFAs, Ramadan and Mörsel (2003b) obtained similar percentages than those found here in the NT, NA and FR cultivars, but the rest of the studied cultivars showed lower values. About PUFAs, the FR and NJ cultivars showed similar values than those obtained by these authors, but the rest of the cultivars showed lower values. All the cultivars analyzed in this study showed higher values of MUFAs than those obtained by Ramadan and Mörsel (2003b).

Regarding cladodes, Abidi et al. (2009) identified nine fatty acids, with linoleic acid predominating; this trend agreed with the current results. However, these authors detected higher values of linolenic acid. The SFAs values obtained by these authors agreed with that of old cladodes of FR and NJ cultivars, but were lower than those of the rest of cultivars in both young and old cladodes. Abidi et al. (2009) reported similar percentages of PUFAs and MUFAs to that of NO cultivar young cladodes, but higher than that of NT cultivar old cladodes and lower than the rest of cultivars in both young and old cladodes.

De Wit et al. (2017) studied the fatty acid profile of prickly pear seed oil (Table 5). They identified 14 fatty acids, of which linoleic acid was the predominant compound; this trend agreed with the current results, but linolenic acid showed lower values in prickly pear seed oil than in the botanical parts analyzed in the present study. Regarding MUFAs and PUFAs, De Wit et al. (2017) obtained higher values in prickly pear seed oil than those obtained in this study in fruits and cladodes. Prickly pear seed oil (De Wit et al., 2017) showed lower values of SFAs, the indexes AI and TI, and a higher value of the U/S ratio as compared to the values obtained in prickly pear fruits and cladodes in the current study.

Another way to verify the current results was to compare them with the fatty acid profiles of different fruits: white mulberry (*Morus alba* L.) and black mulberry (*Morus nigra* L.) fruit, pomegranate (*Punica granatum* L.) seed oil, jujube [*Ziziphus zizyphus* (L.) H. Karst.] seed oil, red flesh pitaya [*Hylocereus polyrhizus* (F.A.C Weber) Britton & Rose] seed oil and white flesh pitaya [*Hylocereus undatus* (Haw.) Britton & Rose] seed oil (Table 5). All these species have in common that they can get easily adapted to arid and semi-arid climatic zones. The values of the indexes AI and TI were also compared with those of other fruits and oils.

According to Sánchez-Salcedo et al. (2016) 12 fatty acids were found in white mulberry and black mulberry fruits. Prickly pear presented higher percentages of oleic acid, linolenic acid and MUFA, but the total PUFAs percentage was higher in white mulberry and black mulberry fruits. Regarding SFAs, the percentage was higher in the different botanical parts of prickly pear, mainly in young cladodes. Besides, white mulberry and black mulberry fruits showed lower values in AI than prickly pear, and a higher value for U/S ratio.

Regarding pomegranate seed oil, five fatty acids were reported (Hernández et al., 2011). Pomegranate seed oil presented the highest percentage of PUFAs (82.25%), whereas prickly pear showed highest values of MUFAs, both oleic and palmitoleic acids, and besides this later compounds was not detected in pomegranate seed oil (Hernández et al., 2011.). The percentage of SFAs was also higher in all the botanical parts of prickly pear. Besides, pomegranate seed oil showed a lower value for AI and a higher value for U/S ratio than prickly pear.

Regarding jujube seed oil, 10 fatty acids were identified by El Aloui et al. (2012). The total content of MUFAs was higher in jujube seed oil than in prickly pear fruits and cladodes. Regarding PUFAs, jujube seed oil showed similar percentages to those of the prickly pear fruits pulp of the different cultivars, higher percentage than those of young cladodes and lower percentages than those of fruits peel and old cladodes. In the case of SFA, jujube seed oil presented lower values than all the botanical parts of prickly pear. Regarding AI, jujube seed oil showed a lowest value than prickly pear and a higher value of U/S ratio.

Regarding red flesh pitaya and white flesh pitaya seed oil, Ariffin et al. (2009) detected seven fatty acids. In this case, both prickly pear, red flesh pitaya and white flesh pitaya seed oil presented similar percentages of MUFA and PUFA, except for MUFA in prickly pear fruits peel and PUFA in young cladodes, which presented lower values. The percentage of SFA was slightly higher in prickly pear fruits (peel and pulp) and old cladodes, but young cladodes presented the highest value of these fatty acids. Both species (red flesh pitaya and white flesh pitaya) showed lower values of TI and AI than prickly pear and higher values of U/S ratio.

The AI values in all botanical parts of prickly pear were higher than those obtained by Castro-Boloñanos et al. (2005) in corn, soybean, olive and sunflower oils. The AI and TI values obtained by Siano et al. (2015) in sweet cherry seed oil are also higher than all those in the botanical parts of prickly pear. Instead, the AI and TI values obtained by Siano et al. (2015) in pomegranate and pumpkin seed oil are similar to those obtained in prickly pear fruit pulp and peel, lower than those obtained in young cladodes and higher than those obtained in old cladodes. Ulbricht and Southgate (1991) obtained higher values of AI and TI in coconut oil and palm oil than those obtained in all botanical parts of prickly pear and lower AI and TI values in olive oil and sunflower oil.

Regarding PCA results, young cladodes of NO cultivar presented a beneficial profile of fatty acids, because they presented a high PUFAs percentage and low SFAs content; thus, NO was the most interesting cultivar for human diet and animal feeding in the case of young cladodes. Concerning old cladodes, FR cultivar seem to be the most interesting cultivar for animal feeding due to their high percentages of MUFAs and PUFAs (palmitoleic and linoleic acid, respectively) and low SFAs content.

5. Conclusions

The results of this research are a valuable contribution on the completion of the nutritional properties of prickly pear: the fatty acid profile of six different cultivars and their botanical parts: fruits (pulp and peel) and cladodes (young and old). The most abundant fatty acids were linoleic (an essential polyunsaturated fatty acid), oleic, and palmitic acids. Regarding fruits, the cultivars NE, NJ and NO were the most interesting, due to their high levels of MUFAs (NE cultivar in fruit peel and pulp) and PUFAs (NJ cultivar in pulp and NO cultivar in peel). The pulp of NE and NJ cultivars could be used for fresh consumption, as well as to produce products such as jams, juices and fruit gummies among others. The peel of NO cultivar was the most suitable for animal feeding. FR cultivar had high MUFAs content in young cladodes and PUFAs in old cladodes; thus, FR was an interesting cultivar for the use of their cladodes both in animal feeding and to produce juices and dehydrated powder for human consumption. Regarding AI and TI, prickly pear fruits (peel and pulp) and old cladodes showed very good values for these indexes; thus, these parts of the prickly pears can positively contribute to good cardiovascular health. From these results, it can be concluded that prickly pear has a beneficial fatty acid profile, because of its high content in PUFAs and MUFAs, and a high potential for their use as food, feed and for pharmaceutical uses.

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Characterization of Bioactive Compounds of *Opuntia ficus-indica* (L.) Mill. Seeds from Spanish Cultivars

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Abstract: *Opuntia ficus-indica* (L.) Mill. is the Cactaceae plant with the greatest economic relevance in the world. It can be used for medicinal purposes, animal nutrition, production of biofuels and phytoremediation of soils. Due to its high content of bioactive compounds, the prickly pear has antioxidant, antimicrobial and anticancer properties. The aim of this study was to determine the polyphenolic, fatty acid and amino acid profile and characterize the antioxidant capacity of seeds of seven Spanish prickly pear cultivars. A total of 21 metabolites, mainly phenolic acids and flavonols, were identified using ultraperformance liquid chromatography photodiode detector quadrupole/time-of-flight mass spectrometry (UPLC-PDA-Q/TOF-MS). Significant differences were found in the phenolic concentrations of the investigated varieties. The highest amount of phenolic compounds (266.67 mg/kg dry matter) were found in the "Nopal espinoso" variety, while the "Fresa" variety was characterized by the lowest content (34.07 mg/kg DM) of these compounds. In vitro antioxidant capacity was positively correlated with the amount of polyphenols. The amino acid composition of protein contained in prickly pear seeds was influenced by the variety. Glutamic acid was the predominant amino acid followed by arginine, aspartic acid and leucine, independent of prickly pear variety. Overall, 13 different fatty acids were identified and assessed in prickly pear seeds. The dominant fatty acid was linoleic acid, with content varying between 57.72% "Nopal ovalado" and 63.11% "Nopal espinoso".

Keywords: prickly pear; UPLC-MS; phenolic compounds; fatty acids; amino acids

1. Introduction

Commonly known as the prickly pear or cactus pear, *Opuntia ficus-indica* (L.) Mill. is the Cactaceae plant with the greatest economic relevance in the world. This plant is mainly known for its fruit, but cladodes are also consumed, mainly in Mexico, which is the country with the largest area under cultivation and also the largest producer [1,2]. Both are consumed fresh, but can also be consumed cooked, canned, dehydrated and as concentrated juice, jams and syrups, among other forms [1,3].

Besides that, the prickly pear has been used for medicinal purposes, animal nutrition, the production of biofuels and phytoremediation of soils, among others [4,5].

The pulp is the edible part of prickly pear fruit and is mainly composed of water (84–90%) and reducing sugars, mainly glucose and fructose (10–15%) [6,7]. The fruit contains a large number of seeds, about 0.24 g/g, constituting about 10–15% of the edible pulp and 30–40% on a dry weight basis [1,7,8]. An edible oil can be obtained from prickly pear seeds, which is rich in polyunsaturated and monounsaturated fatty acids, of which linoleic acid is the predominant fatty acid, followed by oleic acid [9–11]. The consumption of these kinds of fatty acids is related to health benefits and contributes to the improvement of various health conditions such as cardiovascular diseases, obesity and diabetes mellitus, among others [12].

Antioxidant activity is one of the major mechanisms by which fruit and vegetables provide health benefits. The high amounts of polyphenols, which show strong antioxidative properties attributed to their ability to scavenge free radicals and to chelate metal ions involved in their production, contribute to the strong antioxidant activity of prickly pear seeds [8,13]. Besides that, prickly pear seeds contain 11–17% protein, higher than the content in fruit peel and pulp, glutamic acid, aspartic acid, arginine and glycine predominating in its amino acid profile, and are also rich in minerals [11,14,15]. However, the composition of prickly pear seeds can vary among cultivars, varieties and crop environmental factors, among others [16].

The seeds of the prickly pear are usually discarded after the extraction of pulp, providing a large amount of seeds as waste. The study of the composition of these seeds could help to find possible uses in the cosmetic and pharmaceutical industries, animal feed, and also in the human diet as a new source of oil and meal. This work was carried out on the seeds of seven Spanish prickly pear cultivars. The main objectives of this comparative study were (i) to quantify the antioxidant activity and phenolic compounds in these seeds, (ii) to determine the fatty acid profile and (iii) to define the amino acid profile. This research intends to value prickly pears of local origin; the results will provide specific information about the composition of prickly pear seeds, and could be valuable to the food, cosmetic and pharmaceutic industries in order to utilize this byproduct. Besides its health-promoting properties, the prickly pear is also a very profitable crop in Spain, in addition to contributing to the mitigation of climate change in arid and semiarid regions by sequestration. The study of the composition of these cultivars constitutes an advance in the knowledge of their properties and in the elaboration of derived products. Due to the exploitation of the juice, a large amount of waste is left, including the seeds, currently used in animal feed, this work evaluates the composition of the seeds for use as a food supplement and the possibility of them being used in the cosmetic and pharmaceutical industries.

2. Results and Discussion

2.1. Metabolite Identification Using UPLC-MS Analysis

The secondary metabolites of *Opuntia ficus-indica* (L.) Mill extracts were determined using an ACQUITY UPLC system equipped with a PDA detector and G2 Q-Tof micromass spectrometer (Waters, Manchester, UK) operating in negative mode. Figure 1 shows the LC-DAD chromatogram of the "Orito" cultivar. Qualitative analysis results with their UV and mass spectral data are summarized in Table 1.

Two major classes of phenolic compounds were identified—phenolic acids and flavonols. In addition, two organic acids were found and identified.

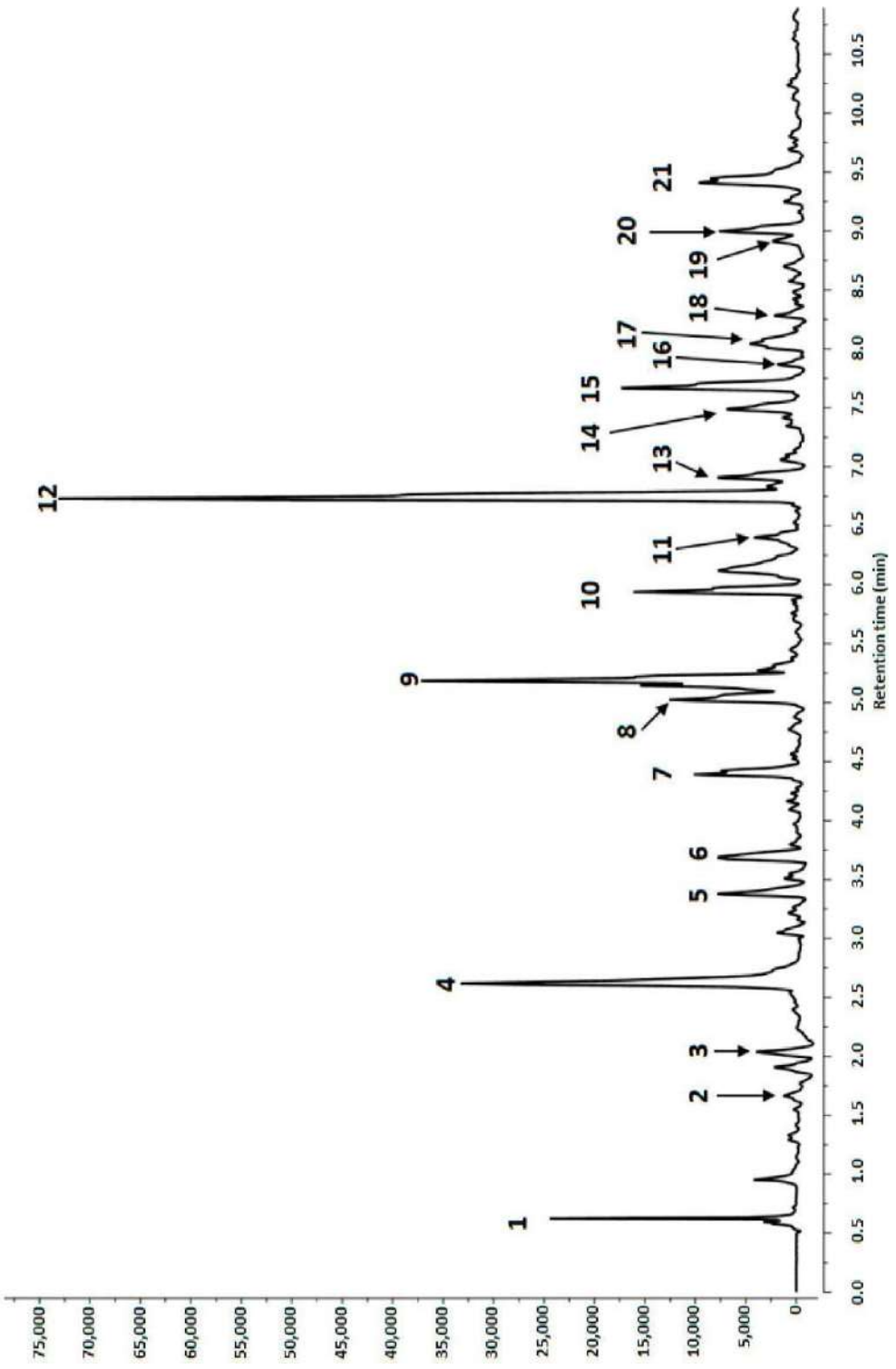

Figure 1. Ultraperformance liquid chromatography (UPLC)–MS chromatogram profile of the "Orito" cultivar at 280 nm. Peak number identities are displayed in Table 1.

Table 1. Retention times, UV–vis spectra and characteristic ions of phenolic compounds and organic acids of the "Orito" cultivar.

Peak No.	Rt	UV [nm]	MS [M − H]⁻ (*m*/*z*) [a]	MS/MS (*m*/*z*) [a]	Tentative Identification
1.	0.62	360	195.0522	177.0404; 159.2073; 129.0194	Gluconic acid
2.	1.66	210	191.0051	110.9974	(Iso)Citric acid
3.	2.04	340	301.0920	179.0521; 151.0274	Quercetin
4.	2.62	220, 275	255.0366	237.0222; 193.0362; 165.0417	Piscidic acid
5.	3.38	299	315.0781	153.0173	Protocatechuic acid hexoside
6.	3.68	325	517.1523	386.1084; 193.0476	Ferulic acid derivative
7.	4.39	220, 275	239.0416	179.0218; 149.0408	Eucomic acid
8.	5.02	325	341.0837	179.0372	Caffeic acid glucoside I
9.	5.18	325	517.1543	193.0480; 175.0378	Ferulic acid diglucoside
10.	5.94	324	341.0697	179.0523	Caffeic acid glucoside II
11.	6.40	325	371.1151	249.9247; 193.0503; 175.0273	Feruloyl gluconic acid
12.	6.73	202, 216, 275	565.1764	339.1087; 327.1086	-
13.	6.91	325	595.2080	329.1040; 197.8092; 175.0987; 193.0476; 162.8393	Ferulic acid derivative
14.	7.49	350	609.1295	301.0355	Quercetin-3-O-rutinoside
15.	7.69	355	463.1399	301.0355	Quercetin-3-O-galactoside
16.	7.75	350	755.1174	623.0457; 315.0475	Isorhamnetin-pentosyl rutinoside
17.	8.04	340	593.1521	315.0511	Isorhamnetin-pentosyl rhamnoside
18.	8.28	350	623.1598	315.0420	Isorhamnetin-3-O-rutinoside
19.	8.91	350	477.1020	315.0455	Isorhamnetin-3-O-galactoside
20.	8.96	350	477.1022	315.0475	Isorhamnetin-3-O-glucoside
21.	9.41	340	519.1130	315.0510	Isorhamnetin acylated hexoside

[a] Experimental data.

Table 1. Retention times, UV–vis spectra and characteristic ions of phenolic compounds and organic acids of the "Orito" cultivar.

2.1.1. Phenolic Acid Derivatives

Four derivatives of ferulic acid (peaks 6, 9, 11 and 13), two derivatives of caffeic acid (peaks 8 and 10) and one derivative each of protocatechuic acid (peak 5), piscidic acid (peak 4) and eucomic acid (peak 7) were identified in prickly pear seeds.

Peaks 6, 9, 11 and 13 showed a similar fragmentation pattern with product ions at *m*/*z* 193 and 175 $[M - H - 18]^-$, corresponding to the loss of a ferulic acid moiety and suggesting that these metabolites are ferulic acid derivatives [17].

In the group of caffeic acid derivatives, two caffeic acid hexoses were detected. Peaks 8 and 10 had pseudomolecular ions at *m*/*z* 341.0837 and 341.0697, respectively, and fragmentation ions at *m*/*z* 179 which correspond to the loss of hexose residues (162u).

Peak number 4 showed a molecular ion $[M - H]^-$ at *m*/*z* 255.0366 with product ions at *m*/*z* 237 $[M - H - 18]^-$, 193 $[M - H - 62]^-$ and 165 $[M - H - 90]^-$, corresponding to the loss of two water, carbon dioxide and carbon oxide residues, and was identified as piscidic acid [18].

Peak number 5 with a pseudomolecular ion at *m*/*z* 315.0781 and pseudomolecular ion at *m*/*z* 153, which corresponded to the loss of a hexoside residue (162u), was identified as protocatechuic acid hexoside [19].

Peak number 7 showed a molecular ion at *m*/*z* 239.0416 and product ions at *m*/*z* 179 $[M - H - 60]^-$ and 149 $[M - H - 90]^-$, and was identified as eucomic acid according to the literature [20].

Phenolic acids and their derivatives have previously been identified in prickly pear fruits and juices. For example, Faraq et al. [20] in their study on three *Opuntia ficus indica* fruit cultivars have identified derivatives of caffeic and ferulic acids. Ferulic and protocatechuic acids have been identified by Guevara-Figueroa et al. [21] in their study on prickly pear cladodes, while Mata et al. [22] have identified among others piscidic and eucomic acids in *Opuntia ficus-indica* juices. Up to now, only ferulic acid had been identified in prickly pear seeds [8], while piscidic, eucomic, protocatechuic and caffeic acid and their derivatives have now been identified in seeds for the first time.

2.1.2. Flavonols

Eight flavonols were detected in prickly pear seed extracts, comprising six isorhamnetin derivatives (peaks 16–21) and three quercetin derivatives (peaks 3, 14 and 15) (Table 1).

The quercetin derivatives were quercetin aglycone, quercetin 3-*O*-rutinoside (rutin), and quercetin-3-*O*-galactoside. Each of the compounds has the typical quercetin fragment at *m*/*z* 301. Peak 3 with a molecular ion $[M - H]^-$ at *m*/*z* 301.0920, was identified as quercetin. Peak 14, with a pseudomolecular ion at *m*/*z* 609.1295, was identified as a quercetin 3-*O*-rutinoside (rutin), and peak 15 with a molecular ion at *m*/*z* 463.1399, was identified as quercetin-3-*O*-galactoside. Quercetin 3-*O*-rutinoside (rutin) and 3-*O*-galactoside are commonly present flavonoids in plants, which have been detected previously, for example, in methanol extracts from the thornless form of Tunisian *O. ficus-indica* [23,24]. Quercetin derivatives have previously been identified in prickly pear fruit (peel and flesh) [20,23] and in its juices [22] and flowers [24], but have not been studied previously in the seeds of this plant.

In the group of isorhamnetin derivatives, isorhamnetin-pentosyl rutinoside (peak 16), -pentosyl rhamnoside (peak 17), -3-*O*-rutinoside (peak 18), -3-*O*-galactoside (peak 19), -3-*O*-glucoside (peak 20) and -acylated-hexoside (peak 21) were found. All of them possess the typical isorhamnetin fragment at *m*/*z* 315 formed by the cleavage of the hexoside residues, i.e., -galactoside (-162u), -rutinoside (-308u) and -acylated-hexosides (-162u-42u), from the isorhamnetin glycosides. Isorhamnetin derivatives are commonly present in various species of prickly pear. They can be found in flowers [24,25], pulp and peel [23]. Isorhamnetin derivatives have also been detected both in the juice [22] and methanolic extracts of *O. ficus-indica* [20], however, they have not been identified in prickly pear seeds.

2.1.3. Organic Acids

Two organic acids—gluconic and (iso)citric acid—were identified in the seeds of the prickly pear (Table 1). Peak 1, with a molecular ion $[M - H]^-$ at *m*/*z* 195.0522, and a typical fragmentation pattern with product ions at *m*/*z* 177 and 159 corresponding to the loss of two water residues (-18u and -36u), was identified as gluconic acid [26]. Peak 2 had a pseudomolecular ion at *m*/*z* 191.0051 and a product ion at *m*/*z* 11.9974 and was identified as (iso)citric acid. Gluconic and (iso)citric acids have previously been identified in *O.ficus indica* fruit extracts [20]. However, these compounds have not been identified in prickly pear seeds.

2.1.4. Other Compounds

Peak 12 had pseudomolecular ion at *m*/*z* 565.1764, and fragmentation ions at *m*/*z* 339.1087 and 327.1086, which corresponded to the loss of 226u and 238u, and was a major peak in prickly pear seeds (Table 1, Figure 1). This compound has previously been detected in *Opuntia ficus-indica* fruit [20], but as in our case, it was not identified.

2.2. Quantitative Analysis of Polyphenols

Quantitative analysis of prickly pear seeds was conducted by external calibration curves using selected reference compounds (Materials and Methods: Section 3.3). The concentration of the individual substances was expressed as mg/kg dry matter (DM) (Table 2).

The analysis showed differences in the content of polyphenols between the tested cultivars. The highest concentration of phenolic acids and flavonols (171.60 and 95.07 mg/kg DM, respectively) was determined in "Nopal espinoso" cultivar (Table 2). "Fresa" cultivar was characterized by the lowest concentration of both polyphenolic groups (19.05 and 34.07 mg/kg DM, respectively). In all samples tested, phenolic acids were the dominant group of phenolic compounds as compared to flavonols, and their total amount was 17% higher.

These results are in agreement with the results presented by Guevara-Figueroa et al. [21], who analyzed the concentration of phenolic compounds in commercial and wild prickly pear cladodes. De Wit et al. [27] obtained slightly higher values, ranging from 74.86 mg/kg to 291.46 mg/kg for seeds from 8 different cultivars of prickly pear. These differences may be due to cultivar and genetic factors, growth conditions, as well as harvesting time, degree of ripeness or fruit processing, and above all, the determination methods [27].

The results obtained show that the proportion and concentration of phenolic compounds in plants are dependent on the anatomical part. The variability of phenolics in plant tissues depends on many factors, such as temperature, UV light and nutrition [28–30].

2.3. In Vitro Antioxidant Activity

The in vitro antioxidant activity of *O. ficus-indica* seeds was measured as the ferric reducing capacity by the FRAP method and free radical scavenging activity (DPPH and ABTS methods) and is listed in Table 3. The results of the DPPH, ABTS and FRAP methods were expressed in the same units, i.e., mmol of Trolox equivalent per kg of prickly pear DM.

Table 2. Phenolic acid and flavonol composition of different varieties of prickly pear (mg/kg of dry matter) *.

Peak No.	[mg/kg]	Fresa	Nopal Alargado	Nopal Espinoso	Nalle	Nopal Ovalado	Nopal Tradicional	Orito
	Phenolic acids							
5.	Protocatechuic acid hexoside	4.57 ± 0.2 ^{a}	7.13 ± 0.2 ^{b}	22.36 ± 1.1 ^{f}	11.37 ± 0.2 ^{c}	11.24 ± 0.2 ^{c}	12.56 ± 0.1 ^{d}	19.03 ± 0.3 ^{e}
6.	Ferulic acid derivative	2.35 ± 0.1 ^{c}	0.00 ± 0.0 ^{a}	0.00 ± 0.0 ^{a}	11.62 ± 0.1 ^{d}	1.08 ± 0.0 ^{b}	0.00 ± 0.0 ^{a}	0.00 ± 0.0 ^{a}
8.	Caffeic acid hexoside I	1.28 ± 0.0 ^{a}	4.35 ± 0.3 ^{b}	12.59 ± 0.2 ^{e}	11.95 ± 0.2 ^{d}	4.09 ± 0.1 ^{b}	5.51 ± 0.1 ^{c}	13.50 ± 0.9 ^{f}
9.	Ferulic acid diglucoside	4.10 ± 0.1 ^{a}	37.07 ± 1.2 ^{c}	108.97 ± 3.5 ^{e}	63.46 ± 3.3 ^{d}	13.59 ± 1.0 ^{b}	31.68 ± 1.2 ^{c}	60.56 ± 2.2 ^{d}
10.	Caffeic acid hexoside II	1.75 ± 0.0 ^{c}	2.41 ± 0.1 ^{d}	1.54 ± 0.0 ^{b}	1.07 ± 0.0 ^{a}	2.40 ± 0.0 ^{d}	2.61 ± 0.0 ^{d}	1.71 ± 0.0 ^{c}
11.	Feruloyl gluconic acid	2.64 ± 0.0 ^{a}	2.43 ± 0.1 ^{a}	14.21 ± 0.5 ^{c}	3.67 ± 0.0 ^{b}	2.20 ± 0.1 ^{a}	3.22 ± 0.0 ^{b}	2.57 ± 0.1 ^{a}
13.	Ferulic acid derivative	2.35 ± 0.1 ^{a}	7.09 ± 0.3 ^{d}	11.93 ± 0.2 ^{f}	6.28 ± 0.5 ^{c}	4.69 ± 0.2 ^{b}	10.16 ± 0.3	7.64 ± 0.4 ^{e}
	Sum of phenolic acids	19.05	60.47	171.60	109.44	39.29	65.75	105.01
	Flavonols							
3.	Quercetin	1.95 ± 0.0 ^{d}	1.21 ± 0.0 ^{b}	0.90 ± 0.0 ^{a}	8.40 ± 0.4 ^{e}	1.52 ± 0.1 ^{c}	2.03 ± 0.0 ^{d}	1.16 ± 0.1 ^{ab}
14.	Quercetin-3-*O*-rutinoside	0.96 ± 0.0 ^{a}	5.95 ± 0.3 ^{c}	8.34 ± 0.2 ^{e}	5.41 ± 0.1 ^{c}	4.18 ± 0.1 ^{b}	7.48 ± 0.6 ^{d}	4.78 ± 0.1 ^{b}
15.	Quercetin-3-*O*-galactoside	1.46 ± 0.1 ^{a}	4.99 ± 0.2 ^{e}	3.58 ± 0.1 ^{c}	4.96 ± 0.1 ^{e}	2.62 ± 0.0 ^{b}	7.13 ± 0.3 ^{f}	4.34 ± 0.3 ^{d}
16.	Isorhamnetin-pentosyl rutinoside	1.97 ± 0.1 ^{a}	2.98 ± 0.2 ^{b}	3.71 ± 0.1 ^{c}	1.60 ± 0.0 ^{a}	3.92 ± 0.1 ^{c}	4.77 ± 0.0 ^{d}	1.67 ± 0.1 ^{a}
17.	Isorhamnetin-pentosyl rhamnoside	1.05 ± 0.0 ^{bc}	1.23 ± 0.1 ^{c}	0.86 ± 0.0 ^{b}	0.94 ± 0.0 ^{b}	0.12 ± 0.0 ^{a}	0.15 ± 0.0 ^{a}	1.03 ± 0.0 ^{bc}
18.	Isorhamnetin-3-*O*-rutinoside	1.23 ± 0.0 ^{b}	1.62 ± 0.0 ^{c}	1.59 ± 0.0 ^{c}	1.10 ± 0.0 ^{b}	0.64 ± 0.0 ^{a}	1.82 ± 0.0 ^{de}	1.94 ± 0.0 ^{e}
19.	Isorhamnetin-3-*O*-galactoside	0.00 ± 0.0 ^{a}	2.06 ± 0.1 ^{e}	3.31 ± 0.1 ^{f}	1.11 ± 0.0 ^{c}	0.61 ± 0.0 ^{b}	1.99 ± 0.1 ^{e}	1.66 ± 0.0 ^{d}
20.	Isorhamnetin-3-*O*-glucoside	2.65 ± 0.2 ^{a}	38.92 ± 1.1 ^{d}	51.03 ± 1.6 ^{e}	22.76 ± 0.8 ^{c}	12.75 ± 0.6 ^{b}	29.81 ± 1.1 ^{cd}	23.93 ± 1.0 ^{c}
21.	Isorhamnetin acylated hexoside	3.75 ± 0.2 ^{a}	17.26 ± 1.0 ^{c}	21.74 ± 1.1 ^{d}	21.21 ± 0.6 ^{d}	3.46 ± 0.1 ^{a}	10.28 ± 0.6 ^{b}	17.69 ± 1.1 ^{c}
	Sum of flavonols	15.02	76.22	95.07	67.49	29.83	65.47	58.21
	Total	34.07	136.69	266.67	176.92	69.13	131.21	163.22

* Values are means ± standard deviation. $n = 6$; Amounts of phenolic acids and flavonols were converted into caffeic acid (caffeic acid derivatives), protocatechuic acid (protocatechuic acid derivatives), ferulic acid (ferulic acid derivatives), quercetin 3-*O*-glucoside (quercetin derivatives), isorhamnetin 3-*O*-glucoside (isorhamnetin derivatives); $^{a-f}$ the same letters within the same row were not significantly different.

Table 3. In vitro antioxidant capacity of different varieties of prickly pear (mMol Trolox/kg of dry matter) *.

[mMol Trolox/kg]	Fresa	Nopal Alargado	Nopal Espinoso	Nalle	Nopal Ovalado	Nopal Tradicional	Orito
DPPH	1.39 ± 0.0 ^{a}	2.62 ± 0.0 ^{b}	4.99 ± 0.0 ^{d}	3.11 ± 0.0 ^{c}	2.57 ± 0.0 ^{b}	1.92 ± 0.0 ^{a}	2.44 ± 0.0 ^{b}
ABTS	7.08 ± 0.1 ^{a}	10.43 ± 0.2 ^{c}	11.67 ± 0.2 ^{e}	11.33 ± 0.1 ^{d}	9.97 ± 0.1 ^{b}	10.04 ± 0.1 ^{b,c}	11.49 ± 0.1 ^{d}
FRAP	3.67 ± 0.0 ^{a}	6.14 ± 0.0 ^{c}	8.89 ± 0.1 ^{f}	6.77 ± 0.0 ^{d}	6.86 ± 0.1 ^{d}	5.9 ± 0.0 ^{b}	7.22 ± 0.1 ^{e}

* Values are means ± standard deviation. $n = 6$; $^{a-f}$ the same letters within the same row were not significantly different.

The highest in vitro antioxidant activity determined by DPPH, ABTS and FRAP methods was observed in the "Nopal espinoso" variety (4.99, 11.67 and 15.64 mmol Trolox/kg DM, respectively), while the "Fresa" variety was characterized by the lowest results—1.39, 7.08 and 3.67 mmol Trolox/kg DM, respectively. Our results were slightly lower than those reported by other authors. Andreu et al. [6] reported that ABTS in vitro antioxidant capacity of prickly pear cladode and fruit was 18.8 mmol Trolox/kg (dw) and 26.8 mmol Trolox/kg (dw), respectively, DPPH in vitro activity was 17.4 mmol Trolox/kg (dw) and 58.0 mmol Trolox/kg (dw), respectively, while FRAP in vitro capacity for cladode and fruit was 85.3 mmol Trolox/kg (dw) and 40.2 mmol Trolox/kg (dw), respectively. These differences may be due to anatomical part of the prickly pear examined. Literature data [31,32] indicate that polyphenols play an important role in antioxidant activity, in particular scavenging DPPH. The content and proportion of phenolic compounds in plants are closely related to the anatomical part. The in vitro antioxidant capacity of the tested seeds was positively correlated with the amount of polyphenolic substances. The results obtained show a high correlation coefficient between the content of polyphenolic compounds and in vitro antioxidant capacity determined by the DPPH, ABTS and FRAP methods (R^2 = 0.77 for DPPH, 0.71 for ABTS and 0.73 for FRAP).

The influence of polyphenolic compounds on antioxidant capacity has been repeatedly described in the literature. The results clearly show that polyphenols play a significant role in shaping antioxidant capacity. Their power to scavenge free radicals depends on their structure and the group to which they belong [33–36]. These results agree with the study presented by Faraq et al. [20] who analyzed the antioxidant effect of *O. ficus-indica* in the crude extracts of pulps and peels. They showed the highest in vitro antioxidant activity in extracts with the highest total phenolic content, when tested using ABTS and DPPH assays [20]. These data were also confirmed by Chougui et al. [8].

2.4. Protein and Amino Acid Composition

The protein content was influenced by the variety of prickly pear (Table 4).

Table 4. Fat and protein content of seeds of different varieties of prickly pear (g/100 g dry matter) *.

[g/100 g]	Fresa	Nopal Alargado	Nopal Espinoso	Nalle	Nopal Ovalado	Nopal Tradicional	Orito
Protein	9.97 ± 0.5 ^{f}	9.45 ± 0.2 ^{d}	9.61 ± 0.2 ^{e}	6.36 ± 0.2 ^{a}	9.97 ± 0.3 ^{f}	7.69 ± 0.4 ^{c}	7.09 ± 0.1 ^{b}
Fat	4.94 ± 0.2 ^{b}	6.17 ± 0.3 ^{d}	5.24 ± 0.3 ^{c}	2.61 ± 0.1 ^{a}	3.25 ± 0.2 ^{c}	5.97 ± 0.3 ^{d}	4.39 ± 0.2 ^{c}

* Values are means ± standard deviation. n = 6; $^{a–f}$ the same letters within the same row were not significantly different.

The "Fresa" and "Nopal ovalado" varieties were characterized by the significantly highest protein content (9.97 g/100 g DM), as compared to the "Orito" variety where this value was the lowest (7.09 g/100 g DM). Several studies have reported that prickly pear seeds are considered a nontraditional source of protein [14,37,38] and the protein content found in these studies was higher compared to the present data. Özcan and Juhaimi [11] and El Mannoubi et al. [39] found that the same seeds contain 4.78% crude protein. These differences may be influenced by growth conditions, variety, genetic factors, harvesting time, soil properties or geographical variations of prickly pear plants.

Analysing the amino acid composition of the protein contained in prickly pear seeds (*O. ficus-indica*), it was found that the variety had a significant effect on the content of individual amino acids and their sum in the tested samples (Table 5).

Protein from the prickly pear seeds of the "Nopal alargado" variety contained the highest values for total indispensable amino acids (IAAs) and total dispensable amino acids (DAAs)—21.60 and 47.36 g/100 g, respectively, while the "Fresa" variety was characterized by the lowest total IAA and DAA content—10.30 and 22.90 g/100 g, respectively. Protein from "Nopal tradicional", "Nopal ovalado", "Orito" and "Nalle" prickly pear varieties was characterized by a similar content of total IAAs and DAAs, on average 18.97 and 43.58 g/100 g, respectively. Glutamic acid was the predominant amino acid followed by arginine, aspartic acid and leucine, independent of prickly pear variety. These results

are in agreement with the study presented by Nassar [38] who analyzed the chemical composition and functional properties of prickly pear seed flour and its protein concentrate. However, a higher total IAA content and therefore higher IAA/DAA ratio (0.65) was noted in comparison to the present study, where this value was found to be on average 0.44, independent of prickly pear variety. These data were also confirmed by Sawaya et al. [40].

The value of the proteins derived from the seeds is determined by the presence of a set of amino acids, including all exogenous amino acids, i.e., lysine, methionine, tryptophan, threonine, valine, leucine, isoleucine and phenylalanine, and the relatively exogenous histidine. However, the most important in nutrition are lysine, sulphur amino acids, threonine, tryptophan, valine and isoleucine. The quality of the protein in the tested seeds was evaluated according to its content of IAAs in comparison to the reference protein pattern of FAO/WHO [41], as shown in Table 5. From the data obtained, it can be observed that none of the tested protein from prickly pear seeds of different varieties contained an adequate amount of all IAAs. In "Nopal tradicional", "Nopal alargado", "Nopal ovalado", "Nopal espinoso", "Orito" and "Nalle" prickly pear varieties, the first limiting amino acid was lysine and the second and third were methionine and cysteine, except for "Fresa" seeds where an inverse relationship was observed. This means that protein from prickly pear seeds is incomplete protein. On the other hand, Sawaya et al. [40] stated that prickly pear protein is a significantly good source of the sulphur amino acids (Met + Cys), which are generally the most limiting amino acids in seed proteins. In this respect, prickly pear seed protein is comparable to sesame protein which is high in sulphur-containing amino acids, containing about 6 g of methionine and cysteine/100 g.

2.5. Fat and Fatty Acid Composition

Table 6 shows the composition of the fatty acids in the fat extracted from the prickly pear seeds being analyzed.

The oil content obtained from the seven cultivars ranged from 2.61% for "Nalle" to 7.69% for "Nopal ovalado" (Table 6). De Wit et al. [9] obtained slightly higher values, ranging from 4.09% to 8.76% for 42 cultivars from South Africa, while those obtained by Labuschagne and Hugo [42] were slightly lower—from 2.24% to 5.59%. These differences may be due to growth conditions, cultivar and genetic factors as well as harvesting time, degree of ripeness or fruit processing [9]. The oil was mainly composed of unsaturated fatty acids, including polyunsaturated fatty acids (PUFA), that is, linoleic acid, and monounsaturated fatty acids (MUFA), mostly oleic acid, with a lower but significant fraction of saturated acids (SFA). Overall, 13 different fatty acids were identified and assessed. The dominant fatty acid was linoleic acid with a content varying between 57.72% ("Nopal ovalado") and 63.11% ("Nopal espinoso"). Linoleic acid ($n-3$) was detected at a concentration lower than 1%, with the exception of "Nopal tradicional" and "Nopal espinoso" varieties. The highest PUFA content was measured in the variety "Nopal espinoso" (64.33%), and the lowest in "Nopal ovalado" (58.74%). Similar levels of PUFA were observed by Cirimina at al. [43] in oil extracted from Sicilian varieties. Among the MUFA, oleic acid occurred in the greatest amounts, from 19.37% (in "Nopal espinoso") to 21.79% (in "Nopal tradicional"). Although there was a slight difference in MUFA content between the varieties analyzed, the average MUFA content was highest in the variety "Nopal tradicional". Two dominant saturated fatty acids were palmitic acid with the share between 12.47% (in "Nopal espinoso") to 15.06% (in "Nopal alargado") and stearic acid, which varies from 2.56% (in "Nopal espinoso") to 4.10% (in "Nalle"). The obtained results are in accordance with those of other researchers. Observed differences between analyzed cultivars could be connected with genetic factors.

Table 5. Amino acid concentration in different varieties of prickly pear seeds (g/100 g of protein) *.

Amino Acids	Fresa	Nopal Alargado	Nopal Espinoso	Nalle	Nopal Ovalado	Nopal Tradicional	Orito	FAO/WHO Reference Pattern (1991)
IAA *								
LEU	2.41 ± 0.04 ^{b}	4.75 ± 0.08 ^{a}	3.55 ± 0.28 ^{a,b}	4.23 ± 0.11 ^{a}	4.04 ± 0.43 ^{a,b}	4.05 ± 0.35 ^{a,b}	4.24 ± 0.19 ^{a}	6.60
Leucine	1.33 ± 0.03 ^{b}	2.54 ± 0.04 ^{a}	1.98 ± 0.12 ^{a,b}	2.45 ± 0.18 ^{a}	2.24 ± 0.24 ^{a}	2.24 ± 0.15 ^{a}	2.32 ± 0.12 ^{a}	2.80
Isoleucine	0.32 ± 0.02 ^{b}	0.56 ± 0.02 ^{b}	0.72 ± 0.21 ^{a}	0.63 ± 0.07 ^{a,b}	0.78 ± 0.14 ^{a}	0.65 ± 0.17 ^{a,b}	0.64 ± 0.09 ^{a,b}	
Methionine	0.22 ± 0.01 ^{b}	0.58 ± 0.05 ^{a}	0.44 ± 0.06 ^{a,b}	0.58 ± 0.04 ^{a}	0.46 ± 0.11 ^{a}	0.60 ± 0.06 ^{a}	0.49 ± 0.05 ^{a}	
Cysteine	0.54	1.14	1.16	1.21	1.24	1.25	1.13	2.50
Cysteine+Methionie	1.32 ± 0.04 ^{b}	3.13 ± 0.09 ^{a}	2.14 ± 0.18 ^{a,b}	2.57 ± 0.10 ^{a}	2.53 ± 0.34 ^{a}	2.54 ± 0.29 ^{a}	2.53 ± 0.11 ^{a}	
Phenyloalanie	1.08 ± 0.08 ^{b}	2.39 ± 0.09 ^{a}	1.83 ± 0.11 ^{a,b}	2.06 ± 0.02 ^{a}	2.17 ± 0.29 ^{a}	2.08 ± 0.16 ^{a}	2.08 ± 0.05 ^{a}	3.40
Threonine	1.93	4.77	3.28	4.63	3.82	4.08	3.89	6.30
Phenyloalanine+Threonine	1.48 ± 0.05 ^{b}	2.59 ± 0.04 ^{a}	2.09 ± 0.17 ^{a,b}	2.41 ± 0.04 ^{a,b}	2.43 ± 0.25 ^{a,b}	2.32 ± 0.21 ^{a,b}	2.39 ± 0.09 ^{a,b}	5.80
Lysine	0.61 ± 0.03 ^{b}	1.64 ± 0.10 ^{a}	1.14 ± 0.11 ^{a,b}	1.42 ± 0.14 ^{a}	1.29 ± 0.25 ^{a,b}	1.54 ± 0.19 ^{a}	1.36 ± 0.09 ^{a,b}	
Tyrosine	1.53 ± 0.25 ^{b}	3.43 ± 0.06 ^{a}	2.60 ± 0.21 ^{a,b}	2.77 ± 0.15 ^{a}	3.00 ± 0.31 ^{a}	2.94 ± 0.10 ^{a}	2.82 ± 0.10 ^{a}	3.50
Valine	10.30	21.60	16.49	19.12	18.94	18.97	18.86	
DAA **								
ASP	2.78 ± 0.23 ^{c}	5.82 ± 0.08 ^{a}	4.42 ± 0.37 ^{a,b}	5.16 ± 0.54 ^{a}	5.33 ± 0.74 ^{a}	4.99 ± 0.40 ^{a,b}	5.05 ± 0.16 ^{a,b}	
Aspartic acid	5.44 ± 0.41 ^{b}	13.85 ± 0.23 ^{a}	9.69 ± 1.33 ^{a,b}	12.02 ± 0.33 ^{a}	11.44 ± 1.73 ^{a,b}	11.83 ± 1.13 ^{a}	11.40 ± 0.36 ^{a,b}	
Glutamic acid	1.40 ± 0.10 ^{b}	2.76 ± 0.07 ^{a}	2.40 ± 0.20 ^{a,b}	2.66 ± 0.22 ^{a}	2.74 ± 0.35 ^{a}	2.55 ± 0.18 ^{a}	2.51 ± 0.08 ^{a,b}	
Serine	1.97 ± 0.13 ^{b}	4.79 ± 0.17 ^{a}	3.66 ± 0.46 ^{a,b}	4.13 ± 0.11 ^{a}	3.65 ± 0.43 ^{a,b}	4.18 ± 0.42 ^{a}	4.27 ± 0.17 ^{a}	
Glycine	2.14 ± 0.03 ^{b}	3.65 ± 0.05 ^{a}	3.18 ± 0.21 ^{a,b}	3.48 ± 0.45 ^{a}	4.02 ± 0.27 ^{a}	3.71 ± 0.23 ^{a}	3.21 ± 0.21 ^{a,b}	
Alanine	1.44 ± 0.39	2.77 ± 0.53	2.43 ± 0.12	2.66 ± 0.12	2.31 ± 0.22	2.67 ± 0.57	2.63 ± 0.58	
Histidine	4.21 ± 0.04 ^{c}	9.58 ± 0.52 ^{a}	6.09 ± 0.67 ^{a,b}	8.08 ± 0.43 ^{a,b}	7.62 ± 1.03 ^{a,b}	8.17 ± 0.85 ^{a,b}	7.93 ± 0.42 ^{a,b}	
Arginine	3.52 ± 0.22 ^{b}	4.15 ± 0.17 ^{a,b}	7.39 ± 0.32 ^{a}	6.89 ± 0.18 ^{a,b}	7.09 ± 0.46 ^{a,b}	5.18 ± 0.16 ^{a,b}	4.71 ± 0.28 ^{a,b}	
Proline	22.90	47.36	39.25	45.08	44.20	43.28	41.75	
IAA/DAA	0.45	0.46	0.42	0.42	0.43	0.44	0.45	
I limiting amino acid	Met+Cys	Lys	Lys	Lys	Lys	Lys	Lys	
II limiting amino acid	Lys	Met+Cys	Met+Cys	Met+Cys	Met+Cys	Met+Cys	Met+Cys	
TP	9.97 ± 0.5 ^{a}	9.45 ± 0.2 ^{c}	9.61 ± 0.2 ^{b}	6.36 ± 0.2 ^{f}	9.97 ± 0.3 ^{a}	7.69 ± 0.4 ^{d}	7.09 ± 0.1 ^{e}	

* Values are means ± standard deviation. $n = 4$; $^{a-f}$ the same letters within the same row were not significantly different; * IAA-indispensable amino acids; ** DAA-dispensable amino acids.

Table 6. Fat content (%) and fatty acid composition (% of total fatty acid profile) of prickly pear seeds as affected by cultivar *.

	Fresa	Nopal Alargado	Nopal Espinoso	Nalle	Nopal Ovalado	Nopal Tradicional	Orito
Fat content (%)	4.99 ± 0.2 ^{b}	6.17 ± 0.3 ^{d}	5.24 ± 0.3 ^{c}	2.61 ± 0.1 ^{a}	7.69 ± 0.2 ^{e}	5.97 ± 0.3 ^{d}	5.55 ± 0.2 ^{c}
Fatty acid (%)							
Miristic acid (C 14:0)	0.01 ± 0.01 ^{a}	0.02 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	0.02 ± 0.01 ^{a}
Palmitic acid (C 16:0)	14.56 ± 0.53 ^{d}	15.06 ± 0.35 ^{e}	12.47 ± 0.41 ^{a}	13.83 ± 0.28 ^{b}	14.33 ± 0.26 ^{c}	13.77 ± 0.82 ^{b}	14.16 ± 0.51 ^{c}
Palmitooleic acid (C 16:1)	0.82 ± 0.28 ^{a}	0.79 ± 0.12 ^{a}	0.78 ± 0.12 ^{a}	0.84 ± 0.15 ^{a}	0.85 ± 0.25 ^{a}	0.91 ± 0.32 ^{a}	0.83 ± 0.30 ^{a}
Margaric acid (C 17:0)	0.02 ± 0.01 ^{a}	0.01 ± 0.00 ^{a}	0.03 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	0.02 ± 0.01 ^{a}	0.01 ± 0.00 ^{a}	0.02 ± 0.01 ^{a}
Stearic acid (C 18:0)	3.50 ± 0.22 ^{b}	3.36 ± 0.17 ^{b}	2.56 ± 0.21 ^{a}	4.12 ± 0.16 ^{c}	4.00 ± 0.27 ^{c}	3.62 ± 0.31 ^{b}	3.88 ± 0.28 ^{c}
Oleic acid (C 18:1)	20.26 ± 0.61 ^{b}	20.48 ± 0.51 ^{b}	19.37 ± 0.37 ^{a}	21.23 ± 0.41 ^{d}	21.64 ± 0.41 ^{e}	21.79 ± 0.54 ^{e}	20.77 ± 0.41 ^{c}
Liloleic acid (C 18:2)	60.04 ± 0.43 ^{d}	59.38 ± 0.66 ^{c}	63.11 ± 0.82 ^{e}	58.11 ± 0.48 ^{c}	57.72 ± 0.70 ^{a}	58.30 ± 0.72 ^{b}	59.29 ± 0.55 ^{c}
Linolenic acid (C 18:3)	0.23 ± 0.10 ^{a}	0.33 ± 0.10 ^{a}	1.10 ± 0.19 ^{d}	0.54 ± 0.07 ^{b}	0.89 ± 0.20 ^{c}	1.01 ± 0.09 ^{d}	0.46 ± 0.10 ^{b}
Arachidic acid (C 20:0)	0.20 ± 0.01 ^{a}	0.21 ± 0.01 ^{a}	0.19 ± 0.03 ^{a}	0.20 ± 0.02 ^{a}	0.20 ± 0.02 ^{a}	0.21 ± 0.02 ^{a}	0.21 ± 0.02 ^{a}
Gondoic acid (C 20:1)	0.09 ± 0.01 ^{a}	0.11 ± 0.01 ^{a}	0.11 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}	0.10 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}
Eicosatrienoic acid (C 20:3)	0.13 ± 0.02 ^{a}	0.15 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}	0.13 ± 0.02 ^{a}	0.14 ± 0.01 ^{a}	0.14 ± 0.01 ^{a}
Behenic acid (C 22:0)	0.11 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}	0.10 ± 0.01 ^{a}	0.08 ± 0.01 ^{a}	0.10 ± 0.10 ^{a}	0.09 ± 0.01 ^{a}
Lignoceric acid (C 24:0)	0.03 ± 0.02 ^{a}	0.01 ± 0.01 ^{a}	0.03 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}	0.02 ± 0.01 ^{a}	0.01 ± 0.01 ^{a}
∑ MUFA	21.17 ± 0.6 ^{b}	21.38 ± 0.33 ^{b}	20.26 ± 0.32 ^{a}	22.16 ± 0.34 ^{d}	22.61 ± 0.29 ^{e}	22.71 ± 0.55 ^{e}	21.72 ± 0.41 ^{c}
∑ PUFA	60.40 ± 0.38 ^{d}	59.86 ± 0.51 ^{c}	64.33 ± 0.50 ^{e}	59.56 ± 0.50 ^{c}	58.74 ± 0.62 ^{a}	59.55 ± 0.72 ^{b}	59.89 ± 0.48 ^{c}
∑ SFA	18.43 ± 0.34 ^{d}	18.76 ± 0.36 ^{e}	15.41 ± 0.31 ^{a}	18.28 ± 0.17 ^{c}	18.65 ± 0.45 ^{e}	17.74 ± 0.21 ^{b}	18.39 ± 0.25 ^{d}

MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids, SFA—saturated fatty acids. * Values are means ± standard deviation. $n = 6$; $^{a-e}$ the same letters within the same row were not significantly different.

3. Materials and Methods

3.1. Reagents and Standards

Acetonitrile, formic acid, methanol, DPPH (1,1-diphenyl-2 picrylhydrazyl radical), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ [2,4,6-tri(2-pyridyl)-s-triazine], caffeic acid and boron trifluoride in methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Quercetin 3-*O*-galactoside, isorhamnetin 3-*O*-glucoside and ferulic acid were purchased from Extrasynthese (Lyon, France). Diethyl ether was purchased from Chempur (Piekary Śląskie, Poland). Ninhydrine, hydrantine, methylcellosolve and sodium acetate buffer were purchased from Ingos company (Prague, Czech Republic).

3.2. Plant Material and Sample Processing

Prickly pear fruits from "Nopal alargado", "Nopal espinoso", "Nopal ovalado" and "Nopal tradicional" cultivars were collected at the experimental field station of Miguel Hernández University, in the province of Alicante, Spain (02°03′50″ E, 38°03′50″ N, and 25 masl). Another three cultivars were collected from private farms of Murcia ("Fresa" cultivar) and Alicante ("Nalle" and "Orito" cultivars). Plant species were identified by an expert botanist from the Department of Plant Sciences and Microbiology, using the protocol by García-Rollán [44].

The harvest of the fruits was done during the summer of 2018 and 2019. Fruits were manually picked at the same ripening stage, and immediately transported to the laboratory. In this way, a total of 30 fruits per cultivar and year were collected. One voucher of each cultivar is kept in the Miguel Hernández University herbarium (#152019). Table 7 presents the characteristics of the analyzed prickly pear cultivars.

Table 7. Characteristics of the analyzed prickly pear cultivars.

Cultivar	Characteristics
Fresa	Red cultivar. High amount of betalains and polyphenols. Weight of the fruit: 100–140 g.
Nalle	Green cultivar. Average weight of the fruit 90−100 g.
Nopal alargado	Green-yellow cultivar without prickles. Weight of the fruit: 120–160 g.
Nopal espinoso	Highly spiny green cultivar. Weight of the fruit 60–80 g.
Nopal ovalado	Green-yellow cultivar. Weight of the fruit: 90–120 g.
Nopal tradicional	Traditional cultivar (orange). Weight of the fruit: 90–120 g.
Orito	Orange cultivar. Average weight of the fruit 110−140 g. High fruit production.

Once in the laboratory, the spines of fruits were removed with a brush under tap water for 2 min, peeled, the fruits were cut into small pieces and submerged in water for a week to make the removal of the pulp easier. After this time, the water was removed, and the seeds were washed under tap water for two minutes to remove the pulp completely. After that, the seeds were placed on blotting paper and were left to dry at room temperature for ten days, and frozen at −80 °C until the time of analysis.

3.3. Identification and Quantification of Polyphenols by the UPLC-PDA-MS Method

For the extraction and determination of polyphenols, a protocol described before by Kolniak-Ostek [45] was followed.

Identification of polyphenols of prickly pear extracts was carried out using an ACQUITY Ultra Performance LC system equipped with a photodiode array detector with a binary solvent manager (Waters Corporation, Milford, MA, USA) with a mass detector G2 Q-Tof micromass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative mode. The separation of individual polyphenols was carried out using a UPLC BEH C18 column (1.7 mm, 2.1 × 100 mm, Waters) at 30 °C.

The samples (10 µL) were injected, and the elution was completed in 15 min with a sequence of linear gradients and constant flow rates of 0.42 mL/min. The mobile phase consisted of solvent A (0.1% formic acid, *v*/*v*) and solvent B (100% acetonitrile). The linear gradient was as follows: 0.0–1.0 min, 99% A, 0.42 mL/min (isocratic), 1.0–12.0 min, 65.0% A, 0.42 mL/min (linear), 12.0–12.5 min, 99% A, 0.42 mL/min (linear), 12.5–13.5 min, 99% A, 0.42 mL/min (isocratic). The analysis was carried out using full-scan, data-dependent MS scanning from *m*/*z* 100–1500. Leucine enkephalin was used as the reference compound at a concentration of 500 pg/mL, and the $[M - H]^-$ ion at 554.2615 Da was detected. The $[M - H]^-$ ions were detected during a 15 min analysis performed within ESI–MS accurate mass experiments, which were permanently introduced via the LockSpray channel using a Hamilton pump. The lock mass correction was ±1.000 for the mass window. The mass spectrometer was operated in negative-ion mode, set to the base peak intensity (BPI) chromatograms, and scaled to 12,400 counts per second (cps) (100%). The optimized MS conditions were as follows: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 100 C, desolvation temperature of 300 °C, and desolvation gas (nitrogen) flow rate of 300 L/h.

Collision-induced fragmentation experiments were performed using argon as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Characterization of the single components was carried out via the retention time and the accurate molecular masses. Each compound was optimized to its estimated molecular mass in the negative mode, before and after fragmentation. The data obtained from UPLC–MS were subsequently entered into the MassLynx 4.0 ChromaLynx Application Manager software (Waters).

The runs were monitored at the following wavelengths: phenolic acids at 320 nm and flavonol glycosides at 360 nm. The PDA spectra were measured over the wavelength range of 200–600 nm in steps of 2 nm. The retention times and spectra were compared to those of the authentic standards.

The quantification of phenolic compounds was performed by external calibration curves ($R^2 > 0.999$), using reference compounds selected based on the principle of structure-related target analyte/standard (chemical structure or functional group). Standard stock solutions were diluted to appropriate concentrations (five calibration points were used in each case) for the plotting of calibration curves. The linearity was obtained by plotting the peak areas versus the corresponding concentrations (ppm) of each analyte. The calibration curve for caffeic acid was used to quantify caffeic acid hexosides. The calibration curve of ferulic acid was used to quantify ferulic acid derivatives. Protocatechuic acid hexoside was quantified with protocatechuic acid calibration curve.

The calibration curves of quercetin, quercetin rutinoside, and 3-*O*-galactoside were used to quantify quercetin derivatives. For isorhamnetin quantification, isorhamnetin 3-*O*-rutinoside and 3-*O*-glucoside were used.

All determinations were done in triplicate ($n = 3$). The results were expressed as milligrams per kg of dry matter (DM).

3.4. Antioxidant Capacity

The total in vitro antioxidant potential of samples was determined using a ferric reducing ability of plasma (FRAP) assay by Benzie and Strain [46] as a measure of antioxidant power. The DPPH and ABTS radical scavenging activities of samples were determined according to the method of Yen and Chen [47] and Re et al. [48]. The powder samples (0.5 g) were extracted with 10 mL of 80% methanol acidified with 1% HCl (*v*/*v*). The extraction was performed by incubation for 20 min under sonication (300 W, 40 kHz; Sonic 6D, Polsonic, Warsaw, Poland) with occasional shaking. This method has proved to be adequate for complete extraction. Next, the slurry was centrifuged at 19,000 g for 10 min, and the supernatant was filtered through a hydrophilic PTFE 0.20 µm membrane (Millex Samplicity Filter, Merck) and used for analysis A standard curve was prepared using different concentrations of Trolox. All determinations were performed in triplicate using a Shimadzu UV-2401 PC spectrophotometer (Kyoto, Japan). The results were corrected for dilution and expressed in µmol Trolox Equivalent per kg of DM.

3.5. Proximate Composition

The total protein content was evaluated according to the Kjeldahl method of the Association of Analytical Chemists [49]. Approximately 1 g of raw material was hydrolyzed with 25 mL concentrated sulfuric acid (H_2SO_4) containing one catalyst tablet in a heat block (Büchi Digestion Unit K-424, Labortechnik AG, Flawil, Switzerland) at 370 °C for 2 h. After cooling, H2O was added to the hydrolysates before neutralization, using a Büchi Distillation Unit K-355 (Athens, Greece) and titration. A nitrogen to protein conversion factor of 6.25 was used to calculate total protein. Fat content was determined according to the standard method of the Association of Official Analytical Chemists International [50]. A sample of 2 g of ground seeds was hydrolyzed using 4N HCl. Fat extraction and solvent (diethyl ether) removal were performed in an automated Soxhlet apparatus B-811 (Büchi Labortechnik AG, Flawil, Switzerland); the extraction time was 180 min.

3.6. Amino Acid Analysis

The amino acid composition of prickly pear seeds was determined by ion-exchange chromatography after 23 h' hydrolysis with 6 N HCl at 110 °C. After cooling, filtering and washing, the hydrolyzed sample was evaporated in a vacuum evaporator at a temperature below 50 °C. The dry residue was dissolved in a buffer of pH 2.2. The prepared sample was analyzed using the ninhydrin method [51,52]. The pH 2.6, 3.0, 4.25, and 7.9 buffers were applied. The ninhydrin solution was buffered at pH 5.5. The hydrolyzed amino acids were determined using an AAA-400 analyzer (INGOS, Prague, Czech Republic). A photometric detector was used, working at two wavelengths, 440 nm and 570 nm. A column of 350 × 3.7 mm, packed with ion exchanger Ostion LG ANB (INGOS) was utilized. Column temperature was kept at 60−74 °C and the detector at 121 °C. The calculations were carried out relative to an external standard. No analysis of tryptophan was carried out.

3.7. Quantitative Evaluation of Protein Quality

The amino acid content in opuntia seeds was expressed on the nitrogen basis (g per 16 g N) and it was compared to a reference protein. The amino acid pattern for high-quality protein established by the Joint Food and Agriculture Organisation/World Health Organisation (FAO/WHO) Committee in 1991. Levels were calculated on the basis of the essential amino acid composition of the chemical scores (CS), according to the Mitchell and Block method [53] and the integrated EAA index [54].

3.8. Fatty Acids Analysis

Fatty acid composition of seeds oil was determined by GC, according to the American Oil Chemists' Society Official Method Ce 1-62 [55]. Boron trifluoride in methanol was used as methylating agent. Fatty acid methyl esters (FAMEs) were analyzed by an Agilent 7820A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA), equipped with a capillary column RTX-2330, 105 m length, 0.25 mm i.d., 0.2 μm film thickness (Restek, Bellefonte, PA, USA). Injector and detector (FID) temperatures were 260 °C and 280 °C, respectively. Column temperature was set to 200 °C for 21 min, then increased to 250 °C at a rate of 10 °C/min; the final temperature was held for 6 min. Helium was used as a carrier gas, at a linear flow rate of 35 cm/sec. Individual FAMEs were identified using the Certified Reference Material (CRM) 47885 (Supelco, Bellefonte, PA, USA). The following fatty acid combinations were calculated: total saturate fatty acids (SFA), total monounsaturated fatty acids (MUFA) and total polyunsaturated fatty acids (PUFA).

4. Conclusions

The research conducted has shown that the seeds of the prickly pear (*O. ficus-indica*) are an excellent source of nutrients and health-promoting substances. Due to the high content of phenolic compounds, they are characterized by strong antioxidant properties. The seeds of the prickly pear are usually discarded after extraction of pulp, providing a large amount of seeds as waste. Prickly pear

seeds can be used as a low-cost source of health-promoting compounds. Additionally, this would contribute to reducing the amount of waste generated during the production process.

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Volatile composition of prickly pear fruit pulp from six Spanish cultivars

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Abstract: This research analyzed the volatile composition of the fruits pulp of six prickly pear cultivars (NT, NE, NO, NA, FR, and ORI) growing in Spain, by headspace solid-phase microextraction and gas chromatography (GC-MS and GC-FID). A total of 35 compounds were isolated, identified, and quantified, with aldehydes, alcohols, and terpenes being the predominant chemical families, and esters, ketones, linear hydrocarbons, and terpenoids being also found. Nonanol, 2,6-nonadienal, 1-hexanol, 2-hexenal, and D-limonene were the predominant compounds. NT and FR cultivars showed the highest concentration of total volatile compounds. On the other hand, NE and NO cultivars presented the lowest concentration. Future studies on sensory evaluation are required to determine the sensory quality of the fruits of these Spanish cultivars.

Keywords: alcohols, aldehydes, gas chromatography, HS-SPME, *Opuntia ficus-indica*

1. INTRODUCTION

Opuntia ficus-indica (L). Mill., commonly known as prickly pear, cactus pear, or nopal cactus, is a tropical or subtropical plant which can grow in arid and semi-arid climates. Prickly pear is native to tropical and subtropical America but at present is naturalized in all continents (Sáenz, 2006). This plant is mainly known by their fruits, popularly named "figs" or "tunas", but their cladodes are also consumed, principally in Mexico, which is the country with the largest area under cultivation (Reyes-Agüero, Aguirre, Carlín-Castelán, & González-Durán, 2013) and also the largest producer (FAO, 2018).

There is ample evidence of the health benefits of prickly pear: it is a source of nutrients and vitamins (Cherkaoui-Malki et al., 2014; FAO, 2018; Feugang, 2007), it shows antioxidant properties due to its phenolic content and antioxidant activity (Ammar, Ennouri, & Attia, 2015; Andreu, Nuncio-Jáuregui, Carbonell-Barrachina, Legua, & Hernández, 2017; Butera et al., 2002; Oumato et al., 2016) and presents medicinal use: anticancer effect (FAO, 2018; Feugang, 2007; Serra, Poejo, Matias, Bronze & Duarte, 2013), treatment of hyperglycemia (FAO, 2018; Frati, Jiménez, & Ariza, 1990; Lopez, 2007) and treatment of high levels of cholesterol (Cherkaoui-Malki et al., 2014; Ennouri et al., 2006) among others. Besides, prickly pear seed oil is rich in tocopherols, which are biologically highly active natural antioxidants, and essential and unsaturated fatty acids (Matthäus & Ozcan, 2011; Ozcan & Al Juhaimi, 2011). Prickly pear has also been studied for other uses and properties, for example CO_2 uptake (Nobel, Pimienta-Barrios, Hernández, & Ramírez-Hernández, 2002; Nobel, Valenzuela-Tapia, Zañudo -Hernández, Pimenta-Barrios, & Rosas-Espinoza, 2004), phytoremediation of soils (Bañuelos & Lin, 2010; Escobar-Alvarado, Vaca-Mier, & Rojas-Valencia, 2018) and biofuel production (Sánchez-Godoy, 2012; Santos et al., 2016).

Sensory analysis data of prickly pear fruits in fresh is limited. By contrast, sensory analysis was performed in processed products from prickly pear fruits, like syrups (Sáenz, Estévez, Sepúlveda, & Mecklenburg, 1998), juices (Atef, Abou-Zaid, Ibrahim, Ramadan, & Nadir, 2013; El-Samahy, El-Hady, Habiba, & Moussa-Ayoub, 2007; Rothman, De Wit, Bothma, & Hugo, 2012), nectars (El-Samahy, El-Mansy, Bahlol, El-Desouky, & Ahmed, 2008), and sheets (Atef et al., 2013; El-Samahy et al., 2007). These studies evaluated color, aroma, acidity, taste, texture, and acceptability among other characteristics.

Volatile compounds directly affect the sensory quality of fruits, whose aroma is composed by a complex group of chemical substances such as alcohols, aldehydes, terpenes, ketones, and esters among others. Arena, Campisi, Fallico, Lanza, and Maccarone (2001) reported that the family predominating the aroma pro-file of this fruit was alcohols; however, Farag, Maamoun, Ehrlich, Fahmy, and Wesjohann (2017), in a more recent study, concluded that short chain aldehydes and acids were the major volatile classes. These compounds generally show a low concentration in fruits and their variability depends on climatological conditions, cultivar, maturity, and storage conditions among other factors (Vázquez-Araújo et al., 2011).

The aim of this study was to determine the volatile composition of fruits pulp of six cultivars of prickly pear, all grown in Spain under homogeneous farming conditions. The information generated will help farmers in selecting and growing those cultivars with the highest contents of volatile compounds.

2. MATERIALS AND METHODS

2.1 Plant material and sample processing

The fruits of six cultivars of *O. ficus-indica* were used for this study. Four of them (NA, NT, NE and NO) were collected at the experimental field station of Miguel Hernández University in the province of Alicante, Spain (02°03 50 E, 38°03 50 N, and 25 masl). FR and ORI cultivars were collected from private farms of Murcia and Alicante, respectively. Plant species were identified by an expert botanist from the Department of Plant Sciences and

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Microbiology, using the protocol by García-Rollán (1981). One voucher of each cultivar is kept in the Miguel Hernández University herbarium (#152019).

The harvest of the fruits was done during the summer of 2017 and 2018. Three different batches of samples were prepared using 10 uniform fruits of each cultivar; fruits were manually picked at the same ripening stage, and immediately transported to the laboratory for preparation and further analyses. In this way, a total of 30 fruits per cultivar were used for the analyses. Once on the laboratory, the spines of fruits were removed with a brush under tap water for 2 minutes, peeled, and fruits of each batch were cut, grinding for 10 s in a grinder (Taurus Aromatic Ver II; Taurus Group, Barcelona, Spain), and frozen at −80 °C until the time of analysis.

2.2 Extraction procedure of volatile aroma compounds

Headspace solid-phase microextraction (HS-SPME) was the method selected to study the volatile composition of the samples under analysis. After several preliminary test to optimize the extraction system, each sample (10 g of the mixture described above) was placed together with 10 mL of water, 1.5 g of salt, and β-ionone as internal standard (10 µL of 1,000 mg/L) into 50 mL vials with polypropylene caps and a polytetrafluoroethylene/silicone septum. Then, a magnetic stirring bar was added, and the vial was placed in a water bath with controlled temperature and automatic stirring. The vials were equilibrated during 5 min at 40 °C in the bath and after that a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane fiber was exposed to the sample headspace for 30 min at 40 °C. Later, desorption of the volatile compounds from the fiber coating was performed in the injection port of the CG-MS during 3 min. Extraction experiments were run in triplicate.

2.3 Chromatographic analyses

The isolation and identification of the volatile compounds was carried out on a gas chromatograph (GC), Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (MS) QP-5050A. The GC-MS system was equipped with a SLB-5 ms capillary column, 95% dimethylpolysiloxane, and 5% diphenylpolysiloxane (Sigma-Aldrich, Spain; 30 m × 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 13 mL/min, in a split ratio of 1:20, and the following temperature program: (a) initial temperature 80 °C; (b) rate of 3.0 °C/min to 170 °C and hold for 1 min; (c) rate of 25 °C/min from 170 to 300 °C and hold for 1.8 min. Injector and detector temperatures were held at 230 and 300 °C, respectively.

Three analytical methods were used for the identification of the volatile compounds: (1) retention indices of each problem compound (retention indices), (2) GC-MS retention times (authentic standard), and (3) mass spectra (authentic chemicals and NIST05 spectral library collection; NIST, 2011). Tentatively identified compounds, based on only mass spectral data, have been also included in this study.

The semiquantification of the volatile compounds was performed on a GC, Shimadzu GC-17A, with a fame ionization detector (FID). The column and chromatographic conditions were those previously reported for the GC-MS analysis. The injector temperature was 300 °C and nitrogen was used as carrier gas (1 mL/min). The relative abundance was obtained from electronic integration measurements using FID.

For the semiquantification of the volatile compounds, β-ionone was added as internal standard (10 µL of 1,000 mg/L) and the areas from all compounds were normalized using its area; this compound was chosen after checking that it was not present in the prickly pear cultivars under study. No standard curves were performed for each one of the quantified volatile compounds, so data included in this study should be considered as semiquantitative. However, relative values are suitable for comparing differences between prickly pear cultivars. All analytical analyses were run in triplicate.

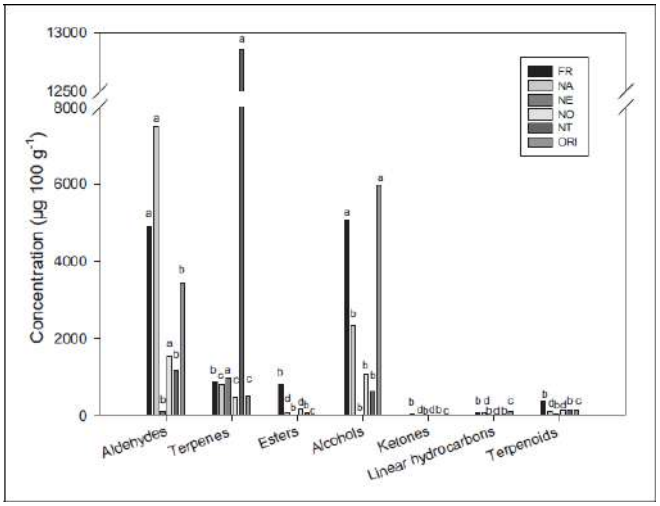

Figure 1–Main chemical families in the studied cultivars of prickly pear fruit pulp. Bars with the same letter within the same cultivar were not significantly different at $P < 0.05$, according to Tukey's multiple range test.

2.4 Statistical analyses

One-way analysis of variance (ANOVA) and multiple-range tests were used for samples comparison. The method used to discriminate among the means (multiple range test) was the Tukey's least significant difference procedure. Significance was defined at P 0.05. Statistical analysis was performed using StatGraphics Plus 5.0 software (2000) (Manugistics, Inc., Rockville, MD). Figure 1, which shows the concentration of each chemical family in the studied cultivars, was drawn using SigmaPlot 11.0 (Systat Software, San José, CA, USA). Besides, principal component analysis (Figure 2) was performed using XLSTAT software version 9 (Addinsoft, 2010).

3. RESULTS AND DISCUSSION

A total of 35 compounds were isolated, identified, and quantified using the HS-SPME technique combined with GC and two detectors (GC-MS and GC-FID). Table 1 shows these compounds with an assigned code and their sensory descriptors according to SAFC ® Flavors and Fragrances Catalog (SAFC, 2011) and the Flavor and Extract Manufacturers Association of the United States (FEMA, 2018). Table 2 shows the concentration of these compounds in µg 100/g and their total content in mg 100/g.

The cultivars which presented the highest concentration of volatile compounds were NT and FR (14.83 and 12.06 mg 100/g, respectively). By contrast, NE and NO cultivars were the cultivars with the lowest total volatile content (1.10 and 3.33 mg 100/g, respectively). The concentration found in Yellow and White cultivars (1.10 and 1.08 mg 100/g, respectively), studied by Arena et al. (2001), were similar to those found in NE cultivar, but the rest of cultivars showed higher values. The concentration of Red cultivar, studied by Arena et al. (2001), was lower than all the cultivars studied in this research.

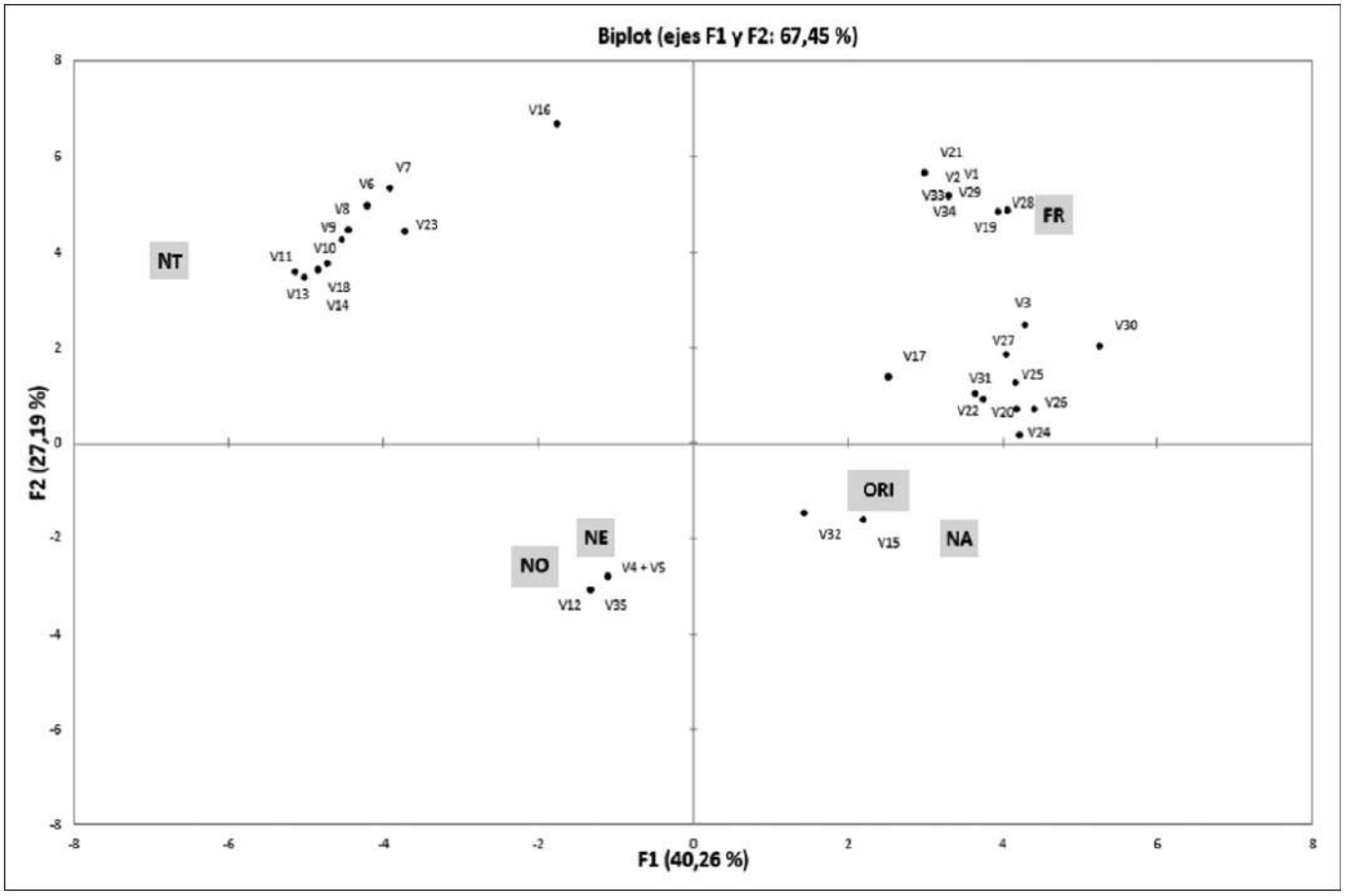

Figure 2–Principal component analysis (F1 and F2) of six cultivars of prickly pear fruit pulp.

The volatile compounds that were isolated can be grouped into seven chemical families:

(a) Aldehydes (n = 10): 2-hexenal, heptanal, 2-heptenal, octanal, *(E)*-2-octenal, nonanal, *(Z)*-2-nonenal, *(E)*-2-nonenal, 2,6-nonadienal (two isomers were found), 2,4-decadienal.
(b) Terpenes (n = 7): β -myrcene, *p*-cymene, D-limonene, (*E*)-β-ocimene, γ -terpinene, α-ocimene and α-farnesene.
(c) Esters (n = 7): 2-methylbutanoic acid methyl ester, methyl-3-hexenoate, ethyl hexanoate, hexyl acetate, ethyl octanoate, methyl-4-decenoate, methyl decanoate.
(d) Alcohols (n = 6): 2-methyl-1-butanol, 1-hexanol, 1-octanol, *(E,Z)*-3,6-nonadien-1-ol, nonanol, 2-nonen-1-ol.
(e) Ketones (n = 1): 1-Penten-3-one.
(f) Linear hydrocarbons (n = 1): 5-undecene.
(g) Terpenoids (n = 2): eucalyptol, linalool.

FR cultivar showed 30 compounds, of which four were cultivar-specific (1-penten-3-one, 2-methyl-butanol, 2-nonen-1-ol, and methyl decanoate). In fruits of the NE and NA cultivars 20 compounds were detected, of which eucalyptol was only detected in NA cultivar and hexyl acetate and α-farnesene were only detected in the NO cultivar. NT cultivar presented 17 compounds and ethyl hexanoate was only found in this cultivar. In the fruits of the ORI cultivar, 16 compounds were detected and 2,4-decadienal was exclusive to this cultivar. NE cultivar showed only 11 volatile compounds.

Volatile compounds common to all cultivars were β -myrcene, *p*-cymene, D-limonene, (*E*)-β-ocimene, γ -terpinene, linalool, nonanal, and 2,6-nonadienal. The volatile profile of fruits of the FR, NO, NT, and ORI cultivars included many aldehydes, whereas those of the NA and NE cultivars had more terpenes. These results did not agree with other authors who reported alco-hols (Arena et al., 2001; Flath & Takahashi, 1978; Oumato et al., 2016) and esters (Rodríguez, Díaz, & Nazareno, 2015) as the most numerous and abundant compounds.

Figure 1 shows the concentration of each chemical family in the studied cultivars. Aldehydes were the predominant compounds in NA and NO cultivars (69.0% and 46.1% of the total concentration of volatile compounds), followed by alcohols in NA cultivar (21.6%). FR showed alcohols and aldehydes as the predominant chemical families (42.0% and 40.5%, respectively), and ORI cultivar presented alcohols as the predominant compounds (58.9%) followed by aldehydes (33.9%). However, in NE and NT cultivars the predominant compounds were terpenes (86.7% in both cases). The results obtained in FR and ORI cultivars agreed with Flath and Takamashi (1978) and Arena et al. (2001), who reported alcohols as the most abundant chemical family. Oumato et al. (2016) studied three cultivars: *Dellahia* presented alcohols as the predominant chemical family followed by aldehydes, which also agreed with the results obtained in FR and ORI cultivar; however, *Aissa* and *Should* cultivars showed aldehydes as the primary chemical family, followed by alcohols, which agreed with the results in NA, FR, and NO cultivars. Rodríguez et al. (2015) obtained hydrocar-bons as the most abundant chemical family; no cultivar followed this pattern in this study.

D-Limonene was the predominant compound in NE and NT cultivars, having a content ranging from 814 µg 100/g in the

Table 1–Aromatic compounds found in prickly pear fruits pulp using headspace solid phase microextraction (HS-SPME).

Code	Compound	Retention time (min)	Retention indexes		Sensory descriptor
			Exp.[a]	Lit.[a]	
V1	1-Penten-3-one[b]	2.627	–	–	Vegetable[d]
V2	2-Methyl-1-butanol[b]	2.853	–	–	Green, malt, onion, wine[d]
V3	Methyl 2-methylbutanoate[b]	3.091	–	–	Apple, fruity, strawberry[d]
V4 + V5	1-Hexanol + 2-Hexenal	3.964	857	850	Green, herbaceous / Almond, fruity[d]
V6	Heptanal	4.464	901	901	Oily, fruity, woody, fatty, nutty[d]
V7	Methyl-3-hexenoate[c]	4.893	929	936	Fruity[e]
V8	2-Heptenal	5.449	954	954	Apple, citrus, fatty, spicy, vegetable[d]
V9	β-Myrcene	6.103	988	987	Anise, fruity, herbaceous, sweet[d]
V10	Ethyl hexanoate	6.235	996	1000	Apple, banana, pineapple, wine-like[d]
V11	Octanal	6.426	1004	1006	Honey, fruity, fatty, citrus[d]
V12	Hexyl acetate	6.517	1007	1010	Apple, cherry, floral, pear, sweet[d]
V13	*p*-Cymene	7.115	1,029	1,027	Citrus[d]
V14	D-Limonene	7.242	1,033	1,033	Citrus, sweet[d]
V15	Eucalyptol	7.464	1,035	1,035	Citrus, herbaceous, fruity, sweet[d]
V16	trans-β-Ocimene	7.558	1,044	1,041	Floral[e]
V17	2-Octenal, (E)-	7.934	1,058	1,059	Spicy, herbaceous, green[d]
V18	γ-Terpinene	8.024	1,061	1,062	Herbaceous, citrus[d]
V19	1-Octanol	8.232	1,069	1,069	Citrus, fatty, woody, waxy[d]
V20	5-Undecene[c]	9.061	1,098	1,090	Not defined in the literature
V21	Linalool	9.181	1,102	1,101	Citrus, floral, sweet[d]
V22	*(E,Z)*-3,6-Nonadien-1-ol[b]	9.250	1,104	–	Melon, green, violet[d]
V23	Nonanal	9.334	1,106	1,104	Citrus, vegetable, nutty, waxy, fatty[d]
V24	*(Z)*-2-Nonenal	10.669	1,143	1,149	Waxy, fatty[d]
V25	*(E)*-2-Nonenal	10.823	1,148	1,156	Waxy, fatty[d]
V26	2,6-Nonadienal (isomer 1)	11.096	1,155	1,160	Vegetable, green[d]
V27	Nonanol	11.351	1,162	1,168	Melon, green, fatty[d]
V28	2,6-Nonadienal (isomer 2)	11.455	1,165	1,160	Vegetable, green[d]
V29	2-Nonen-1-ol[b]	11.551	1,168	–	Melon, waxy, fatty, sweet, violet[d]
V30	α-Ocimene[b]	12.166	1,185	–	Floral[e]
V31	Ethyl octanoate	12.858	1,203	1,206	Apricot, floral, pear, pineapple[d]
V32	2,4-Decadienal	16.685	1,297	1,309	Fatty, citrus, meaty[d]
V33	Methyl-4-decenoate[b]	17.172	1,309	–	Fruity[e]
V34	Methyl decanoate	17.807	1,324	1324	Oily, wine-like, fruity[d]
V35	α-Farnesene	26.220	1,523	1,522	Apple, lavender, lime, woody, green[d]

[a]Lit., literature (NIST 2011); Exp., experimental.
[b]Tentatively identified.
[c]Identified in DB-1 column.
[d]SAFC (SAFC, 2011).
[e]FEMA (FEMA, 2018).

NE cultivar to 11,026 µg 100/g in NT cultivar (these contents represented 69.2% and 72.9% of the volatile compounds profile, respectively). This compound can be described as having lemon, orange, citrus, and sweet notes. However, FR, NA, and ORI cultivars showed nonanol and 2,6-nonadienal (isomer 1) as the most abundant compounds. Nonanol represented 20.6% of the volatile profile in NA cultivar (2,251 µg 100/g), 34.0% in FR cultivar (4,146 µg 100/g), and 58.7% in ORI cultivar (5,946 µg 100/g). 2,6-Nonadienal (isomer 1) showed similar values to those of nonanol in FR cultivar (3,731 µg 100/g, 30.3% of the volatile compounds profile), higher values in NA cultivar (7,193 µg 100/g, 66.1% of the total volatile compounds) and lower ones in ORI cultivar (2926 µg 100/g, 28.7% of the volatile compounds pro-file). These compounds can be described as having green, melon, and fatty notes (nonanol) and vegetable and green notes (2,6-nonadienal). In NO cultivar, the most abundant compounds were 1-hexanol and 2-hexenal, with concentrations of 1,883 µg 100/g, represented 57.2% of the volatile profile both together. 1-Hexanol can be described as having green, herbaceous, wood, and sweet notes and 2-hexenal as having almond, apple, green, plum, sweet, and vegetable notes. These results do not agree with those obtained by Flath and Takahasi (1978), who obtained ethanol as the predominant compound, but in the Should cultivar studied by Oumato et al. (2016). 2-Hexanal was the most abundant compound, which agreed with the results obtained in NO cultivar. Besides, Arena et al. (2001) identified 1-hexanol, together with 2-hexen-1-ol, as the most abundant compounds, which also agreed with the results for NO cultivar.

2,6-Nonadienal, one of the main volatile compound in the FR, NA, and ORI cultivars, has also been found as the principal volatile compound in cucumber (*Cucumis sativus* L.) (Buescher & Buescher, 2001; Kemp, Knavel, & Stoltz, 1974; Schieberle, Ofner, & Grosch, 1990) and it is an important contributor to mango (*Mangifera indica* L.) aroma (Engel & Tressl, 1983; Pino & Mesa, 2006). Nonanol, which was also an important component in FR, NA, and ORI cultivars, was also detected in black tea (*Camellia sinensis* L.; Chen et al., 2019) and *Arctium lappa* L. leaf (Golbaz, Zarei, Garakani, & Mojab, 2018), but in lower concentrations. 1-Hexanol and 2-hexenal, the principal compounds in NO culti-var, were also found in tropical fruits such as guava (*Psidium guajava* L.; Nishimura, Yamaguchi, Mihara, & Shibamoto, 1989; Soares, Pereira, Maio Marques, & Monteiro, 2007) and kiwi (*Actinidia chinensis* Plach.; Bartley & Schwede, 1989; Takeoka, Güntert, Jennings, Flath, & Wurz, 1986).

Table 2–Volatile composition found in fruits pulp of six cultivars of prickly pear (μg 100/g).

Compound	ANOVA	Concentration (μg 100/g)					
		FR	NA	NE	NO	NT	ORI
1-Penten-3-one	*** [a]	47.15 a [b]	N.D. b	N.D. b	N.D. b	N.D. b	N.D. b
2-Methyl-1-butanol	**	9.91 a	N.D. b	N.D. b	N.D. b	N.D. b	N.D. b
Methyl 2-methylbutanoate	***	53.70 a	60.52 a	N.D. b	N.D. b	N.D. b	N.D. b
1-Hexanol + 2-Hexenal	***	123 b	N.D. d	N.D. d	1883 a	N.D. d	20.97 c
Heptanal	*	8.80 b	N.D. c	N.D. c	3.85 b	34.2 a	N.D. c
Methyl-3-hexenoate	**	16.39 b	N.D. c	N.D. c	2.77 b	54.56 a	N.D. c
2-Heptenal	***	16.13 b	N.D. c	N.D. c	5.08 b	117 a	N.D. c
β-Myrcene	***	82.52 b	26.31 b	35.31 b	40.61 b	567 a	47.23 b
Ethyl hexanoate	**	N.D. b	N.D. b	N.D. b	N.D. b	26.24 a	N.D. b
Octanal	***	9.08 a	N.D. c	11.44 b	14.32 b	60.22 a	N.D. c
Hexyl acetate	***	N.D. b	N.D. b	N.D. b	138 a	N.D. b	N.D. b
p-Cymene	***	2.16 c	1.71 c	7.13 b	7.71b	79.12 a	1.2 c
D-Limonene	***	224 c	298 c	814 b	329c	11026 a	10.14 d
Eucalyptol	*	N.D. b	11.3 a	N.D. b	N.D. b	N.D. b	N.D. b
(*E*)-β-Ocimene	***	81.80 a	26.46 c	28.15 c	37.43 bc	95.43 a	50.87 b
(*E*)-2-Octenal	***	66.71 b	7.15 d	N.D. e	3.79 d	19.16 c	141 a
γ -Terpinene	**	17.50 b	30.24 b	73.07 b	36.01 b	1086 a	N.D. c
1-Octanol	**	14.91 a	3.86 b	N.D. c	N.D.c	N.D.c	N.D. c
5-Undecene	*	63.46 b	55.81 b	N.D. c	N.D. c	N.D. c	107 a
Linalool	***	354 a	98.14 b	42.99 c	113 b	127 b	134 b
(*E*,*Z*)-3,6-Nonadien-1-ol	***	50.05 b	89.76 a	N.D. d	14.83 c	8.96 c	7.73 c
Nonanal	***	38.33 b	15.27 c	4.46 c	45.29 b	75.73 a	18.37 c
(Z)-2-Nonenal	**	52.60 b	121 a	N.D. c	N.D. c	N.D. c	37.78 b
(E)-2-Nonenal	***	67.58 b	34.22 c	N.D. d	N.D. d	N.D. d	106 a
2,6-Nonadienal (isomer 1)	***	3731 b	7193 a	69.02 c	491 c	829 c	2926 b
Nonanol	***	4146 b	2251 c	15.1 d	103 d	613 d	5946 a
2,6-Nonadienal (isomer 2)	***	828 a	132 b	4.23 b	29.80 b	15.48 b	166 b
2-Nonen-1-ol	***	785 a	N.D. b	N.D. b	N.D. b	N.D. b	N.D. b
α-Ocimene	***	457 a	403 a	N.D. b	N.D. b	N.D. b	384 a
Ethyl octanoate	**	6.78 a	13.52 a	N.D. b	N.D. b	N.D. b	N.D. b
2,4-Decadienal	***	N.D. b	N.D. b	N.D. b	N.D. b	N.D. b	28.59 a
Methyl-4-decenoate	***	679 a	N.D. c	N.D. c	9.16 b	N.D. c	N.D. c
Methyl decanoate	**	30.90 a	N.D. b	N.D. b	N.D. b	N.D. b	N.D. b
α-Farnesene	**	N.D. b	N.D. b	N.D. b	21.48 a	N.D. b	N.D. b
Total (mg 100/g)	***	12.06 ab	10.87 b	1.10 c	3.33 c	14.83 a	10.13 b

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

[b] Values are the mean of three replications. Values followed by the same letter, within the same row, were not statistically different according to the Tuckey's multiple range test. N.D., nondetected.

The predominant compounds in NT and NE cultivars, D-limonene, and γ -terpinene are also found in high contents in citrus fruits (Moufida & Marzouk, 2003; Reynes, Alter, Brat, Brillouet, & Rega, 2003; Rudaz et al., 2013).

Principal component analyses (PCA) was performed to obtain an easier and complete understanding of the relationship among the studied cultivars and their volatile compounds (Figure 2). The first principal component (F1) accounted for 40.26% and the sec-ond one for (F2) 27.19% of the total variance. It is important to remember that the higher the distance between two parameters, the lower their correlation.

F1 was positively linked with (Z)-2-nonenal, 5-undecene, *(E,Z)*-3,6-nonadien-1-ol, nonanol, 2-methylbutanoic acid methyl ester, α-ocimene, 2,6-nonadienal (isomer 2), 1-penten-3-one, methyl decanoate, and 1-octanol, and negatively with ethyl hexanoate, p-cymene, γ -terpinene, D-limonene, octanal, β -myrcene, 2-heptenal, heptanal, nonanal, and methyl-3-hexenoate. F2 was positively linked with (*E*)-β-ocimene and inversely with 1-hexanol + 2-hexenal, acetic acid hexyl ester and α-farnesene.

The principal component F1 was able to establish differences among samples. FR, ORI, and NA cultivars, which were positioned on the right part of the graph, were correlated with the presence of volatile compounds with green and fatty notes, such as 2,6-nonadienal and nonanol. On the other hand, NT, NO, and NE cultivars were situated of the left of the graph and were positively linked with compounds having citrus, fruity and vegetable notes, mainly D-limonene, 1-hexanol, and 2-hexenal.

4. CONCLUSION

The volatile composition of six cultivars of *O. ficus-indica* was analyzed. Even though prickly pears have not a strong aroma, a total 35 compounds of were isolated, identified, and quantified: 10 aldehydes (for example, 2,6-nonadienal), 8 terpenes (β -myrcene), 7 esters (methyl-3-hexenoate), 6 alcohols (nonanol), 1 ketone (1-penten-3-one), 1 linear hydrocarbon (5-undecene), and 1 terpene (linalool). The cultivars with the highest total concentration of volatile compounds were NT and FR, making them attractive for consumers because, in general, the more volatile content, the higher consumer acceptance. On the other hand, the fruits of the NO and NE cultivars showed the lowest concentration of volatile compounds. Nonanol and 2,6-nonadienal were the pre-dominant compounds in FR, NA, and ORI cultivars, 1-hexanol + 2-hexenal in NO cultivar and D-limonene and γ -terpinene in NT and NE cultivars. *O. ficus-indica* fruits are highly valued for their high health-promoting benefits but sensory evaluation is needed to complete the knowledge of the aroma of this fruit and the effect of the cultivar. Thus, further investigation on the organoleptic attributes of prickly pear will be conducted and were not already done due to the lack of orchards in our surrounding area.

AUTHOR CONTRIBUTIONS

L. Andreu-Coll and L. Noguera-Artiaga were responsible for laboratory work, statistical analysis, and writing the manuscript. A. Carbonell-Barrachina was responsible for experiment design, data processing, and reviewed the manuscript. P. Legua and F. Hernández reviewed the manuscript and were in charge of data analysis.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Influence of Storage on Physiological Properties, Chemical Composition, and Bioactive Compounds on Cactus Pear Fruit (*Opuntia flcus-indica* (L.) Mill.)

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Abstract: Cactus pear (*Opuntia ficus-indica* (L.) Mill.) fruit from 'Orito' cultivar were stored at 2 °C and 90% RH for 28 days plus three days at 20 °C (shelf life, SL). This research analysed the changes in fruit quality parameters (weight loss, firmness, color, titratable acidity, and total soluble solids), ethylene production, respiration rate, antioxidant activity and bioactive compounds (total phenols and carotenoids) of cactus pear fruit during cold and shelf life storage. Under cold conditions, CO_2 production decreased, and ethylene production increased slightly, while under shelf life conditions CO_2 production increased and ethylene production increased more sharply. Firmness increased under cold conditions and did not change during shelf life period. The content of total soluble solids (TSS), titratable acidity (TA), pH, total carotenoids, and lipo-antioxidant activity (L-TAA) remained stable under both conservation conditions. However, hydro-antioxidant activity (H-TAA) increased under both cold and shelf life conditions, and total phenols remained stable during cold storage and increased under shelf life conditions. Besides, weight loss was acceptable under both storage conditions, and color changes were more pronounced under shelf life storage. These results show that the marketability of cactus pear fruit from 'Orito' cultivar was acceptable until the end of the storage under cold and shelf life conditions.

Keywords: prickly pear; storage; shelf life; fruit quality; antioxidants

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1. Introduction

Cactus pear (*Opuntia ficus-indica* (L.) Mill.) is the Cactaceae plant with the greatest economic relevance in the world [1,2]. It is a tropical or subtropical plant original from the arid and semi-arid regions of America [3], which can grow in arid and semi-arid climates [4]. Cactus pear is known by its fruit, commonly named "tunas" or "figs". Mexico is the largest producer and consumer in the world, with the largest cultivation area [2,5]. Italy, South Africa, Chile, Israel, and Spain are also important producers [2]. In addition to the consumption of its fruit, this plant presents a wide range of applications. Some of the more important are cultivation as a forage supplement, consumption of cladodes, medical uses, non-food industrialization (for instance, the production of bioenergetics and cosmetics), and carmine production [2].

The fruit or cactus pear is generally consumed fresh, but they are highly perishable, and usually after nine days of storage at ambient temperature (19 ± 5 °C), the fruit can

show spots and rotting due to decay [6]. This fruit is classified as a non-climacteric fruit, in which cold storage reduces the respiration rate and fruit mass loss, inhibits the growth of microorganisms, and prolongs shelf life [7].

There are some studies that have evaluated the storage of cactus pear under different conditions and treatments, such as effects of storage temperature [8,9], effects of UVB light [10], and cryocauterization [6], among others. However, the success of storage depends on several factors, including the cultivar, storage atmosphere, orchard management practices (especially irrigation and mineral nutrition), and fruit maturity stage [11].

The aim of this study was to evaluate the effect of cold storage and shelf life on physical and physicochemical characteristics and bioactive compounds of fruit from a Spanish cultivar called 'Orito'. Due to the limited of studies evaluating these characteristics in cactus pear fruit, this information will be used to improve the storage of cactus pear fruit and its marketability.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Cactus pear fruit from a commercial farm (38°23′30.7" N, O°40′13.0" W) in Orito (Alicante, Spain) were used for this study. Fruit from 'Orito' cultivar is orange and had an average weight of 125.92±3.87 g. Two thousand fruit were hand-harvested in mid-August 2017 at the commercial ripening stage. The fruit was transported, under cold conditions, to the laboratory for preparation and further analyses. Once in the laboratory, the spines of fruits were removed with a brush, and 540 fruit were selected based on the absence of visual defects and by homogeneous size and color, and randomly divided into 27 lots of 20 fruit, being each a biological replicate.

Three lots were used to evaluate fruit properties at harvest. The rest of the lots were stored in a refrigeration chamber at 2 °C and 85.90% relative humidity (RH). Of these, three lots were taken at seven, 14, 21, and 28 days after harvest, in which all the analyses were carried out. The other three lots were taken and placed at 20 °C for three days to study the shelf life (SL). After each analysis, the fruit were frozen at −80 °C for total antioxidant activity (due to both hydrophilic (H-TAA) and lipophilic (L-TAA) compounds), total phenolics, and total carotenoids. Quality parameters, such as weight loss, color, fruit firmness, total soluble solids (TSS), and total acidity (TA), were measured in three replicates of 20 fruit.

2.2. Ethylene Production and Respiration Rate

Ethylene production and respiration rate were measured by placing each lot in a 2 L glass jar hermetically sealed with a rubber stopper for one hour. One mL of the holder atmosphere was withdrawn with a gas syringe and used to quantify ethylene concentration into a Shimadzu TM GC-2010 gas chromatograph (Kyoto, Japan), with the characteristics explained in Díaz-Mula [12].

Another sample of 1 mL of the same atmosphere was used to quantify respiration rate by measuring CO_2 concentration into a gas chromatograph GC 14B (Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD), with the characteristics explained in Díaz-Mula [12]. Ethylene production and respiration rate was expressed as nmol kg^{-1} s^{-1}. These analyses were made in duplicate; data are the mean ± SE of determinations made in three replicates.

2.3. Fruit Quality Parameters

Each lot of fruit were weighed using a digital balance (model BL-600; Sartorius, Madrid, Spain) to calculate weight loss. Fruit lots were weighed at day zero, and after the storage period (both cold and shelf life), weight loss was determined as the percentage of weight loss in relation to the weight at day zero. Fruit firmness was determined in each fruit as force deformation (N mm^{-1}) by using a flat steel plate coupled with a texturometer (TX-XT2i Texture Analyzer, Stable Microsystems, UK), which employed a force causing a

10% of deformation of the fruit diameter at day zero and 5% the rest of the days. Color, as L*, a*, and b* parameters, were measured with a Minolta colorimeter CR200 model/Minolta Camera Co., Osaka, Japan) by using the CIEL*a*b* System and was expressed as Hue angle ($\tan^{-1}$ (b*/a*)). For these parameters, data are the mean ± standard error (SE) of individual determinations made in three replicates of five fruit.

After these non-destructive determinations, the pulp of the fruit was cut into small pieces in order to obtain a uniform sample of each replicate. A part was employed to measure total soluble solids (TSS) concentration and titratable acidity (TA), and the remaining were immediately frozen at −80 °C until analysis of H-TAA, L-TAA, total phenolics, and total carotenoids were made.

Total soluble solids (TSS) concentration and titratable acidity (TA) were measured in the juice of the homogeneous samples of each lot. TSS was determined in duplicate at room temperature with a digital refractometer Atago Pocket PAL-1 (Atago Co. Ltd., Tokyo, Japan) and expressed as a percentage. TA was also determined in duplicate by titration of 1 mL of juice with 0.1 N NaOH up to pH 8.1 by using an automatic titrator (TitraLab AT1000 series, Hach Tokyo, Japan), and the results were expressed as g of malic acid equivalent per kg $^{-1}$. Ripening index (RI) was calculated as the ratio between TSS and TA. Data are the mean ± SE of three replicates.

2.4. Antioxidant Activity and Bioactive Compounds

Total antioxidant activity (TAA) was determined in duplicate for each lot according to the methodology of Arnao et al. [13], which allows the determination of TAA due to both hydrophilic (H-TAA) and lipophilic (L-TAA) in the same extract. In summary, 5 g of the homogeneous sample of frozen pulp were homogenized in 15 mL of methanol:water (80:20, *v*/*v*) containing 1% of HCl (39%) and 2 mmol L^{-1} of NaF to inactivate polyphenol oxidase activity, and then centrifugated at 15,000 ×*g* at 4 °C for 15 min. For the quantification of L-TAA was used the upper fraction, and the lower one was used to quantify L-TAA, both made in duplicate. The reaction medium included 2,2-azino-bis-(3-ethylbenzothiazoline- 6-sulfonic acid) di-ammonium salt (ABTS), horseradish peroxidase enzyme (HRP), and its oxidant substrate (hydrogen peroxide). Trolox ((R)-(+)-6-hydroxy 2,5,7,8-tetramethyl-croman-2-carboxylic acid) (0–20 nmol) from Sigma (Madrid, Spain) was used as a standard antioxidant to perform a calibration curve for both H-TAA and L-TAA, and results were expressed as mg Trolox equivalents kg^{-1} (fresh weight basis). Total carotenoids were quantified in the lipophilic extract [13] by reading the absorbance at 450 nm in a UNICAM Helios-α spectrophotometer (Cambridge, UK), and were expressed as mg of β-carotene equivalent kg^{-1} fresh weight, considering the $\epsilon^{1\%}_{cm}$ = 2560. Total phenolics were extracted according to Tomás–Barberán et al. [14] using the same extractant described above and quantified using the Folin-Ciocalteu reagent. Briefly, 200 µL of the hydrophilic extract were diluted in the extractant described above and mixed with 2.5 mL of water-diluted Folin–Ciocalteau reagent. The mixture was incubated for 3 min at room temperature. Then, 2 mL of sodium carbonate (75 g L^{-1}) was added, and the mixture was shaken. At last, the mixture was incubated at 60 °C for 5 min, and absorbance was measured at 760 nm. Gallic acid was used for performing a calibration curve. Results were expressed as mg gallic acid equivalent per kg fresh weight. Results were the mean ± SE of measures made in duplicate in each of the three replicates.

2.5. Statistical Analyses

One-way analysis of variance (ANOVA) and multiple-range tests were used for sample comparisons. The method used to discriminate among the means (Multiple Range Test) was Tukey's least significant difference procedure. Significance was defined at $p \leq 0.05$. Statistical analysis was performed using XLSTAT software version 9 [15]. Figures were drawn using SigmaPlot 11.0 (Systat Software, San José, CA, USA) [16].

3. Results and Discussion

3.1. Ethylene and CO_2 Production

The fruit has been defined as climacteric and non-climacteric depending on the pattern in ethylene production and respiration rate. Ethylene is a gas of natural origin that is produced by fruit and vegetables during their metabolic processes. It is related to the growth and maturation of the fruit, inducing changes such as texture, color changes, and tissue degradation. Ethylene is considered the plant hormone responsible for the ripening process in climacteric fruits, such as tomato, apple, and melon, among others. However, non-climacteric fruit, such as pepper and grapes, present in their respiratory pattern, comparably low values of ethylene production and gradual decline production during the ripening process [12].

Classification of climacteric and non-climacteric fruit is not categorical. Some species show both patterns in different cultivars or genotypes, such as strawberry, grape, and citrus fruit [12,17]. Cactus pear fruit was classified as a non-climacteric fruit [18,19] with low respiration rates in comparison to those of other common fruit like avocado, banana, and mango [18]. However, the 'Orito' cultivar showed a suppressed-climacteric pattern in ethylene production and respiration rate, similar to some cultivars of plum [20,21], which showed no increase in respiration rate or in ethylene production related to ripening. Besides, respiration can be affected by the variety, the maturity stage at harvest time, the type of crop, and the environmental conditions, among others [2], and physical damage or decay cause increased respiration and ethylene production rates [18].

With respect to respiration rate in cactus pear fruit, the storage under cold conditions (2 °C) decreased the CO_2 production, changed from 231 nmol kg^{-1} s^{-1} at day zero to 64 nmol kg^{-1} s^{-1} at day seven. Then, the CO_2 production remained stable until the end of cold storage, reaching 51 nmol kg^{-1} s^{-1} at the end of cold storage (Figure 1A). However, when measuring shelf life conditions, CO_2 production increased slightly after 14 d (287 nmol kg^{-1} s^{-1}) and then decreased up to values below the initials at the end of storage (182 nmol kg^{-1} s^{-1} at 28 d) (Figure 1A). Increasing the temperature from 2 °C to room temperature resulted in a greater increase in CO_2 production rate, but after 14 days, the production of CO_2 began to decrease under both conditions. This increase in CO_2 production in response to temperature can also be observed in other cultivars of cactus pear fruit [22] and in other aerial parts as cladodes [23]. Besides, the results obtained of the CO_2 production were in accordance with those obtained by Laksminarayana and Estrella [19] and Corrales-García et al. [24].

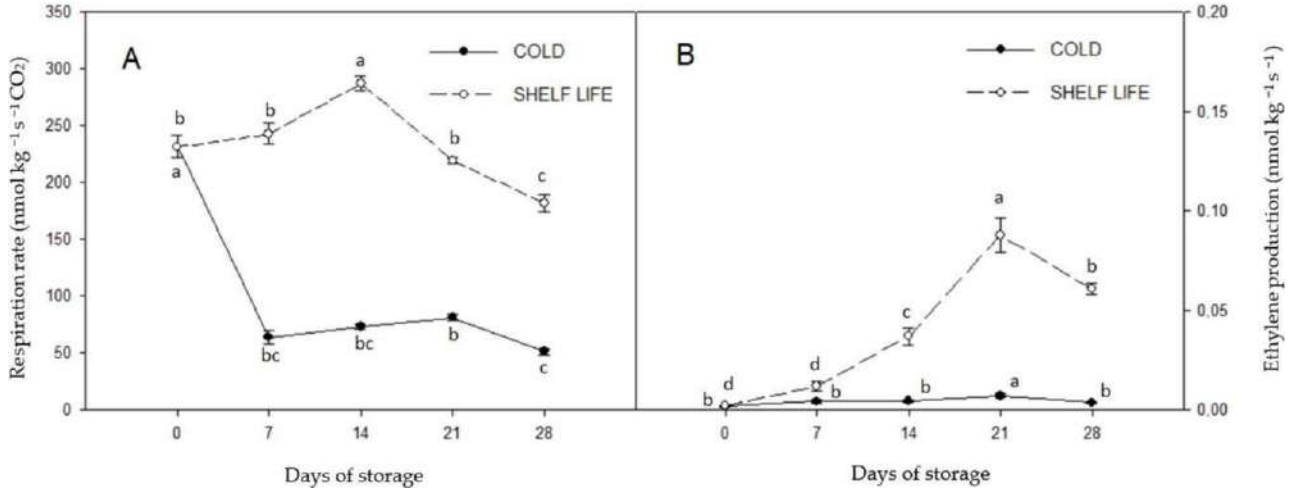

Figure 1. Changes in respiration rate (CO_2) (**A**) and ethylene concentration (**B**) in cactus pear fruit during storage under cold and shelf life conditions. Data are the mean ± standard error (SE) (n = 6). Tukey's test result at a 95% confidence level is shown. Different letters indicate significant differences ($p < 0.05$) during each storage time.

Regarding ethylene production, during cold storage, this compound was slightly increased until day 21 from 0.002 nmol kg^{-1} s^{-1} to 0.007 nmol kg^{-1} s^{-1} and decreased to 0.003 nmol kg^{-1} s^{-1} at the end of storage (Figure 1B). Shelf life storage showed the same trend, but the increase was higher, and at day 21, the ethylene production reached 0.09 nmol kg^{-1} s^{-1} and 0.06 nmol kg^{-1} s^{-1} at the end of storage (Figure 1B). These low ethylene emission rates showed that the cactus pear presented with a suppressed-climacteric pattern in ethylene production, and its metabolism decreased at low temperatures. The results of this study were in accordance with others [22,25], which showed that the ethylene production of cactus pear fruit was low under cold conditions, but the increase in temperature to 20 °C caused an increase in ethylene production up to ten times higher.

3.2. Fruit Quality Parameters

The rate of postharvest water loss in fruits depends primarily on the external vapor pressure deficit, although it can be influenced by other factors such as the intrinsic and extrinsic characteristics of the fruit. Fruit with thick peels, such as citrus fruit, bananas, or cactus pear, can lose a significant quantity of skin moisture without affecting edible quality [12].

In this study, cactus pear fruit showed a low weight loss during the 28 days of storage, both in cold and shelf life conditions. The weight losses at the end of storage were 2.22 ±0.08% in cold storage and 3.71%±0.40 after the shelf life period. Under shelf life conditions, weight loss increased significantly past day 21. However, under shelf life conditions, weight loss was higher between days seven and 21 (Table 1). According to Lamúa [26], in most vegetable species, weight losses above 6–8% cause an irreversible alteration of sensory quality, affecting its commercial quality. Because the weight losses in this study did not reach 4%, cactus pear fruit from the 'Orito' cultivar maintained their quality and marketability. These weight losses under cold conditions were similar in 'Cristalina' and 'Alfajayucan' cultivars and lower than other cultivars studied by López-Castañeda et al. [27]. The 'Copena-Torreoja' cultivar showed more than 10% weight loss when exposed four days at room temperature conditions after cold storage, but the 'Cristalina', 'Picochulo' and 'Burrona' cultivars showed a weight loss of less than 4% under the same conditions, which agree with the results of this study [24]. 'Giallia' cultivar showed 4.1% of weight loss after seven weeks of storage at 6 °C, and 5.7% after seven weeks at 6 °C and three days of a simulated marketing period (shelf life) [22].

Table 1. Fruit quality parameters (total soluble solids (TSS), total acidity (TA), ripening index and weight loss) calculated in cactus pear fruit during conservation under cold and shelf life (SL) conditions. The values represented are the mean.

Days of Storage	0	7	14	21	28	7 + SL	14 + SL	21 + SL	28 + SL
TSS (%)	14.9	14.8	14.5	14.3	14.5	14.1	14.5	14.4	14.0
TA (g malic acid kg^{-1})	0.9	0.8	0.8	0.9	0.9	0.8	0.9	0.9	0.9
Ripening index	168 b	185 a	180 a	160 b	161 b	176 a	161 b	160 b	155 b
Weight loss (%)	0 g	0.28 f	1.05 e	1.97 c,d	2.22 b,c	1.13 e	1.82 d	2.43 b	3.71 a

The different letters within the rows indicate significant differences according to the Tukey's test ($p < 0.05$).

Changes in fruit texture during postharvest storage are due to dehydration and changes in the components of the middle lamella and primary cell wall, which causes fruit softening. These processes depend on the class of fruit and even of the cultivar [12]. Cold storage of cactus pear fruit from 'Orito' cultivar increased firmness from day seven, reached values of 11, 990 n m^{-1}. However, no significant differences were found under shelf life conditions, in which firmness showed values between 9.17–10.1 n mm^{-1} (Figure 2). At the end of storage, firmness increased 16.6% under cold conditions and decreased 3.58% under shelf life conditions with respect to day zero. No visual chilling injuries were detected during cold storage of 'Orito' fruit (data not shown). Excess of fruit softening limited shelf life, storage, because could increase the physical damage during management and make fruit more susceptible to pest and diseases. In this sense, cactus pear fruit from the 'Orito'

cultivar showed an acceptable quality and marketability because the loss of firmness did not occur during cold storage, but rather its increase and firmness loss during shelf life conditions was very low compared to that other fruit such as tomato (55%), apricot (72%), or lemon (26%) under similar conditions [28]. The results of this study are in accordance with other authors [22,25] who obtained that cold storage prevented firmness loss in cactus pear fruit, and this rapidly declined when fruit was kept at 20 °C.

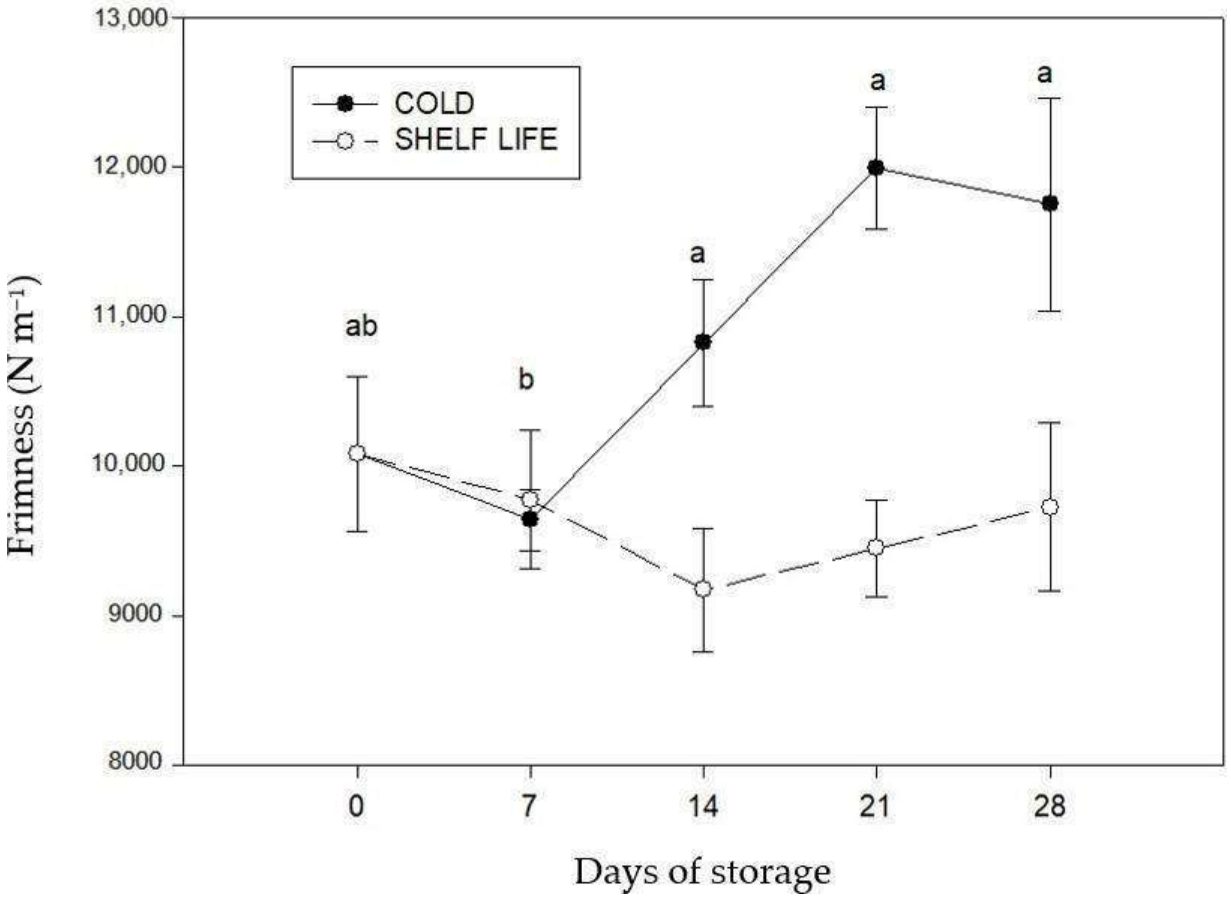

Figure 2. Changes in respiration rate (CO_2) (a) and ethylene concentration (b) in cactus pear fruit during storage under cold and shelf life conditions. Data are the mean ± SE (n = 6). Tukey's test at a 95% confidence level is shown. Different letters indicate significant differences ($p < 0.05$) during each storage time.

Levels of sugars are an important factor in determining the taste of ripe flesh fruit. In cactus pear fruit, the main sugar is glucose, followed by fructose, with levels at harvest in a range of 103–144 g L^{-1} of glucose and 57–88 g L^{-1} of fructose [29]. The measure of total soluble solids (TSS) is important to estimate the sugar content in fruit and to determine its degree of sweetness and thus estimate consumer acceptance, along with volatile compounds, which were studied in 'Orito' cultivar showed mainly green and fatty notes [30]. However, the perception of taste by consumers is not only linked to these parameters, and TA is also an important factor. Thus, the ripening index (TSS/TA) is used to estimate the degree of fruit acceptance [12]. In this study, the values of TSS and TA remained stable during both shelf life and cold conditions because cactus pear, in this parameters, showed a non-climacteric fruit pattern, in which the concentration of nutrients remains in the fruit without substantial changes during storage [2], while in climacteric fruit such as kiwifruit or nectarine, the content of TSS increased and TA decreased during postharvest, although these changes are considerably dependent of the fruit species and cultivars [12,28]. The 'Orito' cultivar showed TSS content between 14% and 14.9% (Table 1),

similar to the results obtained by Andreu et al. [31]. Values of TSS of >12–13% are required to ensure that the fruit has good quality, so the TSS content of the 'Orito' cultivar was appropriate [3]. TA showed values close to 0.9 g×kg^{-1} (Table 1), the same as those obtained by Schirra et al. [22] in the 'Gialla' cultivar and slightly higher than those obtained by Graça-Miguel et al. [32] in the 'Orange', 'Green', and 'Rossa' cultivars. This caused the ripening index (RI) in cactus pear fruit to remain at about 155–185 (Table 1).

Colored fruit has always been part of the human diet and helps us to identify food and evaluate its palatability. In addition to defining the aesthetic value of fruit, color predetermines consumers' expectations of flavor and taste, modulates appetite, and is a major issue for the food industry. However, color may be altered during fruit storage through the action of light, temperature, and oxygen, among others. The CIEL*a*b* System (International Commission on Illumination, Vienna) has been adopted by the food industry for measuring the color of products and color changes during storage [28]. The L* parameter, which reflects color luminosity, did not show significant differences during cold storage but did during shelf life conditions, decreased from an initial value of 57.8 ± 0.40 to 54.2 ± 0.82 after 28 days (data not shown). Regarding the Hue angle, there were no significant differences in this parameter under cold conditions. However, under shelf life conditions, the Hue angle decreased after seven days and stayed constant until the rest of storage, from an initial value of 75.2 ± 0.08 to 67.7 ± 1.59 after seven days (data not shown). Decreases in the Hue angle are related to peel darkening in fruit. The trend of these parameters was in accordance with that obtained by other authors [8,33], who analyzed changes in the color of *Opuntia ficus-indica* and *O. albiarpa* fruit under cold and shelf life conditions.

3.3. Bioactive Compounds and Antioxidant Activity

There is ample evidence about the health benefits of cactus pear fruit consumption, mainly due to its antioxidant activity [31,34,35]. Phenolic compounds are a group of secondary natural metabolites in plants that represent the strongest antioxidants in plant foods [28]. During the cold storage of cactus pear fruit, total phenol content remained stable, with values between 640–810 mg kg $^{-1}$ (Figure 3). These results were in accordance with those obtained by Coria-Cayupán et al. [36] in the fruit of the 'Yellow' cultivar of *Opuntia megacantha*. However, during the shelf life period increased after seven days (903 mg kg $^{-1}$) and decreased at the end of storage (690 mg kg $^{-1}$) (Figure 3). The concentration of these compounds was in accordance with those obtained by Moussa-Ayoub et al. [37] but was lower than those obtained by Ramírez-Ramos et al. [38]. The variation of the content of phenolic compounds may be due to various factors, such as agronomic practices, environmental conditions, the pre- and postharvest management of fruit, and the reduction of these compounds during fruit ripening [38]. Anorve-Morga et al. [39] analyzed changes in phenolic compounds under different storage temperatures in cactus pear fruit and concluded that during storage, there was an increase in phenol content, which was directly influenced by temperature, which could explain the results of these study.

The antioxidant capacity of fruit can be carried out separately on hydrophilic and lipophilic extracts to evaluate if antioxidant activity is derived from water-soluble (H-TAA) or lipo-soluble (L-TAA) molecules [28]. Both cold and shelf life storage increased H-TAA, reached the maximum concentration after 21 days in both storage conditions (Figure 4A). This trend had been reported in non-climacteric fruit such as citrus and plum fruit under cold storage [40,41]. However, this behavior was the opposite in jujube fruit, a climacteric fruit, whose H-TAA decreased significantly with respect to day zero under cold conditions [42]. By contrast, L-TAA in cactus pear fruit, which was significantly lower than H-TAA, remained stable during cold and shelf life storage periods, showed values around 90 mg kg^{-1} (Figure 4B). These results suggested that hydrophilic compounds contributed more than lipophilic compounds in the antioxidant capacity of cactus pear fruit.

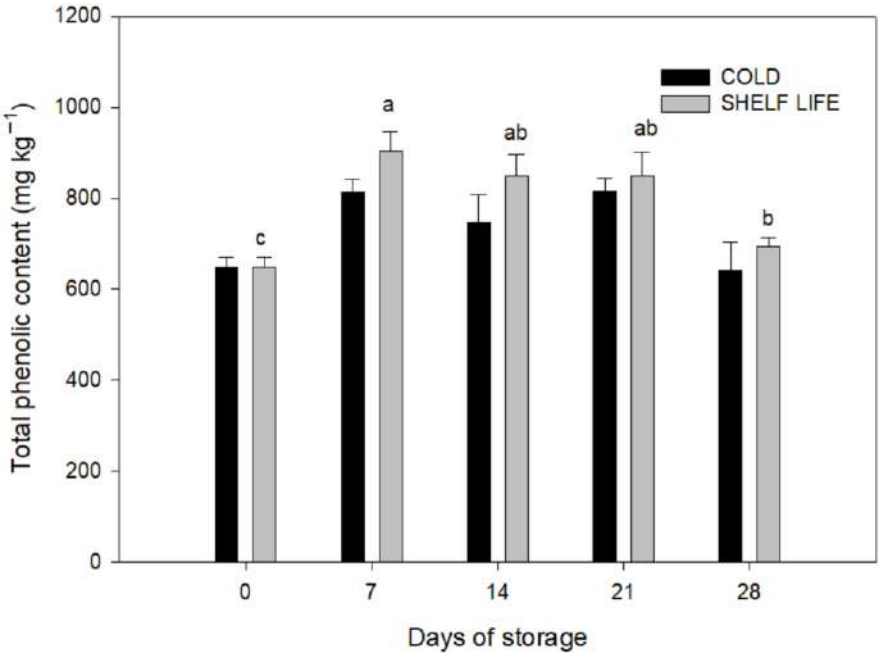

Figure 3. Total phenolic content changes in cactus pear fruit during cold and shelf life storage. Data are the mean ± SE (n = 6). Tukey's test at a 95% confidence level is shown. Different letters indicate significant differences (p < 0.05) during each storage time.

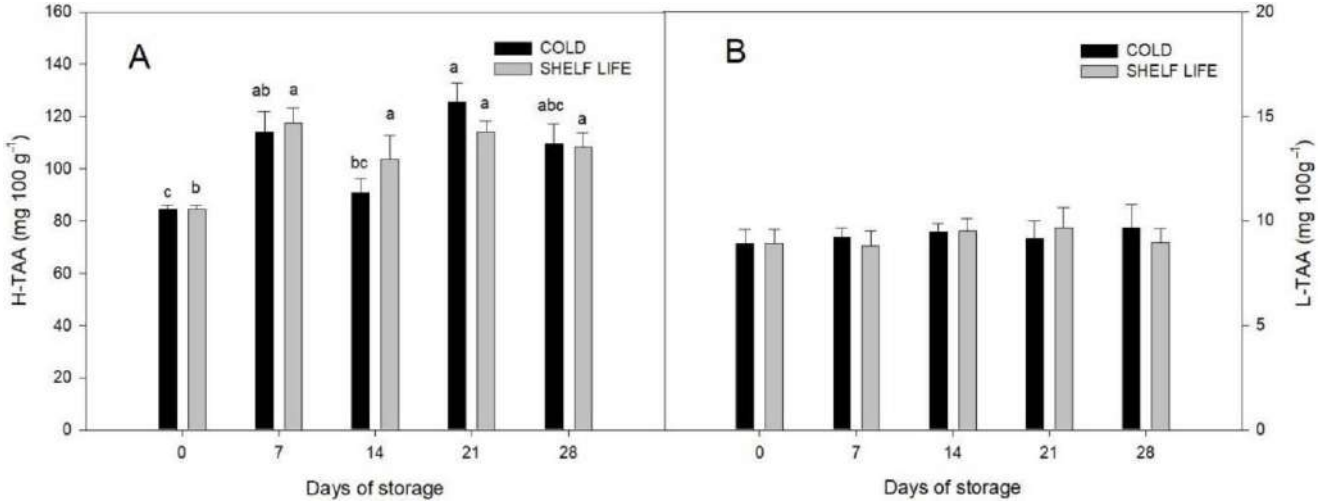

Figure 4. Changes in H-TAA (**A**) and L-TAA (**B**) during storage under cold and shelf life conditions of cactus pear fruit. Data are the mean ± SE (n = 6). Tukey test's at a 95% confidence level is shown. Different letters (a,b,c) indicate significant differences (p < 0.05) during each storage time.

Carotenoids are lipophilic compounds that are responsible for most yellow to red color of fruit and present antioxidant properties. Cactus pear fruit showed a very low concentration of these compounds (1.20 mg kg^{-1} on average, data not shown). These compounds showed a similar trend to L-TAA, without changes during storage under cold and shelf life conditions. The concentration of carotenoids in this study was lower than those obtained by Kuti [43] in a green-skinned cactus pear cultivar. Oranges, which are non-climacteric fruit, did not show changes in carotenoid concentration during cold storage, similar to the behavior of 'Orito' fruit [44].

4. Conclusions

Based on the results of this study, the 'Orito' cultivar showed a suppressed-climacteric fruit profile because of its ethylene production and respiration rate during storage. The storage under cold conditions (2 °C, 85–90% HR) maintained fruit quality parameters in optimal values for up to 28 days. Besides, fruit quality parameters were acceptable during shelf life storage; however, cold conditions were more appropriate. These results showed that the marketability of cactus pear fruit from the 'Orito' cultivar would be possible up to 28 days after harvesting. Thus, further investigation is required to evaluate how long it is possible to preserve the marketability of this fruit and experiment with other conditions, such as modified atmosphere packaging.

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Phytochemical Profile of *Opuntia flcus-indica* (L.) Mill Fruits (cv. 'Orito') Stored at Different Conditions

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Abstract: This research analyzed the phytochemical profile of prickly pear fruits from 'Orito' cultivar stored under cold conditions (2 °C, 85–90% RH) and shelf-life conditions at room temperature (stored at 20 °C for three days after cold storage) for 28 days, mimicking the product life cycle. A total of 18 compounds were identified and quantitated through HPLC-DAD-MS/MS (High-Performance Liquid Chromatographic -Diode Array Detector- Mass Spectrometry) analyses. Phenolic acids such as eucomic acid and betalains such as indicaxanthin were the predominant chemical families, and piscidic acid was the most abundant compound. During cold storage, the content of eucomic acid isomer/derivative and syringaresinol increased, and citric acid decreased, which could be caused by the cold activation of the phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) enzymes. However, no significant differences were found in the content of these compounds during shelf-life storage. These results showed that 'Orito' fruit marketability would be possible up to 28 days after harvesting, retaining its profile, which is rich in bioactive compounds.

Keywords: prickly pear; HPLC-MS; betalains; phenolic acid; lignans; antioxidants

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1. Introduction

Prickly pear is a sweet flavory fleshy berry with thick peel and many seeds, varying in size, shape, and color. This fruit has excellent nutritional properties, is low in calories and high in bioactive compounds, such as betalains, phenolics and vitamins, which show antioxidant activity and are related with health benefits and the prevention of some chronic diseases, due to their anti-inflammatory, antidiabetic, neuroprotective and antiproliferative activities, among others [1–3].

Besides its consumption as a fresh fruit product, prickly pear presents a wide range of applications, including animal feeding, developing of processed products such as juices and jams, and production of bioethanol and biogas. Due to the high concentration of bioactive compounds, especially (poly)phenolic compounds, prickly pear can be used in the nutraceutical, pharmaceutic, and cosmetics industries [4–6]. The (poly)phenolic composition of prickly pear fruits and derived products has been well described, an

includes phenolic acids, flavonoids, and lignans, among others [2,7]. In addition, fruits present high amounts of betalains [2].

Postharvest conservation affects quality characteristics of fruit and vegetables because they continue their metabolic changes after harvesting. In non-climacteric fruits, these changes can be undesirable and cause quality losses [8,9]. For prickly pear fruit, chilling injuries, microbial growth, loss of firmness and weight loss are the major deterioration factors that affect this fruit after harvesting and during postharvest conservation, its quality depending pretty much on handling and storage conditions [8,10].

Although prickly pear was classified as a non-climacteric fruit, classification of climacteric and non-climacteric fruit is not absolute, and genotypes and cultivars of some species can show both patterns [11,12]. 'Orito' fruit, the commercial Spanish cultivar studied in this work, showed a suppressed climacteric pattern in ethylene production and respiration rate similar to some cultivars of plum, which showed no increase in respiration rate or in ethylene production associated with ripening [13–15]. In a previous work we demonstrated that 'Orito' fruit maintained its quality parameters in desirable values up to 28 days, both in cold conditions (2 °C, 85–90% relative humidity, RH) and during shelf-life storage, whereas the total phenolic content increased during the shelf life conditions [13]. However, to this point in time there are no studies evaluating the (poly)phenolic composition of prickly pear fruits during storage. The aim of this work was to evaluate the impact of storage conditions and shelf-life on the bioactive compound profile of 'Orito' fruit, with a particular focus on the (poly)phenolic fraction. The results obtained will contribute to select the best shelf-life and storage conditions to improve marketability of the fruits and to develop further strategies focused on the valorization of the phytochemical profile of this species.

2. Materials and Methods

2.1. Plant Material and Sample Processing

Fruits of a commercial cultivar of *Opuntia ficus-indica* (L.) Mill., called 'Orito', were used for this study. The fruits were hand-harvested in mid-August 2017 in a commercial farm (38°23′30.7" N, 0°40′13.0" W, Orito, Alicante, Spain). The fruit was collected at the commercial ripening stage and was carried in cold to the laboratory for sample preparing and further analyses. At the laboratory, fruits were brushed to remove the spines. Next, 540 fruits were chosen based on homogeneous size, color, and by the absence of visual defects. These fruits were indiscriminately divided into 27 lots of 20 fruit, each beinga biological replicate.

Three lots were used for evaluating the fruit properties at. The rest of the lots were stored in a refrigeration chamber at 2 °C and 85–90% RH (cold conservation). Of these, three lots were reserved for evaluation at 7, 14, 21, and 28 days after harvest. Besides, the other three lots for each time point were taken and disposed of at 20 °C for three days to study shelf life (SL). The pulp of the fruits was cut into small pieces to achieve a uniform sample of each biological replicate and were directly frozen at −80 °C. After freezing, samples were freeze-dried in an Alpha 2.4 freeze drier (Christ Alpha 2.4; Braum Biotech, Osterode am Harz, Germany) for 24 h at a reduced pressure of 0.220 mbar. The temperature in the drying chamber was −25 °C, while the heating plated reached 15 °C. At the end of the freeze-drying, the samples were powdered and packed in vacuum until analysis. The information about samples (code and description) is summarized in Table 1.

Table 1. Description of samples of *O. ficus-indica* (L.) Mill analyzed in this study.

Code	Description
D0	Day 0 (harvest)
D7	seven days in cold conservation
D7SL	seven days in cold conservation + three days at room temperature to study shelf life
D14	14 days in cold conservation
D14SL	14 days in cold conservation + three days at room temperature to study shelf life
D21	21 days in cold conservation
D21SL	21 days in cold conservation + three days at room temperature to study shelf life
D28	28 days in cold conservation
D28SL	28 days in cold conservation + three days at room temperature to study shelf life

2.2. *Extraction of Phytochemical Compounds*

Phytochemicals were extracted following the protocol of Mena et al. [2]. Briefly, 200 mgof freeze-dried powder were mixed with 1 mL of 80% aqueous methanol acidified with formic acid (1%). This mixture was sonicated for 25 min, and the mixture was then centrifuged at 10,480 g for 5 min at room temperature. After supernatant collection, two additional extractions were executed for each sample with an additional 0.5 mL of the extraction solvent, as described above. All three supernatants were pooled and filtered through a 0.45 µm Millipore filter (Billerica, MA, USA) before HPLC-MS analysis.Final extracts presented a concentration of 0.1 g dw mL^{-1}. Each sample was extracted in triplicate.

2.3. *HPLC-DAD (High-Performance Liquid Chromatographic-Diode Array Detector) Analysis*

The extracts were analysed in a HPLC Dionex Ultimate 3000 equipped with a C-18 LiChrospher® 100 RP-18 (5 µm) column (250 4.0 mm) (Sigma-Aldrich, San Luis, MO, USA) operating at 35 °C, coupled to a DAD-3000 detector (Thermo Scientific, MA, USA). The mobile phase consisted of water-formic acid (0.5% v/v) (eluent A) and acetonitrile (90%) + formic acid (0.5%) + water (eluent B) at a flow rate of 0.3 mL/min with an injection volume of 20 µL. The gradient used is summarized in 'Supplementary Materials Table S1' section.

2.4. *HPLC-DAD-MS/MS Analysis*

Samples were also analysed by an HPLC-DAD-MS/MS system: a Waters Alliance 2695 (Waters®, Dublin, Ireland) separation module with an autosampler (20 µL injection volume), a quaternary pump and a solvent degasser, coupled to a Photodiode Array Detec- tor Waters 996 PDA (Waters, Dublin, Ireland) scanning wavelength absorption between 210 and 600 nm. A LiChrospher® 100 RP-18 5 µm column at 35 °C (stabilized by a col- umn oven) was used. Tandem mass spectrometry (MS/MS) detection was carried out with a Micromass® Quattro Micro triple quadrupole (Waters, Dublin, Ireland), using an electrospray ionization source in both positive (ESI+) and negative (ESI-) modes. A full scan mode (m/z: 60–1100) record was applied for the mass spectra of the compounds separated by HPLC, using a collision energy of 20 eV. The HPLC gradient method and elu- ents are described in 'Supplementary Materials Table S2' section. The MS/MS conditions, as source temperature, capillary and source voltages have been previously described by Katsinas et al. [16]. For data acquisition and processing, MassLynx® 4.1 software (Waters,Dublin, Ireland) was used.

2.5. *Statistical Analyses*

One-way analysis of variance (ANOVA) and multiple-range tests were used for sam- ples comparison. The data were compared throughout cold storage and under shelf life conditions independently (from day 0 to day 28) and each day was independently com- pared under cold and shelf life conditions. The method used to discriminate among the means (Multiple Range Test) was the Tukey's least significant difference procedure.

Significance was defined at $p \leq 0.05$. Statistical analysis was performed using XLSTAT software version 9 (Microsoft Corporation, Redmond, WA, USA) [17].

3. Results and Discussion

The exhaustive analysis of *O. ficus-indica* fruit pulp phytochemical composition allowed the tentative identification of 18 compounds (Table 2). Taking into account the compounds identified in prickly pear fruit pulp, betalains (four compounds, namely 14,15 and 17), phenolic acids (nine compounds, of which 3–7 were phenylpyruvic acids and 8 and 13 were hydroxycinnamic acids), lignans (four compounds, 9–12) were the most relevant classes of phytochemicals. In addition, some other compounds such as organic acids (compounds 1 and 2), an amino acid (16) and a prenylflavonoid (compound 18) were detected. These compounds were identified based on their retention time, fragmentation patterns obtained from mass spectra and by comparing their mass spectral characteristics with the available literature.

Table 2. Retention time (RT) and characteristic MS ions of phytochemical compounds identified in prickly pear fruits during cold and shelf life storage.

Id.	Compounds	RT (min)	Percursor Ion [M-H]$^-$ or [M]$^+$ (*m/z*)	λmax (nm)	Product Ions † (*m/z*)	References
1	*L*-Malic acid	8.10	133†	272	71	[2]
2	Citric acid	11.17	191	466, 270	111, 87, 85, 43, 41, 67, 57	[2]
3	Piscidic acid	19.20	255	466, 274	73, 107, 165, 58, 93, 133, 179	[2,18]
4	Dicaffeoylferulic acid isomer 1	26.08	517	278	187, 239	[19]
5	Eucomic acid	27.75	239	274	107, 133, 149, 177, 179, 87	[2,18,20]
6	Dicaffeoylferulic acid isomer 2	27.82	517	274	239, 198	[19]
7	Eucomic acid isomer/derivative	29.89	239	268	179, 91	
8	Ferulic acid derivative	30.45	517	268	175, 193, 235	[2]
9	Guaiacyl(t8-O-4)guaiacyl-hexoside isomer 1	30.45	537	268	195, 324, 165	[2]
10	Guaiacyl(t8-O-4)guaiacyl-hexoside isomer 2	32.26	537	273	375	[2]
11	Secoisolariciresinol-hexoside	35.19	523	276	361, 447	[2]
12	Syringaresinol	46.80	417	275	181, 387, 166, 123	[2]
13	Feruloyl derivative	57.98	562	321,285	-	[2]
14	Betaxanthin- proline (indicaxanthin) isomer 1	19.85	309	480	106, 70, 263, 217	[21,22]
15	Betaxanthin-proline (indicaxanthin) isomer 2	20.51	309	480	106, 70, 263, 217	[21,22]
16	Tryptophan	31.61	205	278	118, 146, 132, 170	[23]
17	Phenylalanine-betaxanthin	37.28	359	480	315, 313, 131	[22]
18	Prenylnaringenin (trihydroxy-8-prenylflavanone)	38.27	341	275	137, 175, 251, 119, 311	[24]

† Compounds 1–13 were identified in negative ionization mode, while compounds 14–18 were detected in positive mode. RT, retention time.

Piscidic acid (component 3) was the compound which showed the largest area (Table 3) and some authors reported this acid as the most abundant compound in prickly pear fruits [21]. This compound showed no significant changes during cold and shelf life storage, and neither did eucomic acid (component 5). Piscidic acid is a chelator of iron which shows strong antioxidant activity, and its presence is unusual in nature, being restricted to crassulacean acid metabolism (CAM) and succulent plants [21,22]. Although there are no data about the antioxidant properties of eucomic acid, Mata et al. [22] suggested that, due to their structure, it may be similar to that of piscidic acid.

Table 3. Average of area (mAU*min) for each component detected in O. *ficus-indica* (L.) Mill. samples at 280 nm, except components 14–15 (474 nm), during cold and shelf life storage.

Id.	Component		D0	D7	D14	D21	D28
1	L-Malic acid	After cold storage	7.67 ± 0.86 A †	6.23 ± 0.68 Aa	11.32 ± 2.50 Aa	8.71 ± 2.58 Aa	8.0 ± 2.38 Aa
		After shelf life	7.67 ± 0.86 A †	7.93 ± 1.25 Aa	15.39 ± 2.09 Aa	10.87 ± 3.15 Aa	6.04 ± 3.20 Aa
2	Citric acid	After cold storage	45.3 ± 3.94 B	44.62 ± 2.01 Ba	29.45 ± 4.05 Aa	24.50 ± 4.18 Aa	28.58 ± 6.65 Aa
		After shelf life	45.3 ± 3.94 A	40.40 ± 3.2 Aa	40.18 ± 4.92 Ab	40.21 ± 4.04 Ab	32.49 ± 4.52 Aa
3	Piscidic acid	After cold storage	166.85 ± 25.99 A	172.18 ± 5.59 Aa	161.62 ± 11.07 Aa	158.07 ± 7.99 Aa	173.45 ± 3.33 Aa
		After shelf life	166.85 ± 25.99 A	180.19 ± 17.40 Aa	162.25 ± 4.10 Aa	150.47 ± 16.59 Aa	171.55 ± 26.44 Aa
5	Eucomic acid	After cold storage	43.77 ± 13.25 A	42.10 ± 1.67 Aa	37.81 ± 2.88 Aa	42.66 ± 6.14 Aa	37.66 ± 3.32 Aa
		After shelf life	43.77 ± 13.25 A	47.84 ± 4.71 Aa	42.90 ± 12.43 Aa	30.96 ± 2.81 Aa	40.47 ± 7.38 Aa
7	Eucomic acid isomer/derivative	After cold storage	19.85 ± 1.45 A	24.19 ± 0.97 Ba	25.52 ± 0.54 Ba	24.32 ± 0.73 Ba	25.25 ± 0.89 Ba
		After shelf life	19.85 ± 1.45 A	24.16 ± 1.67 Aa	25.84 ± 1.27 Aa	22.77 ± 3.58 Aa	24.54 ± 2.20 Aa
8	Ferulic acid derivative	After cold storage	50.35 ± 2.97 AB	49.55 ± 2.35 Aa	58.00 ±1.66 Ba	56.66 ± 2.77 ABa	52.49± 2.21 ABa
		After shelf life	50.35 ± 2.97 A	63.56 ± 7.57 Ab	57.95 ± 6.30 Aa	59.43 ± 3.40 Aa	57.81 ± 6.68 Aa
12	Syringaresinol	After cold storage	27.28 ± 1.61 A	31.48 ± 0.37 Ba	33.99 ± 0.67 Ba	31.42 ± 0.71 Ba	33.43 ± 0.83 Ba
		After shelf life	27.28 ± 1.61 A	30.77 ± 2.56 Aa	32.74 ± 0.20 Aa	29.87 ± 4.35 Aa	34.15 ± 3.14 Aa
13	Feruloyl derivative	After cold storage	6.79 ± 2.11 A	5.74 ± 1.20 Aa	8.38 ± 1.97 Aa	8.25 ± 1.03 Aa	6.55 ± 1.27 Aa
		After shelf life	6.79 ± 2.11 A	11.55 ± 1.77 Aa	7.38 ± 1.55 Aa	9.42 ± 2.16 Aa	8.77 ± 2.92 Aa
14–15	Betaxanthin-proline (indicaxanthin) isomers 1 and 2	After cold storage	21.00 ± 1.03 A	25.55 ± 1.62 Aa	23.42 ± 3.13 Aa	23.66 ± 1.01 Aa	23.52 ± 2.14 Aa
		After shelf life	21.00 ± 1.03 A	27.72 ± 4.29 Aa	25.67 ± 1.32 Aa	21.46 ± 2.04 Aa	22.15 ± 1.15 Aa
16	Tryptophan	After cold storage	19.21 ± 6.51 A	20.03 ± 8.77 Aa	18.21 ± 3.21 Aa	14.60 ± 0.53 Aa	12.17 ± 1.03 Aa
		After shelf life	19.21 ± 6.51 A	15.73 ± 1.25 Aa	21.71 ± 2.65 Aa	14.85 ± 3.25 Aa	18.22 ± 2.81 Aa

† Data are the mean ± standard error SE ($n = 3$). The different capital letters within the rows (A, B, AB) indicates significant differences according to the Tukey test ($p < 0.05$) throughout cold and shelf life storage. Lowercase letters within the columns (a, b) indicate significant differences according to the Tukey test ($p < 0.05$) each day independently compared under cold and shelf life conditions.

No major changes were observed for most of the phytochemicals during the cold stor- age of 'Orito' fruit, while three-day shelf life after storage did not change the phytochemical profile of prickly pear fruits (Table 3). The content of eucomic acid isomer/derivative (7) and syringaresinol (12) increased, and the content of citric acid (2) decreased (Table 3). Compounds 7 and 12 increased their content starting on day seven of cold storage, and from this moment they remained stable until the end of cold storage. However, in these compounds no significant differences were found in the comparison of cold and shelf life in the same day of storage. Ferulic acid derivative (8) decreased its content after seven days of cold storage, increased by day 21, and then decreased slightly again at the end of cold storage.

Compound 12, which content increased under cold storage, pertained to the family of lignans, a group of secondary metabolites recognized as phytoestrogens and present ina great variety of plants, including seeds and some botanical berries as prickly pear. They are polyphenolic compounds related with the phenylalanine metabolism and show an- tioxidant properties related to anti-inflammatory, antitumoral and antiviral effects, among others [25,26].

Phenolic acids, as compounds 3–8 and 13, are aromatic secondary plant metabolitesand are also widely spread throughout vegetables. They are related to color, sensory qualities, nutritional and antioxidant properties. There is some evidence of their role in the inhibition of oxidative damage diseases such as coronary heart disease, stroke and cancers [27,28]. Most of them did not change from being stored, which agrees with the results of this study, in which only compounds 7 and 8 changed during cold storage, and all of them remained stable under shelf-life conditions.

Betalains, as compounds 14, 15 and 17, are water-soluble pigments responsible for the red or yellow color of fruits and other botanical parts such as flowers and leaves of species belonging to the order of Caryophyllales, in which prickly pear is included. These compounds, which remained stable under cold and shelf-life conditions, also show antioxidant activity and may provide health benefits to consumers [29,30].

Betalains, phenolic acids and lignans are compounds related with the phenylalanine metabolism. Phenylalanine is an aromatic amino acid which is a precursor or secondary metabolite in plants, serving as a building block of many compounds essential to plant structure, reproduction, defense, and communication [31]. Phenylalanine ammonia-lyase (PAL) is an enzyme regulating the synthesis and accumulation of phenolic compoundsin plants. This enzyme is stimulated by cold, thus, the variation of phenolic content in fruits during cold storage might be influenced by its activation. Some studies reported the increase of PAL activity during cold storage, in parallel to the increase in phenolic compounds in strawberry, artichokes and blueberries [32–34].

Citric acid (2) content decreased during cold storage. This decrease could be attributed to the effect of the enzyme polyphenol oxidase (PPO), which may affect the concentration of other organic acids and is stimulated by cold storage [35].

There were no detected chilling injuries in 'Orito' fruit as they were found in other cultivars such as 'Copena-Torreoja', as studied by Corrales-García et al. [10]. In this sense, their results [10] showed 100% injury from chilling after the first month of cold storage. Other cultivars studied by these authors showed chilling injuries after two or three months of cold storage. Although visually 'Orito' fruit did not show such chilling-injury signs as browning, either under cold nor shelf life conditions, PAL and PPO enzymes are related to physiological disorders during cold storage in some fruits such as apples, mandarin-fruitsand plums [33,35,36]. More studies are required to evaluate the chilling-injury sensitivity of the 'Orito' cultivar after longer cold storage.

As previously mentioned, all of the identified compounds remained stable during shelf-life storage. This behavior could be attributed to the non-stimulation of these enzymesunder shelf-life conditions. For instance, PAL activity and concentration decreased when mandarin fruits were exposed to non-chilling temperatures [36].

4. Conclusions

Based on the results of this study, 'Orito' fruits maintained their phytochemical com-position during three-day shelf-life storage after cold storage, while the content of some betalains, phenolic acids and lignans increased during cold storage. These results, along with a previous study [13], showed that the marketability of prickly pear fruit from the 'Orito' cultivar can be possible up to 28 days after harvesting. Further investigation is required to evaluate the changes on the (poly)phenolic and betalain profile of these cac- tus fruits under other postharvest conditions, such as modified atmosphere packaging. In addition, assessing the role of well-known enzymes in the metabolism of these phyto- chemicals may lead to mechanistic insights useful to better handling postharvest reactionsin CAM fruits.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/ 10.3390/foods11020160/s1, Table S1: Gradient used in HPLC analysis; Table S2: Gradient used inHPLC-DAD/MS/MS analysis.

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Economic estimation of cactus pear production and its feasibility in Spain

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ABSTRACT

This paper explores economic opportunities of *Opuntia* cultivation in Spain regarding fresh food production (comparing production structures of Mexico, Italy and Spain), cactus pear non-food uses (exploiting its bio-functional, medicinal, nutraceutical and cosmetic properties) and environmental issues related to climate change mitigation through soil carbon sequestration. Cactus pear production structures and costs are different in the three countries: Mexico (939.77 €), Italy (4.055.1 €) and Spain (9453.77 €). Spain does not present a real productive sector but only isolated farms. *Opuntia* is an interesting opportunity for non-food production due to the amount of its bioactive compounds. Main components (μg g^{1} dried weight) are: kaempferol (34), myrcetin (65), isorhamnetin and derivatives (590), luteolin (8.4), ferulic acid and derivatives (1,050), and catechin (50). Obtaining these compounds could be a way of increasing cactus pear production profitability and creating jobs and value in rural areas. Cactus pear cultivation is a successful tool to mitigate climate change in arid and semiarid regions considering adequate farm and cultivation practices and systems. This crop is often located in high rurality areas, cultivated by small and micro-farmers. Cactus pear cultivation can be an effective tool for rural development in European arid and semiarid areas regarding production, job creation and environmental issues.

1. Introduction

Opuntia is a genus of plants of dicotyledonous angiosperm *Cactaceae* family (which includes ~1500 species) and part of natural environment and agricultural systems in arid areas. They are native to America, where they grow wild from the south part of the USA to the Patagonia. Cactus pear is cultivated worldwide (America, Asia, Europe, Africa and Oceania) as it grows in arid and semi-arid pedoclimatic zones and is the most important economic cactus species (Inglese et al., 2002).

Opuntia (*O. ficus-indica* or *O. amyclaea*) was one of the first species that came from the New World. It arrived into Europe through Spanish conquerors to make profit of unproductive soils in the south of the Iberian Peninsula (1548–1570). The idea was to cultivate it as food for carmine cochineal (*Dactylopius coccus* Costa) used to produce dyes. The plan failed but *Opuntia ficus-indica* soon found its place as a wild plant, natural fencing between land boundaries, cattle feed and human food.

Although *Opuntias* have been used as an important subsistence crop in many communities worldwide, fruit consumption remains limited to local ethnic markets with little export. Only Mexico, Italy, Chile, South Africa and Argentina produce it commercially (Reyes-Agüero et al., 2013) and cactus pear benefits from good marketing strategies in Italy, Mexico, the USA and South Africa (Inglese et al., 2002).

Mexico is the world largest producer (45% of world production), followed by Italy (12.2%) and South Africa (3.7%) (ISTAT Data Bank, 2013). In Mexico, the cactus pear sector creates employment and income in areas where few other crops can be produced (Timpanaro et al., 2015); ~20,000 families live from its cultivation (Gallegos-Vazquez et al., 2013). The planted area covers 50,000–70,000 ha, and the gross annual production ranges 300,000–500,000 t; it is the 5th fruit crop in the country.

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The 2nd world producer (and leading world exporter) is Italy, with 7000–8300 ha producing 78,000–87,000 t yearly (Timpanaro et al., 2015), while South Africa farms 1500 ha and produces 15,000 t. Chile (1100 ha and 8000 t), Argentina (800 ha and 7500 t), and USA (200 ha and 4000 t) also have significant figures, while countries such as Bolivia, Brazil, Jordan, Egypt, Pakistan, Israel, Tunisia, Algeria, Morocco and Spain also cultivate this plant (Inglese et al., 2017). However, limits of statistics collection hinder getting an accurate image of cactus pear production in these countries. Furthermore, fruit relatively low economic and social importance makes difficult that world organizations (EU, FAO, OECD, World Bank, etc.) supply consistent economic data about areas, production, employment, gross sales, export figures, etc.

There has been a general improvement in orchard techniques over the last years but there is also a lot of work to be done to change pro-ducers' perception and convince them that cactus pear can produce high yields and good quality if it receives right care and attention. It is critical to forget the wrong idea that cactus pear needs few inputs for good re-sults; this wrong believe has led to bad management of cactus pear plantations and poor fruit quality. Thus, current knowledge remains scarce and limited scientific information arrives to producers (Inglese et al., 2002). However, the Italian experience proves that rational or-chard management can give high returns and high quality fruit with relatively low management costs. To improve productivity and fruit quality, there must be greater awareness of environment and orchard management effects on fertility, fruit growth and ripening. It is also important to establish fruit quality standards and implement proper orchard design and management.

Consequently, providing the latest technical and scientific informa-tion about crop cultivation and post-harvest management, productivity levels and especially fruit quality standards to farmers should allow cactus pear competing on an equal basis with other agricultural products on international markets. Furthermore, attracting new consumers to cactus pears and creating higher demand requires consistent high-quality fruit availability (Inglese et al., 2017).

Increased fruit productivity is easier to achieve than improved fruit quality. Thus, special attention should be given to all horticultural practices potentially affecting fruit quality, at both pre- and post-harvest stages. In this sense, cactus pear fruit is usually consumed fresh, but increasing market demand for health-promoting food has prompted food technologists to develop techniques to increase its shelf life and to develop new and attractive products (Barba et al., 2017).

Regarding sales and profits, there is potential for development through a wide range of applications, including forage complement (Inacio et al., 2020; Monteiro et al., 2018), human consumption (including cladodes) both fresh and processed food, bio-functional, medicinal, nutraceutical and cosmetic uses, dyes production and bio-energy (Inglese et al., 2017). Fruits could be important commercially as they are well appreciated by consumers and have excellent nutritional properties (Cefola et al., 2014). Fruits are consumed fresh and used for food product manufacture such as juices (Ennouri et al., 2006), alcoholic beverages, jams and natural liquid sweeteners (Saenz, 2000). Regarding its polyphenols, vitamins and other specific compounds composition, cactus pear is an excellent candidate for nutritional diet and therapeutic recommendations (El-Mostafa et al., 2014). So, benefits from its culti-vation are more than just fresh fruit production (Isaac, 2016).

First, there is a vast potential for non-food uses, exploiting its bio-functional, medicinal, nutraceutical and cosmetic properties. Prickly pears chemical and nutritional components have been recently studied (Antunes-Ricardo et al., 2015; Andreu et al., 2017; Melgar et al., 2017; Andreu-Coll et al., 2019; Mena et al., 2018) and their extracts hold antiulcerogenic, anti-inflammatory, antidiabetic, antioxidant, anti-cancer, neuroprotective, hepatoprotective and antiproliferative activ-ities (Santos Diaz et al., 2017). Besides they are a good source for red and yellow food coloring agents (García-Cayuela et al., 2019). Another interesting possibility, it is production of bioethanol and biogas from cladodes. However, due to Spanish production structure, this potential has not been yet analyzed.

Table 1 shows quantities of compounds with bio-functional, medic-inal, nutraceutical and cosmetic properties in several crops, with *Opuntia* playing a key role; however, no economic value analysis of cactus pear cultivation based on the production of these compounds has been done until now.

Second, environmental issues related to agriculture must be considered. Among agriculture environmental implications, climate change mitigation through soil carbon sequestration (SCS) is a key question. SCS is an affordable and cost-effective way to mitigate agri-culture effect in climate change (Glenk and Colombo, 2011). Countries that signed the Kyoto Protocol of the United Nations Framework Convention on Climate Change agreed to lower CO 2 emissions to the atmosphere or increase removal and storage rates. The interest in C sequestration and trading as mechanisms for both environmental pro-tection and poverty alleviation in developing countries has increased considerably in the last decades (Perez et al., 2007). Arid Mediterranean agriculture possesses a SCS potential. The case of olive tree cultivation is well documented; changing practices in favor of more sustainable agricultural procedures (Nieto et al., 2012) has been proved to be suc-cessful in increasing SCS (Rodríguez-Entrena & Arriaza, 2013). Furthermore, implementing these soil-management practices also im-proves soil structure (Castro et al., 2008), reduces water losses, prevents soil erosion (Nieto et al., 2012), and preserves biodiversity and land-scape amenity aspects (Glenk & Colombo, 2011). Overall, they increase agricultural land adaptive capacity against adverse climate change im-pacts (Frelih-Larsen et al., 2008).

Cactus pear is one of the few agricultural options due to the edaphic and climatic conditions in many areas, presenting advantages over other agricultural activities because of practices that attenuate, avoid and even restore damage to the productive ecosystem (Nefzaoui et al., 2014).

Bautista-Cruz et al. (2018) compared C–CO2 emission patterns and total organic carbon (TOC) in a central Mexico highland. They compared different management systems including cactus without and with com-posted manure mulching and soil in oak-pine forest. Their results showed that cactus crop is presently contributing effectively to soil TOC.

An important question is how to achieve that these agricultural sustainable practices, that mitigate climate change, become part of producers' way of cultivating. From a policy perspective, Agri-Environment Climate Scheme (AECS) have been regarded as the most suitable instrument to increase agriculture environmental performance and could represent an interesting tool for SCS strategy development in agriculture (Colombo & Rocamora-Montiel, 2018).

ROAECS (Results Orientated Agro-Environment Climate Scheme) is a type of agro-environmental scheme based on the idea of paying farmers, not for performing management actions, but for achieving specific environmental goals (Burton & Schwarz, 2013). ROAECS encourage farmers innovation, drawing on their experience and local knowledge to achieve improved and more cost-effective results (Colombo & Rocamora-Montiel, 2018). A key factor to ensure reliability in ROAECS development is the existence of measurable and objective indicators (Burton & Schwarz, 2013), which must be clearly measurable, attributable to specific management actions, not in conflict with agri-cultural goals and consistent with ecological purposes.

In this sense, sequestration of carbon (SOC) can be measured and monitored through various laboratory and field methods by using appropriate sampling procedures (Colombo & Rocamora-Montiel, 2018). In cactus pear orchards, SOC indicator totally fulfils the mentioned requirements opening opportunities to ensure sustained in-come and a moderate environment impact. Cactus pear plantations could be part of a strategy to lessen CO2 atmosphere accumulation in arid and semi-arid areas implementing ROAECS. They can function as a water reserve and as a carbon reservoir offering a cost-effective contri-bution to climate change mitigation from the agricultural sector reducing soil erosion and water pollution.

Table 1

Quantities of compounds with bio-functional, medicinal, nutraceutical and cosmetic properties in several crop (μg g^{-1} dw)

Compound	*Opuntia ficus-indica*	*Opuntia joconostle* (1)	*Ziziphus jujube* (2)	*Stenocereus pruinosus* (3)	*Stenocereus stellatus* (3)	*Punica granatum* (4)
Kaempferol	34.1	139	39.17	nd	3.78	nd
Myrcetin	65	nd	nd	nd	nd	nd
Isorhamnetin and derivatives	590	nd	nd	2.53	nd	nd
Luteolin	8.40	nd	nd	nd	nd	nd
Ferulic acid and derivatives	1050	70	nd	8.8	36.8	nd
Catechin and derivatives	50.0	346	29.9	nd	nd	nd
Guaiacyl and derivatives	165	nd	nd	nd	nd	nd
Syringic acid and derivatives	165	33.9	nd	nd	nd	nd
Sinapic acid and derivatives	1140	nd	nd	nd	nd	nd
Quercetin and derivatives	91.1	225	148	3.53	7.14	nd
Narigin and derivatives	75.0	nd	nd	nd	22.5	nd
4-Hydroxy-benzoic acid	665	104	nd	nd	nd	nd
Eriodictyol derivative	nd	nd	2.67	nd	23.3	nd
Phloretin-3$_0$,5$_0$-di-glucoside	nd	nd	1.62	nd	nd	nd
Polymeric proanthocyanidins	nd	nd	1631	nd	nd	nd
Caffeic acid and derivatives	nd	nd	nd	34.3	29.4	135
p-coumaric acid and derivatives	nd	nd	nd	9.8	17.8	114
Gallic acid	nd	113	nd	nd	nd	175
Vanillic acid	nd	178	nd	nd	nd	nd
Elagic acid	nd	nd	nd	nd	nd	231
Rutin	nd	53.6	nd	nd	nd	nd
Taxifolin acetylhexoside	nd	nd	nd	5.25	14.6	nd
Total polyphenols	3426	1175	2254	64.7	131	655

(1) Cortez-García et al. (2015); (2) Wojdyło et al. (2016); (3) García-Cruz et al. (2017); (4) Elfalleh et al. (2011); nd = not detected.

Third, the crop is often located in high rurality areas, cultivated by small and micro-farmers. This makes it attractive from a strategic viewpoint and it should be seriously considered in public policy devel-opment actions, especially in arid and semi – arid areas.

Considering all previous considerations, this study had 3 aims: (i) economic evaluation of cactus pear production structure and costs in Mexico (main world producer), Italy (main world exporter) and Spain, special attention will be paid to establish the main economic and market features precluding *Opuntia* successful implementation in Spanish rural arid areas; (ii) economic analysis of cactus pear bio-functional, medici-nal, nutraceutical and cosmetic properties; and (iii) economic estimation of carbon soil sequestration schemes possibilities in cactus pear pro-duction considering environmental issues.

2. Tools used for calculating the estimates

2.1. Economic evaluation of cactus pear production structure

First, production environment for Mexico, Italy and Spain was compared. Then, economic evaluation of cactus pear production struc-ture was done through cost accounting (Romero et al., 2006). All op-erations are considered self-financing to avoid introducing financial variables. Economic assessment does not include fixed costs because these costs can introduce bias that do not affect the production process.

Data from other countries were obtained through published research (Basile et al., 2002; Losada et al., 2017). Average value of 1.0 € equal to 1.129 US$ is considered during 2017 (European Central Bank, 2018) for comparisons with Losada et al. (2017) and 1.259 for comparisons with Timpanaro & Foti (2014). Information was updated using inflation in-formation from European Central Bank (2018).

Spanish production information was obtained through *in situ* in-terviews in three steps: (i) open interviews with farmers; (ii) question-naires sent by post; and, (iii) audits and information validations with specific questions directed to interviewees. This data collection covered 3 full seasons in Spain.

The total variable production cost was established and was included in working assets costs. Opportunity costs were calculated as the next-best alternative use of working capital in risk-free financial assets; 2.0% interest rate was assumed, depending on money current cost and inflation adjustment.

Production variables obtained from secondary data and interviews (Table 2) were used to calculate costs and incomes. Differences in cat-egories are due to the different processes undertaken for getting infor-mation and to country cultivation techniques differences. Gross income and total variable costs can be calculated by using contribution margin (CM), which is the margin used before considering depreciation and fixed costs. CM is calculated by taking the difference between gross in-comes (GI) and incremental costs or variable costs (IC).

2.2. Economic analysis of cactus pears bio-functional, medicinal, nutraceutical and cosmetic properties

Data about the contents of components with bio-functional, medic-inal, nutraceutical and cosmetic properties found in cactus pears has been reviewed and will be presented in tables together with economic data regarding their cost and estimated prices. Market prices of these compounds were obtained through a questionnaire among main producers.

Table 2

Cactus pear production cost structure (€ ha $^{-1}$).

Item	Mexico (1) (€ ha $^{-1}$)	Italy (2)	Spain
Tools	198.40		
Weeding	163.86		77.37
Pruning	54.91		55.26
Fertilization	55.80	383.4	
Fumigation (pests)	69.08	142.35	389.47
Others		25.8	
Pruning, *scozzolatura*, fruit thinning		1330.65	
Other cultivation operations		393	
Harvest	326.83		442.11
Transport	181.57		
Mechanized operations		394.5	
Brooms	4.42		
Straw	51.37		
Gloves	8.85		
Cost of crates	2.65		
Watering		309.45	344.24
Thinning			221.05
Insurances and taxes		383.25	260.52
Wages and salaries		326.7	7663,42
TOTAL	**939.77**	**4055.1**	**9453.77**

(1) Adapted from Losada et al. (2017); (2) adapted from Basile et al. (2002) and Timpanaro & Foti (2014).

Then, an estimation of the quantities that could be obtained from 1 ha of cactus pear in Spain was calculated considering production data obtained in questionnaires carried out to producers.

2.3. Economic estimation of cactus pear production value considering environmental issues

An estimate of cactus plant CO2 accumulation will be presented based on scientific literature together with the price to be paid for car-bon sequestration, which was calculated considering not only carbon sequestration but also the benefits on the environment generated by its cultivation.

To estimate the exchange surface of each plant, 50 of them were measured in width, height and length. Then, number of cladodes *per* plant was counted and 20 of each were measured in height and length to estimate their surface area. Plant average area and average cladode surface were calculated to estimate exchange surface and CO2 daily net intake *per* m^2 and day. Cactus plant weight was calculated counting cladodes per plant and weighting 25 of them; roots were not considered.

3. Discussion on economic estimates for the potential implementation of *Opuntia ficus-indica* in Spain

3.1. Characterization of fruit productive environment

3.1.1. Mexico

Mexico has introduced significant changes in cactus pear production recently, including drip irrigation in semi-arid areas, cultivation in less arid areas (central highlands and some subtropical regions in central south and western parts of the country), use of mechanical fruit-cleaning technologies, improved packing materials and modern commercial presentations. According to Losada et al. (2017), Mexican orchard size ranges 1–20 ha, with predominant size being 1–3 ha (64% of producers), 23% from 4 to 8 ha (23%), and 12–20 ha (9%). The main produced variety is pale green Alfayuca (*Opuntia amyclaea*). The distance between plants and rows goes from 4 to 6 m and orchard age from 20 to 70 years. They are pruned when they reach 1.5–2.0 m height (to facilitate fruit picking) (February–April). Fertilization is mainly done using triple 17 (17 N - 17 P2O5 - 17 K 2O) and urea (46 N), without a clear period for inorganic fertilizer use. Organic material is used, once or twice *per* year, mainly as manure because it is free and only transport cost applies, 15–60 kg of dry manure *per* plant (500 t ha^{1}) depending on availability and orchard age. Prickly pear is very prone to pests and diseases. Pro-ducers constantly try to avoid them especially during post-harvest (Inglese et al., 2017). The production cycle, in a commercial orchard, starts in March (after frosts), increases in April, rises significantly during June and falls in September, October and November. The yield *per* ha is 10–15 t.

3.1.2. Italy

Italy represents an atypical example of *Opuntia ficus-indica* appreci-ation. Cactus pear has been exploited since the 18th century but with no commercial purposes, such as farm fencing and emergency fodder. Cactus pear is mainly cultivated in southern regions: Sicily, Sardinia, Calabria and Apulia. However, cultivation concentrates on Sicily (96%) with 4 important geographical areas: San Cono, Volcano Etna, Rocca-palumba and Santa Margherita Belice. The most cultivated cultivars are “Gialla”, “Rossa” and “Bianca”, with “yellow” varieties predominating (~75%), followed by “red” and “white” ones. Sicilian orchard average size is less than 3 ha (Basile et al., 2002), with a plantation density from 300 to 900 plants *per* ha. Rainfall is 600 mm per year and under irri-gation, the yield can reach 25 t *per* ha. Traditional use of simple and complex (binary and ternary) mineral fertilizers is common and coun-tered by a generalized use of stable manure or other organic manures. Weeding with glyphosate for fighting the fruit fly and scabious rust with products based on dimethoate in conventional cultivation are also frequent. Mechanical weeding, use of traps (organic au-xiliaries) and natural insecticides in organic cultivation are other techniques. Running a cactus pear plot requires a relatively high number of labor hours, although recent technical progress has made possible a partial reduction of tasks. However, due to their specific nature of some cultivation operations, such as pruning, *scozzolatura* and thinning, must be done manually. Apulia production is around 2650 t in 320 ha, mainly in Foggia province (North Apulia) with selected (spineless) cultivars. Initially, Sicilian cactus pear production was exported to the continent. This economic success was reinforced by *scozzolatura* technique. This ancient practice, developed by Italians at the beginning of the 18th century, consists of cutting off May first flowering production. The plant is forced into a second more abundant flowering during full summer period (July/August). It delays fructification, allowing autumn har-vesting, producing better quality fruits than in the regular August season (Inglese et al., 2017). Autumn harvest (August–November) represents 90% of total production.

3.1.3. Spain

In Spain cactus pear is cultivated only in few family plantations in Andalusia, Murcia, Almeria and the Balearic Islands, with Lanzarote (Canary Islands) having a small production of red dye (Inglese et al., 2017). *O. ficus-indica* regular crops cover around 185 ha with an esti-mation of ~131,360 disseminated plants. Orchard average size is 15 ha, with a plantation framework of 2 m 7 m (between plants and rows, respectively) and 714 plants *per* ha. As a cultivated plant, prickly pear life is approximately 20 years. Orchards are irrigated 4 times a week (2–3 h *per* ha) during May and June through drip irrigation in dry years, using municipal-treated wastewater. Pruning, weeding, thinning and harvesting are done manually. Pruning is only for renewal purpose (daily, for 15–30 min *per* ha) and weeding is only made in the streets (once a year); these labors do not require many working hours. But, thinning requires more work, being done during a full month for 5–6 h *per* ha daily. Harvesting requires more time, because it is a very delicate labor due to fruit spines or prickles. To facilitate this labor, long-arm tongs are used and it is usually done early in the morning, preventing prickles from getting rigid and inserting into the farmer body. Prickles removal and packaging (13–14 kg boxes) are also done manually; a person can pack 30–75 kg of fruit *per* h, considering removal, accom-modation and fruit weighing. In general, no fertilizers nor organic matter are used. About phytosanitary products, main active substances are dimethoate (1.5%) and chlorpyrifos (2%) for preventing Mediter-ranean fruit fly (*Ceratitis capitata*) and cochineal (*Dactylopius coccus*), respectively. Chlorpyrifos treatment is done approximately once a month but not during the harvest period (August and September). In contrast, dimethoate treatment is carried out every two weeks from the second half of July to the end of September. Official Spanish production is around 720 t *per* year (MAPA, 2018), but real production is difficult to quantify.

3.2. Cost analysis

Table 2 shows cactus pear production cost analysis for Mexico, Italy and Spain, and shows clear differences among countries. With the main costs being harvest, pruning/*scozzolatura*/thinning and wages/salaries in Mexico, Italy and Spain, respectively.

3.3. Analysis of the gross economic profit margin

Mexican average production *per* ha is approximately 12.8 t ha^{1} (400 crates), with a selling price of 3.2 € *per* crate (Losada et al., 2017); this gives a total of 1280 € *per* ha planted and a profit of ~340 € *per* ha.

Italian average production *per* ha is approximately 15.1 t ha^{1} (Basile et al., 2002). Timpanaro & Foti (2014) calculate farm incomes consid-ering fruit market value in 2013 at different producing areas. By combining average yields and prices, average farm incomes vary from

4756 € ha^{-1} for "*Belice Valley*" to 6672 € ha^{-1} for "*San Cono Hills*" (þ40% of the minimum). Thus, the average income is 5714 € *per* ha, leading to an average profit of 1659 € *per* ha.

Spanish average production *per* ha is 234 t ha^{-1} (range 195–273). Prices ranges between 1.05 and 1.8 € *per* kg. Average income is 555,255

€ *per* ha leading to an average profit of 545,801 € *per* ha.

3.4. Economic analysis of cactus pears production regarding bio-functional, medicinal, nutraceutical and cosmetic properties

Table 3 shows average content of bio-functional, medicinal, nutra-ceutical and cosmetic components in cactus pears according to pub-lished research. Table 4 summarizes average commercial quantities and prices, average quantity for the main bio-functional, medicinal, nutra-ceutical and cosmetic components and value (€) of 1 g of cactus (dry weight, dw) according to its composition.

The next step in the analysis is looking at the cost of obtaining these compounds in prickly pear. These processes and their costs depend on the type of plant material and compound to be extracted. There are no studies on these and in-depth cost analysis should be done considering the estimated value shown in Table 4. This will give an estimate of the viability of cultivating *Opuntia* for these purposes.

3.5. Economic estimation of cactus pear production value considering environmental issues

Most plants open stomata at dawn, taking CO2 from the atmosphere, which is incorporated into various products of photosynthesis. Diurnal opening of stomata leads to an inevitable loss of water from leaves and meristematic stems. CO2 intake and water loss occur mainly at night in *Opuntias*, when temperature is lower and humidity is higher, reducing water loss. CO2 intake and *Opuntia* biomass accumulation depend on

Table 3
Average quantity of bio-functional, medicinal, nutraceutical and cosmetic components in cactus pears (μg g ^{1} dried weight, dw).

Compound	Average content (μg g ^{1} dw)	References
Kaempferol	34.04	El-Mostafa et al. (2014), García-Cayuela et al. (2019), Mena et al. (2018)
Myrcetin	65	Mena et al. (2018)
Isorhamnetin (and derivatives)	590	El-Mostafa et al. (2014), García-Cayuela et al. (2019), Mena et al. (2018), Yeddes et al. (2013)
Luteolin	8.4	El-Mostafa et al. (2014)
Ferulic acid (and derivatives)	1050	Mena et al. (2018)
Catechin	50	Mena et al. (2018)
Guaiacyl(t8-*O*-4) guaiacyl-hexoside	105	Mena et al. (2018)
Guaiacyl(8-*O*-4) syrinigyl(8–8) guaiacyl-hexoside	60	Mena et al. (2018)
Syrinigyl(t8-*O*-4) guaiacyl	60	Mena et al. (2018)
Sinapic acid (and derivatives)	1140	Mena et al. (2018)
Quercetin (and derivatives)	91.1	El-Mostafa et al. (2014), García-Cayuela et al. (2019), Mena et al. (2018), Yeddes et al. (2013)
Narigin (and derivatives)	75	Mena et al. (2018)
Syringaresinol	105	Mena et al. (2018)
4-Hydroxy-benzoic acid	665	García-Cayuela et al. (2019)
Piscidic acid	18865	García-Cayuela et al. (2019)
Betaxantins	196	Cano et al. (2017); García-Cayuela et al. (2019)
Betacyanins	328	Albano et al. (2015); Cano et al. (2017), García-Cayuela et al. (2019)

Table 4
Average commercial quantities and prices, average quantity (in micrograms in a gram of cactus pear dry weight) for the main bio-functional, medicinal, nutra-ceutical and cosmetic components and value of each g of cactus (dry weight) according to its composition.

Compound	Weight	Average price (€)	Average price (€ μg ^{1})	Average content (μg) in 1 g dw of cactus pear	Value (€) of 1 g dw of cactus pear
Kaempferol (520-18-3)	20 mg	213.68	0.010684	34.04	0.36
Myricetin (529-44-2)	20 mg	238.11	0.011906	65	0.77
Rhamnetin (90-19-7)	10 mg	198.42	0.019842		
Fisetin (528-48-3)	10 mg	195.37	0.019537		
Isorhamnetin (480-19-3)	10 mg	204.53	0.020453	589.87	12.06
Myrcene (123-35-3)	100 mg	134.32	0.0013432		
Galangin (548-83-4)	20 mg	225.89	0.0112945		
Kaempferide (491-54-3)	10 mg	177.05	0.017705		
Luteolin (491-70-3)	10 mg	189.26	0.018926	8.4	0.16
Ferulic acid (537-98-4)	1 g	134.32	0.134320	1050	141.04
Gossypetin (489-35-0)	10 mg	265.58	0.026558		
4-Coumaric acid (501-98-4)	1 g	134.32	0.00013432		
3-Coumaric acid (14755-02-3)	1 g	134.32	0.00013432		
2-Coumaric acid (614-60-8)	1 g	134.32	0.00013432		
(þ)- Catechin (154-23-4)	10 mg	186.21	0.018621	50	0.93
Morin (480-16-0)	20 mg	195.37	0.0097685		

environmental conditions, mainly soil water content, air temperature, light and various soil elements. Allegra et al. (2015) and Pimienta-- Barrios et al. (2005) quantified this CO2 intake (Table 5); their data considered close-to-optimal temperatures, wet soil and indicated photosynthetic photon flow (PPF), and showed that *O. ficus-indica* takes 550 mol CO2 m^2 daily.

Considering data from 3 Spanish orchards, as an average, a 5-years-old *O. ficus-indica* plant has 75 cladodes with an average cladode area of 0.09 m^2 (0.45 m 0.21 m). This leads to a plant average area of 7.8 m^2. The average plant density is 714 plants *per* ha. Thus 1 ha will contain 5060.47 m^2 of cladodes, implying that 1 ha of *O. ficus-indica* can take 2,783,261 mol CO2 *per* d (63.25 kg d ^{1}). An *O. ficus-indica* plant is fully productive when it is 5 years old and can reach 20 years of full pro-duction. Consequently, 1 ha of *O. ficus-indica* can take ~462 t of CO2 during its complete productive life.

The number of cladodes were counted in 50 plants to estimate a cactus plant weight. Three cladodes *per* plant were weighted. A young plant (6–8 years old) presents 150 cladodes with an average weight of 2.5 kg each. An adult plant (20 years old) reaches, as average, 250 cladodes, leading to ~625 kg *per* adult plant. According to El-Mostafa et al. (2014), García-Cayuela et al. (2019), Mena et al. (2018) average water quantity of cactus pear is 80%. Thus, an adult cactus pear plant has 125 kg of dry mass.

Gomez-Casanovas et al. (2007) indicate that C content in a cladode is 36.2%. Thus, an adult plant has 45.25 kg of C (~166 kg of CO2). As a result, an adult cactus pear plant fixes 8.29 kg of CO2 *per* year through its

Table 5
Average temperature (T), daily total photosynthetic photon flow (PPF), soil water potential and CO_2 daily net intake for cultivated CAM plants in monitored laboratory conditions.

CAM plants	Day/night average air T (ºC/ºC)	PPF (mol m^2 d^{-1})	Soil water potential (MPa)	CO2 daily net intake (mol m^2 d^{-1})	CO_2 net intake periods			CO_2 daily net intake contribution (%)	
					Day	Night	Total	Day	Night
Agave salmania	25/15	22	0.2	481	5	12	17	3	97
Agave tequilina	15/10	22	0.1	298	6	12	18	30	70
O. ficus-indica	25/10	20	0.1	550	3	12	15	10	10
Sten. queretaroensis	28/15	19	0.2	317	6	12	18	14	14

Source: Adapted from Allegra et al. (2015) and Pimienta-Barrios et al. (2005).

cladodes. This value should be revised and checked with in-depth studies, but it allows estimating the value of 1 ha considering environ-mental issues.

Bautista-Cruz et al. (2018) showed how cactus crop can contribute effectively to soil accumulation of organic carbon. Thus, cactus pear cultivation can be a successful way to mitigate climate change in arid and semiarid regions. Obviously, the amount of CO2 remaining in the soil will depend on the agricultural practices applied by the farmers. A major concern is how to implement and achieve that sustainable prac-tices helping to mitigate climate change become part of farmers' way of cultivating. As presented, ROAECS are agro-environmental schemes based on the idea of paying landowners for achieving specific environ-mental outcomes. ROAECS could be designed to adapt cactus pear production and management practices defining measurable and objec-tive indicators consistent with ecological goals. SOC in cactus pear farms can be an effective indicator as it totally fulfils the stated requirements. Cactus pear plantations can function, not only as a water reserve, but as a carbon reservoir in arid and semi-arid regions offering a cost-effective agricultural contribution to climate change mitigation. Furthermore, it will reduce soil erosion and water pollution.

Carbon price is an issue to be analyzed. According to Point Carbon (Reuters, 2014), carbon price estimates would remain below 10 € during 2015 and 2016, dropping below 5 € in 2020, but rising steeply up to around 50 € by 2030. Carbon was marketed in recent years within the EU Carbon Trading Scheme, starting from a value close to 30 € *per* t of CO2 in 2008 (Carbon Market Watch, 2014), since 2012 price has persistently been under 10 € t *per* t of CO2 until March , 2018 and being over 20 € *per* t since December , 2018. Given carbon price ranges and the uncertainty over prices might apply, various scenarios should be considered. 2020 current average value, according to European bourse for Unit Allowances and Carbon Credits (SENDECO2, 2020), is around 25 € *per* t of CO 2. A 20 € value could, therefore, represent an average estimate during 2020–2030, but as previously stated, there can be no guarantee for future carbon prices (UK-Department of Energy and Climate Change, 2013).

4. Conclusions

This study had 3 aims and conclusions will be summarized for each one of them:

1 *Economic evaluation of cactus pear production structure and costs in Mexico, Italy and Spain*. Production structure is different in each producing country, with Spain not being a real productive sector but consisting of isolated farms. The high price that the product reaches (and the profitability) is because the demand is much higher than the production. On the other hand, Italy presents a developed cactus pear producing sector. Spanish producers should look at Italy before growing to avoid problems derived of increasing production without real and effective distribution channels and mature demand.

2 *Economic analysis of cactus pear bio-functional, medicinal, nutraceutical and cosmetic properties*. Quantities of compounds with bio-functional, medicinal, nutraceutical and cosmetic properties in several crops have been analyzed; *Opuntia* holds the highest contents of several of these compounds. Average quantities and average prices of these highly demanded components compared with average quantity (in μg) of these components in 1 g of dried cactus pear have been pre-sented. Further research should look at the cost of obtaining these compounds. Obtaining these compounds could increase profitability of cactus pear production and, thus, promote a comprehensive development of the rural areas in which the production takes place.

3 *Economic estimation of applying carbon soil sequestration schemes in cactus pear production considering environmental issues*. Cactus pear cultivation is a successful tool to mitigate climate change in arid and semiarid regions. Farm and cultivation practices and systems are key aspects of how cactus crop can contribute effectively to improve soil carbon sequestration. To better develop this issue, measurable and objective indicators consistent with ecological goals are required. Such indicators should also be used to establish sustainable pro-duction and management practices. Under a policy perspective, these indicators should be embraced in a ROAECS to boost their adoption among farmers.

As a general conclusion, cactus pear cultivation can be an effective tool for rural development in European arid and semiarid areas regarding production, job creation and environmental issues.

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Chapter

Valorization of Prickly Pear [*Opuntia ficus-indica* (L.) Mill]:Nutritional Composition, Functional Properties and Economic Aspects

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Abstract

Opuntia ficus-indica (L.) Mill, usually named prickly pear or nopal cactus, is the Cactaceae plant with the greatest economic relevance in the world. It is a tropical or subtropical plant, native to tropical and subtropical America, which can grow in arid and semiarid climates. Prickly pear is mainly known by its fruits, popularly named "tunas" or "figs," but their cladodes are also consumed, principally in Mexico, which is the country with the largest cultivated area and the largest pro-ducer. There is ample evidence of the health benefits of prickly pear: it shows high antioxidant activity, it is a source of nutrients and vitamins and it presents medici- nal uses, among others. Furthermore, prickly pear presents other uses, including cosmetics, biofuel production, animal nutrition and soil phytoremediation.

Keywords: cactus pear, fruit, antioxidant activity, health benefits, peel, pulp

1. Introduction

Opuntia ficus-indica L. Mill, usually known as prickly pear, cactus pear or nopal, is a tropical or subtropical plant that belongs to the Cactaceae family, originally from arid and semiarid regions of America. This plant can grow in arid and semi- arid climates, being the Cactaceae plant with the greatest economic relevance in the world [1]. It produces an edible and highly flavored fruit, known as "cactus pear," which is a berry with numerous seeds and thick peel, enclosing a delicately flavored pulp [2]. Their cladodes are also consumed, mainly in Mexico, which is the country with the largest area under cultivation and the largest producer [3, 4] but it is also cultivated in the United States, Spain, Italy, South Africa and Argentina, among other countries [5, 6]. Prickly pear fruit is commonly consumed in fresh, but it can also be consumed as juices, jam, syrups and other processed products. They are widely employed in Latin America. The current demand of prickly pear in Spain is increasing [4, 6].

There are ample evidences of the health benefits of consumption of prickly peardue to its source of nutrients and vitamins [4, 7, 8] and antioxidant properties due to its content of bioactive compounds [2, 9, 10]. Additionally, prickly pear presents medicinal uses: it is used in treatment of hyperglycemia and high levels of cho- lesterol [7, 11, 12] and its consumption is linked with lower incidence of coronary diseases and some types of cancer [8, 13], among others.

This chapter is focused on the nutritional composition, bioactive compounds and economic aspects of prickly pear fruits through a compilation and synthesis of theavailable studies. With this, the authors intend to contribute to the knowledge of *O. ficus-indica* and also to promote new scientific research and industrial use of this crop.

2. Nutritional composition

Table 1 shows nutritional composition of prickly pear pulp and peel. Prickly pear fruit pulp has high content of protein, lipids and moisture but low content of total fiber and ash comparing to the peel.

About sugar profile, glucose and fructose are the predominant ones in both peel and pulp. On average, fruit pulp shows high content of glucose (123 g L^{-1}) andfructose (71.7 g L^{-1}) than peel (91.0 and 52.0 g L^{-1}, respectively) [9].

Prickly pear fruit also stands out for its mineral contents. Potassium is the major macronutrient in pulp (199–410.7 mg 100 g^{-1} dw), followed by calcium (12.4–49.1 mg 100 g^{-1} dw) and magnesium (18 mg 100 g^{-1} dw). Fruit peel presents magnesium (18.6–987 mg 100 g^{-1} dw), calcium (49.04–951) and potassium (320–549 mg 100 g^{-1} dw) as the major macronutrients [14, 15]. Fruit pulp shows lower level of sodium (0.70–1.09 mg 100 g^{-1} dw) than peel (1.8–951 mg 100 g^{-1} dw)[14–16]. Iron, manganese and copper are the major microelements in fruit peel and pulp [14, 15]. The mineral pattern depends on the fruit origin and crop factors [15].

Constituents	Unit	Pulp	Peel	References
Moisture	%	90.66	88.92	[15]
Titratable acidity	g citric acid L^{-1}	0.23–1.60	0.61–3.40	[9]
Total soluble solids	° Brix	10.7–15.7	8.03–15.4	[9]
pH	—	5.41–6.01	4.83–5.59	[9]
Energy	kcal 100 g^{-1} dw	361	169	[17]
Protein	% dw	1.62	1.53	[15]
Lipids	% dw	0.56	0.32	[15]
Total fibers	% dw	4.65	5.83	[15]
Ash	% dw	2.60	3.40	[15]
Fructose	g L^{-1}	57.8–88.0	27–81.8	[9]
Glucose	g L^{-1}	103–144	57–128	[18]

Table 1.
Nutritional composition of prickly pear fruit pulp and peel.

3. Bioactive compounds

Table 2 shows the main bioactive compounds present in prickly pear fruit peel and pulp. These are betalains (betanin and indicaxanthin), flavonoids, phenolics, vitamin C and carotenoids.

Compounds	Unit	Pulp	Peel	References
Vitamin C	mg 100 g^{-1} fw	28–79.2	59.8	[2, 14, 19, 20]
Total flavonoids	mg rutin equivalents g^{-1} fw	0.2–0.7	1.4–2.8	[21, 22]
Total phenolic content	mg rutin equivalents g^{-1} fw	2–2.5	5.4–6.2	[21]
Carotenoids	μg g^{-1} fw	2.56–3.79	12.58–16.93	[2, 6]
Indicaxanthin	mg 100 g^{-1} fw	2.61–39.6	—	[19, 20]
Betanin	mg 100 g^{-1} fw	0.10–1.04	—	[20]

fw, fresh weight.

Table 2.
Principal bioactive compounds in prickly pear fruit pulp and peel.

Betalains are water-soluble pigments (containing nitrogen) that are responsible for the red or yellow color of fruits, flowers, roots and leaves of plants belonging to the order of *Caryophyllales*, in which Cactaceae plants are included. [19]. Prickly pear fruits are characterized by various colors due to the combination of two betalain pigments, the purple-red betanin and the yellow-orange indicaxanthin [20]. These compounds make prickly pear fruits a good source of bioactive compounds with anti-oxidant properties, which may have beneficial effects on the consumer's health [19].

Flavonoids are a group of secondary metabolites of plants implicated in fruit and flower coloration, photosensitization and energy transfer, among others. Flavonoids present high antioxidant activity that helps to neutralize damaging free radicals and to prevent oxidative stress in the human body [21, 22]. Prickly pear fruits contain more flavonoids in the peel than in the pulp and there are fewer flavonoids than phenolic compounds (**Table 2**) [21].

Vitamin C is an essential nutrient for humans that provides a high antioxidant activity and prevents against oxidative stress in humans [14, 20, 21]. The content of this vitamin depends on the cultivar among other factors, being higher in red cultivars, which show higher concentration of vitamin C than some common fruits such as apple, peach and grapes [2].

Carotenoids are organic pigments that belong to isoprenoid group and are widely distributed among fruits. They are responsible for most yellow, orange and red colors in vegetables. These pigments contribute to the appearance and attrac- tiveness of a fruit. They can also perform as antioxidants [2, 6]. Concentration of carotenoids in prickly pear fruits is slightly lower than that reported for other fruits but it confirms the observation that yellow-colored fruits present higher concentra-tions than colored fruits [2].

4. (Poly)phenols and phenolic profile

Polyphenols are an important group of natural compounds, founded in plants and characterized by the presence of more than one phenol group in their structure. These molecules are considered to be of high scientific and therapeutic interest, because they help to prevent degenerative diseases, cardiovascular diseases and cancers, among others, due to their antioxidant activity [21, 23].

In general, the peel of prickly pear fruits is richer than pulp in total phenolic content [21, 24, 25] (**Table 2**). The profile of individual (poly)phenolic compounds depends on the cultivar [18]. Generally, predominant compounds in prickly pear fruit pulp and peel are ferulic acid derivatives,

Compound	Pulp [18]	Peel [18]	Pulp [5]	Peel [5]	Pulp and peel [26]
Protocatechuic acid-hexoside	x	x			
Piscidic acid			x	x	x
Caffeic acid 4-O-glucuronide					x
4-Hydroxybenzoicacid derivative			x	x	
p-Coumaric acid 4-O-glucoside					x
Myricetin-hexoside	x	x			
Ferulic acid derivative	x	x			x
Ferulic acid-hexoside	x	x			
Guaiacyl(t8-O-4)guaiacyl-hexoside	x	x			
Sinapic acid-hexoside	x	x			
Syrinigyl(t8-O-4)guaiacyl	x	x			
Quercetin-hexoside-pentoside	x				
Quercetin-rhamnose-hexoside-rhamnose		x			
Rutin-pentoside		x			
Syrinigyl(t8-O-4)guaiacyl	x				
Kaempferol-di-rhamnose-hexoside		x			
Kaempferol-glucosyl-rhamnoside			x	x	x
Kaempferol 3-O-(2”rhamnosyl-galactoside)7-O rhamnoside					x
Taxifolin					x
Isorhamnetin-rhamnose-rutinoside	x	x			
Isorhamnetin glucosyl-rhamnosyl-rhamnoside			x	x	
Isorhamnetin glucosyl-pentoside			x	x	
Isorhamnetin glucosyl-rhamnoside			x	x	x
Quercetin-hexoside-pentoside	x	x			
Isorhamnetin derivative	x	x			

Compound	Pulp [18]	Peel [18]	Pulp [5]	Peel [5]	Pulp and peel [26]
Dihydrosinapic acid hexoside	x	x			
Quercetin-3-O-rutinoside (rutin)		x	x	x	
Secoisolariciresinol-hexoside	x	x			
Quercetin-hexoside	x	x			
Kaempferol-rutinoside		x			
Syringaresinol	x	x			
Naringenin-hexoside	x	x			
Isorhamnetin-rutinoside	x	x			
Isorhamnetin-3-O-glucoside					x
Isorhamnetin diglucoside					x
Isorhamnetin-C-hexoside		x			
Eucomic acid					x
Naringin	x	x			
Guaiacyl(8-O-4) syrinigyl (8-8) guaiacyl-hexoside	x	x			
Feruloyl derivative	x				
Trihydroxy-methoxy-flavonol	x	x			

Table 3.
Phenolic compound found in prickly pear fruit peel and pulp in the most recent studies.

isorhamnetin and derivatives, sinapic acid and derivatives, and quercetin and derivatives [5, 18, 24]. Other compounds found in these botanical parts are kaempferol, myricetin, luteolin, catechin, naringinand syringaresinol, among others [5, 18, 24].

The presence of the phenolic compounds in prickly pear fruit peel and pulp, due to its antioxidant activity, makes this fruit an important product that can contribute to prevent human degenerative diseases such as cancer, diabetes, hypercholesterol-emia, arteriosclerosis or cardiovascular and gastric diseases [21, 25]. **Table 3** shows some compounds found in the most recent studies [5, 18, 26] about phenolic profile of prickly pear fruit peel and pulp.

5. Sugars and organic acid composition

Citric and malic acids are the major organic acids present in prickly pear fruit pulp and peel. Other organic acids, such as oxalic, tartaric, quinic, shikimic and fumaric acids, are present in traces. Citric acid ranges from 1.60 to 3.20 g L^{-1} in fruit peel and shows values from 0.30 to 1.61 in pulp g L^{-1} [9]. Malic acid shows concentrations between 1.04 and 2.20 g L^{-1} in peel and 1.20 and 2.10 g L^{-1} in pulp. However, cladodes show higher values of these acids (71.8 g L^{-1} of malic acid and

37.7 g L^{-1} of citric acid) and also contain succinic acid (43 g L^{-1}) [9]. This is due to the CAM metabolism of *O. ficus-indica*, especially in the cladodes. Organic acids are accumulated in the vacuole during night and suffer a reciprocal reserve carbohy- drates accumulation during the daytime phase [27].

Organic acids in fruits are in lower concentration in comparison with cladodes; however, fruits, especially pulp, are characterized by high sugar content. Some authors [9] studied the concentration of glucose and fructose in fruits and their results show that glucose predominates over fructose in both fruit peel and pulp (123 g L^{-1} of glucose and 91 g L^{-1} of fructose in pulp versus 91 g L^{-1} of glucose and 52 g L^{-1} of fructose in fruit peel). However, other studies [28] show that glucose, fructose and sucrose concentration is higher in fruit peel than in pulp. These resultsindicate that concentration of sugars may depend on the cultivars.

Sugar concentration in prickly pear fruit makes it a good source of energy and a natural source of sweetness for food preparations. Besides, fructose contributes to the typical sweet taste of this fruit, due to its high wetness compared with glucose and sucrose [29].

6. Volatile compounds

Volatile compounds influence the sensory quality of fruits. Their aromas are formed from a complex group of chemical substances such as aldehydes, alcohols, ketones, terpenes and esters, among others. These compounds usually show a low concentration in fruits and their variability depends on cultivar, climatological conditions, maturity and storage conditions, among other factors [30]. In prickly pear fruit pulp, the content of these compounds varies from 3.33 mg 100 g^{-1} to 14.86 mg 100 g^{-1} [31].

Even though prickly pears have no strong aroma, up to 61 compounds have been identified [32]. In a recent research [31], the studied cultivars showed aldehydes and terpenes as the most numerous compounds. Both chemical groups and alcohols were the most abundant compounds. However, other studies reported alcohols [32–34] and esters [35] as the most numerous and abundant compounds. Some predomi- nant compounds are D-limonene (citrus notes), 2,6-nonadienal (vegetable notes), nonanol (green, melon and fatty attributes), 2-hexenal (almond, apple green, sweet and vegetable notes), and 1-hexanol (green and sweet notes), among others [31–33].

Although prickly pear fruits are highly valued for their health-promoting benefits, sensory analysis is needed to complete the knowledge of aroma of this fruit and the effect of the cultivar [31].

7. Fatty acids

The consumption of monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, respectively) has been stated to provide health benefits. It also contrib- utes to the improvement of various health conditions regarding obesity, cardiovas-cular diseases, diabetes mellitus and even some types of cancer

[13, 36].

Prickly pear fruit pulp and peel showed important percentages of MUFAs and PUFAs. In fruit pulp, MUFAs ranged from 16.9 to 40.2% (as % of total of fatty acid profile) and PUFAs ranged from 35.2 to 53.9%. Fruit peel showed slightly lower values of MUFAs (6.90–31%) but higher ones in PUFAs (37.0–63.2%) [37]. Furthermore, prickly pear seed oil showed high percentages of PUFAs, recorded at levels between 57.90 and 63.29%, and MUFAs, ranged from 19.81 to 23.30% [38].

The most abundant compounds in fruit pulp, peel and seed oil were linoleic(C18:2), oleic (C18:1) and palmitic (C16:0) acids [15, 37]. Prickly pear fruit peel showed higher percentages of linoleic acid than fruit pulp (41.2 and 29.2% respectively), but pulp presented higher percentages of oleic acid than peel (26.8% in pulp and 14.4% in peel).

Both peel and pulp showed similar percentages of palmitic acid [37].

8. Health benefits: antioxidant activity

Antioxidant activity is one of the major mechanisms by which fruits and veg- etables provide health benefits. Fruits and vegetable are also able to inhibit excessive oxidation due to free radicals, which are in the form of reactive oxygen species
[9]. Prickly pear is rich in antioxidant product, containing phenolic compounds, carotenoids, betalains and vitamin C, all of which could be directly responsible for the health benefits [39]. Antioxidant activity in prickly pear fruit and peels may be affected by environmental factors, cultivar, genetic diversity, phenotype, agronomic practices, environmental and climatic conditions and processing of the fruit, among others [40]. Besides, the processing method and the extraction solventaffect antioxidant activity of *O. ficus-indica* extracts [26].

Antioxidant activity can be measured by different methods depending on the various mechanisms of antioxidant action. For example, some authors [2, 6, 8–10, 26, 41] studied antioxidant activity by DPPH, $ABTS^{\cdot+}$, FRAP and ORAC methods. DPPH method consists in the elimination of DPPH radical by antioxidant

Method	Unit	Pulp	Peel	References
ABTS	mmol Trolox kg^{-1} dw	6.40–30.6	14.7–36.9	[9]
	μmol Trolox g^{-1} fw	6.70	—	[10]
DPPH	mmol Trolox kg^{-1} dw	58.4–60.1	54.8–59.6	[9]
	μmol Trolox 100 g^{-1} fw	108.85–122.47	141.60–141.80	[6]
FRAP	mmol Trolox kg^{-1} dw	15.0–32.3	40.2–116	[9]
	μmol Fe (II) g^{-1} dw	18.42–137.65	58.70–175.44	[26]
ORAC	mmol kg^{-1} fw	3.68–8.16	—	[41]
	μmol Trolox g^{-1} fw	26.3	—	[2]

dw, dry weight; fw, fresh weight.

Table 4.
Antioxidant activity of prickly pear fruit pulp and peel by different methods.

compounds present in the extracts, which determines its ability to capture radicals. The ABTS method captures the cationic $ABTS^{\cdot+}$ radical. FRAP method measures the ability to reduce Fe^{3+} in the sample. ORAC method measures the ability of the sample to scavenge peroxyl radicals.

Table 4 shows the antioxidant activity of *O. ficus-indica* depending on the method and the part analyzed (pulp and peel). The scavenging activity of DPPH, $ABTS^{\cdot+}$ and FRAP methods is higher in fruit peel. This trend can be observed in other fruits like pomegranate [42], guava fruit [43] and berries [44]. The consump- tion of fruits with high antioxidant activity, such as prickly pear fruits, is related to preventing degenerative diseases such as cancer, diabetes, hypercholesterolemia, arteriosclerosis or cardiovascular and gastric diseases [21, 25].

9. Processed products

One of the oldest ways to preserve highly perishable fruits is through different processing systems. Although it is necessary to do more research in preservation of prickly pear fruit and use it out of the harvest period, there are some pro- cessed products obtained from prickly pear fruit. The main ones are juices and nectars, marmalades and jams, dehydrated sheets, sweeteners, alcohol and wines [29, 45].

Juices and nectars from prickly pear fruit are mostly water. They contain appre- ciable amounts of sugars, vitamins and mineral salts (mainly potassium, calcium and sodium). They also are a good source of bioactive substances such as phenolic compounds, betalains, vitamin C and β-carotene. These products show different percentages of fruit pulp (15–75%), citric acid (0.3%), sucrose and water [45, 46].

Marmalades and jams are usually prepared from ripe fruits with high sugar content. In their manufacturing, it is important to control the sugar/pulp ratio, type and quantities of acidifying agents and the percentage of added pectin (thickening agent). Prickly pear fruit pulp already contains pectin, responsible for the viscosity of the pulp, which is a positive element toward the production of juices, marma- lades and jams [45, 47].

Regarding prickly pear dehydrated sheets, there are different formulations and methods for their elaboration, mixing pulp in different sucrose ratios (0–10%). The thickness of the sheets is usually 5–15 mm. The preparations need to be spread and then dried at 60–70°C for at least 44 hours. Some authors mix prickly pear pulp with other fruits, like quince or melon pulps [45, 48, 49].

Sweetener liquid preparation from prickly pear fruit pulp implies enzymatic clarification of pulp juice, its decoloration and its vacuum concentration until 60°Brix (56% of glucose, 44% of fructose approximately). The obtained product shows a density and water activity similar to that of honey and marmalades and its characteristics are similar to other sweetener liquids currently marketed [45, 50].

Alcoholic beverages from *O. ficus-indica* are less known than those from other processed products. Some authors, for obtaining prickly pear wine, inoculated their juice with *Saccharomyces cerevisiae* and added SO_2 (10 mg L^{-1}) and citric acid for obtaining a pH 3.3, and then performed fractional distillation [51]. Besides, prickly pear fruit pulp can be added to other alcoholic beverages such as yakju, increasing the levels of alcohol, sugars and antioxidant activity [52].

Prickly pear seed oil is another potential product that can be obtained during fruit processing. Linoleic acid is the main fatty acid, and the percentages of PUFAs and MUFAs reach 63.29 and 23.30%, respectively [38]. Besides, other physical and chemical characteristics, such as refractive index, iodine number and saponification number, make it similar to other vegetable oils such as corn or grape seed oil [45].

1. Economic evaluation of prickly pear fruit production

Nowadays, *O. ficus-indica* cultivation is developed in at least 18 countries in arid and semiarid areas. The extension of this crop is more than 100,000 ha [53]. This does not include naturalized plants or plants cultivated for home consumption.

Prickly pear has been used since the sixteenth century as an important subsistence crop in many communities of Africa, Asia, Europe and America, although fruit consumption remains limited to local ethnic markets and there is little export. Only Mexico, Italy, Chile, South Africa and Argentina produce cactus pear in a com mercial way [3].

Mexico is the world's largest producer of prickly pear, accounting for 45% of world production [3, 4]. Other important producing countries of prickly pear are Italy (12.2%) and South Africa (3.7%). The rest of the production is in Argentina, Chile, Bolivia, Peru, Colombia, United States of America, Morocco, Algeria, Libya, Tunisia, Egypt, Jordan, Pakistan, Israel, Greece, Spain and Portugal [3, 4].

Regarding Mexico, the planted area covers around 50,000–70,000 ha and the gross annual production is 300,000–500, 000 tones. It is the fifth fruit crop in the country and about 20,000 families obtain some income from cactus pear cultivation. Vegetable production, featured by small plots of land cultivation, supposes an additional 12,000 ha of cultivated area [54]. In this country, the cultivation of prickly pear presents the advantage that it produces employment and income in areas where few other crops can be produced [55].

Italy is the second world producer and the principal world exporter of cactus pear, mostly concentrated (96%) in Sicily with 7000–8300 ha producing about 78,000–87,000 tones per year [55]. South Africa's 1500 hectares produces about 15,000 tones. Other countries where cactus pear is cultivated are South Africa (1500 ha, 15,000 tones of fruit production), Argentina (1650 ha), Brazil (500,000 ha), Chile (934 ha), Peru (5000 tones of fruit production) and California (120 ha) [4]. However, it is difficult to quantify areas and production of prickly pear crop because it is a crop with low economic and social importance in most of the countries, so that there are not consistent economic data about it [4].

In Mexico, the main producer, the average production is approximately 12.8 t ha^{-1} (400 crates), which are sold at an average price of 3.2 euros each crate. This gives a total of 1280 euros per hectare, and the profit is approximately 340 per hectare, because the costs of tools, weeding, pruning, fertilization, fumigation, harvest and transport, among others [56]. In the case of Italy, the average production is approximately 15.1 t ha^{-1}, the incomes are 5.71 euros per hectare on average and the average profit perhectare is 1658.88 euros [55, 57]. In Spain, average production per hectare is 234 t ha^{-1}, and the average price is 1.42 euros per kilogram. Prices depend on the moment of the season and go from 1.8 euros per kilogram to 1.05 euros. This implies an averageincome of 555, 254.7 euros per hectare. So, average profit is 545, 801 euros per hectare.

Besides, prickly pear fruits show a high amount of compounds with biofunctional, nutraceutical and cosmetic properties, above crops like *Opuntia*

joconostle, Ziziphus jujube, Stenocereus pruinosus, Stenocereus stellatus and *Punica granatum* [58– 61]. However, no economic value analysis of the cactus pear cultivation based on obtention of these biofunctional, medicinal, nutraceutic and cosmetic com- pounds has been done. These compounds reach a value in the marketplace of 213.68
€ per 20 mg in the case of kaempferol or 204.53 € per 10 mg in the case of isorham-netin, and both are present in prickly pear fruit, among others.

10. Other aspects

Besides the health benefits of fruit consumption, *O. ficus-indica* presents other multiple applications in different areas:

- There are studies about the antigenotoxic capacity of the cladodes against mycotoxin zearalenone (mycotoxin F-2, produced by some species of *Fusarium*) in mice [62, 63].

- Cladodes of *O. ficus-indica* could be used to produce biofuels, specifically bioethanol and biogas [64, 65].

- Due to its clotting power, cladodes could be used as a natural coagulant to remove turbidity and color in raw waters, with a yield of 65 g of coagulant perkg of cladodes [66].

- Some studies showed that supplementing the feeding of goats with cladodes and fruit peels may be an important resource to reduce their water intake, without detrimental effects on digestion, growth and meat quality [67, 68].

- Pigments of red and purple prickly pear cultivars could be used in food indus- try as additives in products like sweets, desserts and dairy products. These additives were obtained by microencapsulation technique of betalains [69, 70].

- Due to its Crassulacean acid metabolism (CAM), *O. ficus-indica* has been stud- ied for its ability of endure prolonged drought and CO_2 uptake, which can help to mitigate effects caused by desertification and global climatic change [71, 72].

- *O. ficus-indica* could be used in phytoremediation of contaminated soils with Se, Pb and other contaminant substances [73, 74].

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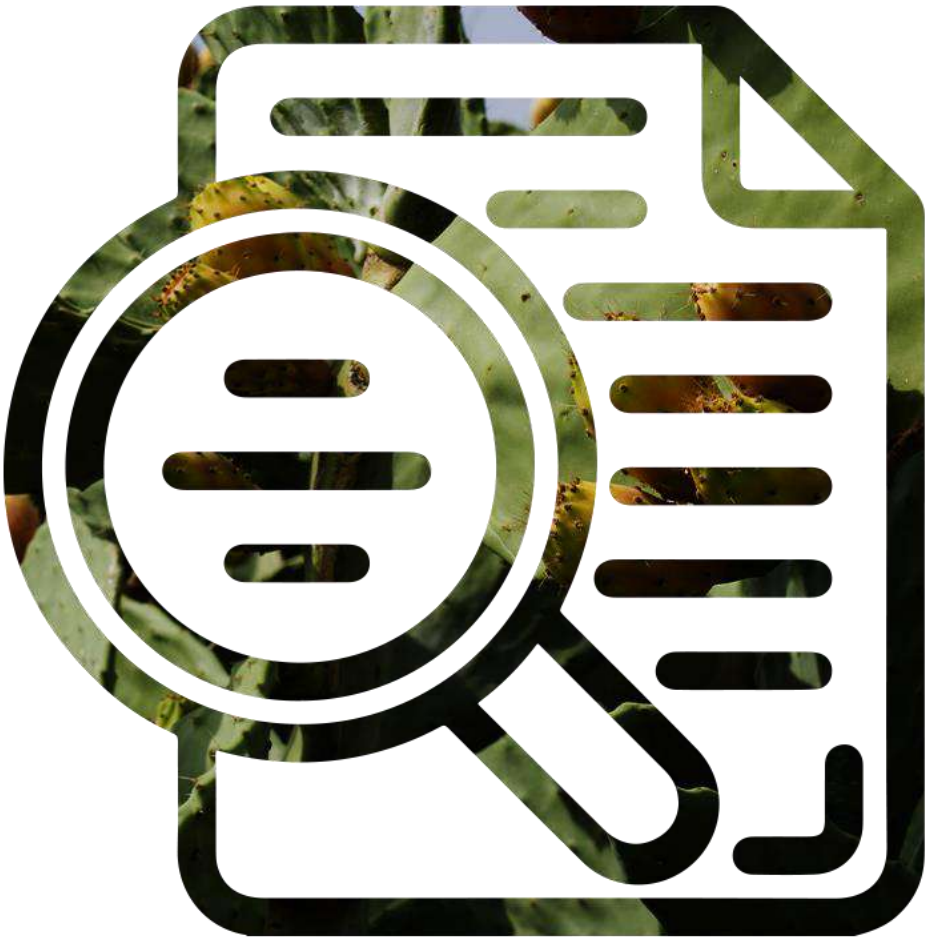


8. RESULTS AND DISCUSSION

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

Objective 1: Phytochemical, nutraceutical, and functional characterization of the fruit, cladodes, and seeds

The results of this objective are reflected in the next publications:

- 1st publication: *Antioxidant properties and chemical characterization of Opuntia ficus-indica Mill. cladodes and fruits.* In this publication, the following parameters were studied in fruits (peel and pulp) and cladodes (young and old): total soluble solids (TSS), pH, total titratable acidity (TA), organic acids and sugars, phenolic content, and determination of antioxidant activity by three different methods ($DPPH^{\cdot}$, $ABTS^{\cdot+}$ and FRAP).
- 2nd publication: *Phytochemical characterization of different prickly pear (Opuntia ficus-indica (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis.* In this publication, identification of phytochemicals and quantification of major (poly)phenolic compounds were determined in fruits (peel and pulp) and cladodes (young and old) of prickly pear.
- 3rd publication: *Fatty acid profile of fruits (pulp and peel) and cladodes (young and old) of prickly pear [Opuntia ficus-indica (L.) Mill.] from six Spanish cultivars.* In this publication, fatty acid composition (% of total fatty acid profile) were studied in fruits and cladodes.
- 4th publication: *Characterization of bioactive compounds of Opuntia ficus-indica (L.) Mill. seeds from Spanish cultivars.* In this publication, the following parameters were analyzed in prickly pear seeds: metabolite identification (phenolic acids and derivatives, flavonols, organic acids and other compounds), quantitative analysis of polyphenols, antioxidant activity by three different methods ($DPPH^{\cdot}$, $ABTS^{\cdot+}$ and FRAP), protein and amino acid composition (g 100 g^{-1} protein) and fat and fatty acid composition (% of total fatty acid profile).
- 9th publication: *Valorization of prickly pear [Opuntia ficus-indica (L.) Mill]: nutritional composition, functional properties and economic aspects.* This publication is a book chapter which contains a review of the results of this

section.

8.1 Results of phytochemical, nutraceutical, and functional characterization of fruit (pulp and peel) and cladodes

8.1.1 Total soluble solids, pH, total titratable acidity, and moisture

The values of TSS obtained in fruit pulp showed that the cultivars 'NT', 'NE', 'NO', 'NJ' and 'FR' are near o within the values established as reference for fruit quality (>12-13º Brix) (Cerezal & Duarte, 2005; Sáenz, 2006). 'NE' was the cultivar which showed highest values (15.7 º Brix), and 'NA' presented the lowest ones (10.7º Brix).

Due to the cladode nature, TA were higher in cladodes than in fruit, especially in old cladodes. The obtained values were in the range 0.2 – 3.4 g citric acid L^{-1} for fruits and 1.3-5.05 g citric acid L^{-1} for cladodes. However, the pH values showed less variation, ranging from 5.2 to 6.02 values, similar to those reported by Celis-Fabian (2009). About the moisture, cladodes showed high content than fruits, (92.8% on average in cladodes and 82.2% in fruits).

8.1.2 Organic acids and sugars

Cladodes showed higher organic acid content than fruits, being malic acid was the predominant organic acid in cladodes, followed by succinic and citric acids, while in fruits malic and citric acids showed much lower values than cladodes and succinic acid were not detected. 'NE' and 'FR' cultivars showed the highest values in the contests of citric and malic acid, especially in old cladodes. These difference between fruits and cladodes are due to the CAM metabolism of *O. ficus-indica* since, especially in cladodes, organic acids accumulate in the vacuole during night phase, mainly malic acid, and suffer a reciprocal reserve carbohydrates accumulation during the daytime phase (Zenteno-Ramírez et al., 2015). Values of citric and malic acids in cladode were higher in old than in young cladodes, probably due to the accumulation that has been generated during cladode ripening.

About sugars, fruit and showed higher values than cladodes. Fruit pulp was predominant in glucose and fructose, and the amount of these sugars was greater than in the peel of the studied cultivars. 'NE' and 'NO' cultivars showed highest values in glucose content, but 'FR' and 'NE' cultivars presented highest values in the fructose content, especially in fruit pulp. These results agreed with previously reported data by Zenteno-Ramirez et al. (2015) and are greater than those reported

for glucose and fructose in other fruits like wolfberry, grapefruit pulp and passion fruit (Alves de Oliveira et al., 2014; Zhao et al., 2015; Zheng et al., 2016).

8.1.3 Total phenolic content and antioxidant activity

The average values of TPC are 18.9 g GAE kg $^{-1}$ and 14.8 g GAE kg $^{-1}$ (dw) in young and old cladodes respectively, and for fruit, 18.2 GAE kg^{-1} and 7.8 GAE kg^{-1} (dw) in peel and pulp, respectively. 'FR' cultivar presented significantly higher values of TPC in young cladodes (35.6 GAE kg^{-1} (dw)), and 'FR' and 'NE' cultivars showed the highest values in the peel fruit (19.2 GAE kg^{-1} and 18.2 GAE kg^{-1} (dw) respectively). Phenolic compounds are characterized by their antioxidant activity, so TPC content correspond with the antioxidant effect.

For considering the different mechanism of antioxidant action, the antioxidant activity of prickly pear botanical parts was conducted by three complementary methods: DPPH$^{\cdot}$, ABTS$^{\cdot+}$ and FRAP. The scavenging activity of DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ methods was higher in fruits than in cladodes, especially in peel. 'FR' cultivar showedthe highest value in peel fruit (60.1 mmol Trolox Kg $^{-1}$ (dw)). These results agreed with several authors who reported a higher antioxidant activity in peel than in the pulp fruits, such as pomegranate, guava, and berries (Calín-Sánchez et al., 2013; Marquina et al., 2008; Oszmianki et al., 2016). Regarding the FRAP method, cladodes presented the highest values, mainly young cladodes of 'FR' cultivar. Comparing these results together with the values obtained in TPC, it is concluded that the young cladodes and peel fruit have higher antioxidant activity than old cladodes and pulp fruit.

8.1.4 Identification of phytochemicals in Opuntia ficus-indica cladodes and fruits

The exhaustive analysis of prickly pear cladodes and fruit phytochemicals composition allowed the tentative identification up to 41 compounds. The most relevant class of phytochemicals was flavonoids, followed by phenolic acids and lignans. In additions, some other compounds such as betalains and organic acids were detected. Most of the compounds were identified in all the botanical parts analyzed, while some compounds were detected only in some of them. For example, betalains were only detected in pulp and peel of 'FR' cultivar, the only one that present red colour.

8.1.5 Quantification of the major (poly)phenolic compounds in prickly pear fruits and cladodes

The highest (poly)phenolic content was found in young cladodes, following by old cladodes, peel, and fruit pulp, respectively. The highest concentration in young cladodes was found in 'FR' cultivar (14.3 mg g^{-1} (dw)), while 'NE' showed the highest concentration in old cladodes (12.4 mg g^{-1} (dw)). In the fruit, 'NT' presented the highest concentration in fruit peel and 'NJ' cultivar the highest concentration in fruit pulp (7.1 mg g^{-1} and 5.1 mg g^{-1} (dw), respectively). Other authors also reported higher amount of (poly)phenolic compounds in fruit peel than in pulp (Moussa-Ayoub et al., 2014; Yeddes et al., 2014).

The profile of individual (poly)phenolic compounds for each botanical part was dependent on the cultivar. In young cladodes were quantified 26 compounds, being flavonoids (in particular, flavonols) the main (poly)phenolic compounds. Myricetin-hexoside was the predominant compound in young cladodes in all the studied cultivars, except 'NE'. Besides, young cladodes also showed high amounts of some isorhamnetin derivatives, rutin, and ferulic acid-hexoside. With respect to old cladodes, 25 compounds were quantified, and as in young cladodes, flavonols were also the major group of (poly)phenolic compounds. Old cladodes were also characterized by the presence of high amounts of isorhamnetin glycosides, myricetin hexoside and ferulic acid-hexoside. Regarding the fruit, phenolic acids were the predominant compounds over flavonols. In fruit peel were quantified 26 compounds, of which ferulic acid-hexoside, sinapicvacid-hexoside, dihydrosinapic acid-hexoside, and isorhamnetin-rutinoside were the predominant compounds. Prickly pear fruit pulp showed 21 quantifiable phenolics, being ferulic acid derivative the predominant compound. Betalains were not quantified due to the lack of commercially available, pure reference standards.

Although other authors studied the (poly)phenolic profile of prickly pear, mainly betalains, (Guevara-Figueroa et al., 2010; Mata et al., 2016; Moussa-Ayoub et al., 2014; Serra et al., 2013; Yeddes et al., 2014), this study provides an exhaustive characterization of the pythochemical profile of the aerial parts of *O. ficus-indica.* The concentration of these compounds depends on genetic, environmental conditions and botanical part, and even if the study of the (poly)phenolic composition of different parts of *O. ficus-indica* and the effect of genotypic

differences in the (poly)phenolic profile had been previously investigated (Moussa-Ayuoub et al., 2014; Stintzing et al., 2005; Yeddes et al., 2014), these results provide novel information about individual phenolics on the basis of different botanical parts and genotypes grown under the same environmental conditions.

The content in (poly)phenolic compounds on the six studied cultivars agreed with previous reports on *Opuntia* fruits (Moussa-Ayoub et al., 2014; Yeddes et al., 2014). Besides, these results showed the presence of up to 9 flavonols and other phenolic scaffolds in the fruit pulp, while other authors reported a lack of flavonols (Moussa-Ayoub et al., 2014) and a few isorhamnetin derivatives (Kuti, 2004; Yeddes et al., 2014) in this fruit part. These inconsistences may be attributed to geographic and genotypic differences or to the sensitivity and accuracy of the methodological approaches used. In any case, these results represent a step forward in the definition of bioactives contained in the main edible part of this plant.

Other authors also reported higher amount of (poly)phenolic compounds in fruit peel than in pulp (Moussa-Ayoub et al., 2014; Yeddes et al., 2014). Previous studies (Fernández-López et al., 2010; Kuti, 2004; Mata et al., 2016; Moussa-Ayoub et al., 2014; Stintzing et al., 2005 Yeddes et al., 2014) reported in fruit peel mainly flavonols and some phenolic acids, while these results extend the number of molecules presents in this fruit part. About cladodes, their (poly)phenolic profile were previously reported in other studies, which showed flavonols and phenolic acids (Guevara-Figueroa et al., 2010; Msaddak et al., 2017). The results presented in Mena et al., (2018) described for first time the presence of flavonones and lignans, increasing the number of bioactive compounds in cladodes. The presence of higher content in young cladodes in comparison with old cladodes may be explained by changes in the physiology of the cladodes because of the age and maturation stage (El-Mostafa et al., 2014; Rodríguez-García et al., 2007).

8.1.6 Fatty acid composition

In fruits, polyunsaturated fatty acids were the prevailing group of fatty acids. The pulp of prickly pear is the edible and most valued part of the fruit by humans, and peels are mainly used for animal feeding. Eight fatty acids were identified in fruit pulp and nine of these compounds were identified in fruit peel. Linoleic acid (C18:2),

which is a polyunsaturated and essential fatty acid, was the most abundant fatty acid in fruit peel and pulp, followed by oleic acid (C18:1) and palmitic acid (C16:0), which agreed with other authors (El-Beltagi et al., 2019; Ramadan & Mörsel, 2003a). In fruit pulp, 'NJ' was the cultivar which showed the highest values of PUFA and U/S ratio, and the lowest values of AI and TI, thus, 'NJ' was the cultivar having the most beneficial profile of fatty acids in fruit pulp. Regarding fruit peel, 'NT' can be considered as the cultivar which the most beneficial fatty acid profile, due to it showed the lowest values for AI and TI and the highest one for U/S ratio, even if 'NO' cultivar showed the highest percentage of PUFA.

In fruit peel, all the studied cultivars, except 'NE', showed higher values of PUFAs than those reported by Ramadan & Mörsel (2003b). In the case of MUFA, 'NT' and 'NE' cultivars presented similar values to those obtained by these authors and the rest of the studied cultivars showed higher values. El-Said et al. (2011) also studied fruit peel and identified linoleic acid as the predominant fatty acid, which agreed with our results, but the cultivars analyzed in our study showed higher percentages of PUFA and lower percentages of MUFA, except 'NE' cultivar, which showed lower values of PUFA and higher values of MUFA.

The obtained results of PUFAs in fruit pulp were lower than those studied by El-Beltagi et al. (2019) in fruit pulp oil, but MUFA percentages where higher than the obtained by these authors. Regarding the content of SFA, 'NO', 'NE' and 'NJ' cultivars showed similar values to those obtained by El-Beltagi et al. (2009), and 'NT', 'NA' and 'FR' cultivars showed lower values. The 'NT' and 'NE' cultivars presented higher values in MUFA than those reported by these authors, but the rest of the cultivars presented lower values. However, all the studied cultivars showed higher values of MUFA than those obtained by Ramadan and Mörsel (2003a) in fruit pulp. However, 'FR' and 'NJ' cultivars showed similar values than those obtained by these authors, but the rest of the cultivars showed lower values. About SFA, 'NT', 'NA' and 'FR' cultivars showed similar values than those obtained by Ramadan and Mörsel (2003a), and the rest of the studied cultivars showed lower values.

Regarding cladodes, they are mainly used for animal feeding, but in some countries, young cladodes are also included in human diets. Seven fatty acids were detected in old cladodes and nine fatty acids were identified in young cladodes. Linolenic acid was the most abundant compound in old cladodes, and palmitic acid (C16:0) was the predominant fatty acid in young cladodes. Old cladodes showed higher percentages of monounsaturated and polyunsaturated fatty acids than young cladodes. 'FR' was the cultivar with the most beneficial fatty acid profile in old cladodes, due to it showed lowest values of AI, TI and the highest value of the U/S ratio and highest percentage of PUFAs. About young cladodes, 'NJ' and 'NO' cultivar presented similar values for AI, TI (the lowest values) and U/S ratio (the highest values), so they were the cultivars with the most beneficial fatty acid profile in young cladodes.

There are no previous reports in the scientific literature comparing old and young cladodes of prickly pear. Abidi et al. (2009) studied the fatty acid profile of cladodes, but they did not differentiate between young and old cladodes Linoleic acid was the predominant fatty acid, with agreed with the results in old cladodes. These authors obtained lower percentages of PUFA and MUFA than all the cultivars, except 'NO', which showed similar percentages, and 'NT', which showed lower values than the cultivars studied by these authors. Regarding SFA, the values obtained by Abidi et al. (2009) agreed with that of old cladodes of 'FR' and 'NJ' cultivars but were lower than those of the rest of cultivars in both young and old cladodes.

In summary, regarding fruits, the cultivars 'NE', 'NJ' and 'NO' were the most interesting, due to their high levels of MUFAs in the case of 'NE' cultivar (fruit peel and pulp) and PUFAs in 'NJ' cultivar (fruit pulp) and 'NO' cultivar (fruit peel). The pulp of 'NE' and 'NJ' cultivars could be used for fresh consumption, as well as to produce products such as jams, juices, and fruit gummies among others. The peel of 'NO' cultivar was the most suitable for animal feeding. About cladodes, 'FR' cultivar had high MUFAs content in young cladodes and PUFAs in old cladodes; thus, 'FR' wasthe most interesting cultivar for the use of their cladodes both in animal feeding andto produce juices and dehydrated powder for human consumption. Regarding AI and TI, prickly pear fruits (peel and pulp) and old cladodes showed very good values

for these indexes; thus, these parts of the prickly pears can positively contribute to good cardiovascular health.

8.2 Characterization of phytochemical, nutraceutical, and functional properties of seeds

8.2.1 Metabolite identification using UPLC-MS analysis

Two major classes of phenolic compounds were identified: phenolic acids and flavonols. In addition, two organic acids were found and identified (gluconic and (iso)citric acids). These organic acids were previously reported by other authors in fruit extracts (Farag et al., 2020), but they have not been identified in prickly pear seeds.

Four derivatives of ferulic acid, two derivatives of caffeic acid (caffeic acid hexosides) and one derivative each of protocatechuic acid (protocatechuic acid hexoside), piscidic acid and eucomic acid, were identified in prickly pear seeds. Phenolic acids and their derivatives have previously identified in pricky pear fruits and juices (Farag et al., 2020; Guevara-Figueroa et al., 2010; Mata et al., 2016). However, up to now, only ferulic acid had been identified in prickly pear seeds (Chougui et al., 2013), while piscidic, eucomic, protocatechuic and caffeic acid and their derivatives have now been identified in seeds for the first time.

About flavonols, eight of these compounds were detected in prickly pear seed extracts. Six of them were isorhamnetin derivatives (isorhamnetin-pentosly rutinoside, -pentosyl rhamnoside, -3-O-rutinoside, 3-O-galactoside, 3-O-glucoside and -acylated-hexoside) and three compounds were quercetin derivatives (quercetin aglycone, quercetin 3-O-rutinoside (rutin), and quercetin-3-O-galactoside). Quercetin derivatives have previously been identified in prickly pear fruit peel and pulp, in its juices and in flowers, but have not been studied previously in the seeds of this plant. Isorhamnetin derivatives are present in various species of prickly pear, and these compounds were detected by other authors in flowers, pulp,peel, juices and in methanolic extracts of *O. ficus-indica*, however, they have not been identified in prickly pear seeds.

8.2.2 Quantitative analysis of polyphenols

'NE' cultivar showed the highest concentration of phenolic acid and flavonols (171.60 and 95.07 mg kg^{-1} DM, respectively). However, 'FR' cultivar presented the lowest concentration of both polyphenolic groups. Phenolic acids were the dominant group of phenolic acids in all the cultivars as compared to flavonols and their total amount was 17% higher. These results agreed with the results reported by other authors in cladodes (Guevara-Figueroa et al., 2010). However, our results were slightly lower than those obtained by de Wit et al. (2019), who studied prickly pear seeds. These differences may be due to the cultivar and genetic factors, growth conditions, as well as harvesting time, degree of ripeness or fruit processing, and above all, the determination methods (De Wit et al., 2019).

8.2.3 In vitro antioxidant activity

Regarding antioxidant activity, 'NE' was the cultivar which showed the highest in vitro antioxidant activity determined by $DPPH^{\cdot}$, $ABTS^{\cdot+}$ and FRAP (4.99, 11.67 and 15.64 mmol Trolox kg^{-1} DM, respectively), while 'FR' cultivar was characterized by the lowest results (1.39, 7.08 and 3.67 mmol Trolox kg^{-1} DM, respectively). Our results were slightly lower than those reported by other authors, may be due to the anatomical part of the prickly pear examined (Andreu et al., 2018).

8.2.4 Protein and amino acid composition

Results of this study showed that the protein content and amino acid composition of protein in prickly pear seeds (*O. ficus-indica*) depends on the cultivar. Regarding protein content, 'FR' and 'NO' were the cultivars which showed the highest concentration (9.97 100 g^{-1}), and 'Orito' cultivar was the cultivar which showed the lowest value (7.09 g 100 g^{-1}). Other authors (El-Mannoubi et al., 2009; Özcan & Juhaimi, 2011) reported lowest content of protein in prickly pear seeds, may be due by different growth conditions, variety, genetic favors, harvesting time or geographical variations of prickly pear plants, among others. However, 'NE' cultivar showed the highest values for total indispensable amino acids (IAAs) and total dispensable amino acids (DAAs) – 21.60 and 47.36 g 100 g^{-1}, respectively. 'FR' was the cultivar which presented the lowest total IAA and DAA content (10.30 and 22.90 g 100 g^{-1} respectively). Glutamic acid was the predominant amino acid in prickly pear seeds, followed by arginine, aspartic acid, and leucine, independent of prickly

pear cultivar. These results agreed with other authors who analyzed the protein concentrate of prickly pear seed flour (Nassar et al., 2008), but in other studies the content of total IAA was higher (Nassar, 2008; Sawaya et al., 1983). However, the results of this study showed that none of the tested protein from prickly pear seeds of different cultivars contained an adequate amount of all IAAs, because they showed lysine, methionine and cysteine as limiting amino acids. On the other hand, Sawaya et al. (1983), determined that prickly pear seed protein is a good source of the suphur amino acids (Met + Cys), which are the most common limiting amino acid in seeds protein.

8.2.5 Fat and fatty acid composition

Prickly pear seeds oil content ranged from 2.61% for 'Nalle' cultivar to 7.69% for 'NO' cultivar. These results were slightly higher than those obtained by Labuschange & Hugo (2019) and slightly lower than those obtained in the cultivars studied by De Wit et al (2018). These differences may be due to growth conditions, cultivar, genetic factors, harvesting time, degree of ripeness of fruit processing, among others (De Wit et al., 2018). In prickly pear seed oil, 13 different fatty acids were identified and assessed, being PUFA and MUFA were the predominating fatty acids. The main fatty acid was linoleic acid, an essential polyunsaturated fatty acid, followed by oleic acid, a monounsaturated fatty acid. 'NE' cultivar was the cultivar that showed the highest content of PUFA (64.33%) also linoleic acid (63.11%). However, there was slight differences in MUFA content between the analyzed cultivars, being 'NT' cultivar the one that showed the highest average MUFA content (22.71%). About SFA, palmitic acid and stearic acid were the predominant fatty acids in this group. The obtained results were agreed with those of others research (Ciriminna et al., 2017; De Wit et al., 2018; Labuschange & Hugo, 2019). Differences between analyzed cultivars could be related to genetic factors.

Objective 2: Sensory analysis

The results of this objective are reflected in the next publications:

- 5th publication: *Volatile composition of prickly pear fruit pulp from six Spanish cultivars.* In this research, the aromatic compounds and the concentration of volatile compounds found in prickly pear fruits pulp were analyzed.
- 9th publication: *Valorization of prickly pear [Opuntia ficus-indica (L.) Mill]: nutritional composition, functional properties and economic aspects.* This publication is a book chapter which contains a review of the results of this section.

8.3 Volatile compounds analysis

A total of 35 compounds were isolated, identified and quantified in prickly pear fruits pulp of the six studied cultivars, which can be classified into seven chemical families: aldehydes, terpenes, esters, alcohols, ketones, linear hydrocarbons and terpenoids. The volatile profile of the 'FR', 'NO', 'NT' and 'ORI' cultivars included many aldehydes, whereas those of the 'NA' and 'NE' cultivars had more terpenes. Other authors reported alcohols (Arena et al., 2001; Flath & Takahashi, 1978; Oumato et al., 2016) and esters (Rodríguez et al., 2015) as the most numerous and abundant compounds.

Regarding the concentration of volatile compounds, aldehydes were the predominant compounds in 'NA' and 'NO' cultivars (69.0% and 46.1% of the total concentration of volatile compounds), alcohols were the predominant compounds in 'FR' and 'ORI' cultivars (58.9% and 42.0% respectively), followed by aldehydes in 'FR' cultivar (40.5%). However, the 'NT' and 'NE' cultivars showed terpenes as the predominant volatile compounds (86.7% in both cases). The results obtained in 'FR' and 'ORI' cultivars agreed with other authors (Arena et al., 2001; Flath & Takamashi, 1978) who reported alcohols as the most abundant chemical family. The results reported by Oumato et al. (2016) in 'Dehallia' cultivar agreed with the results obtained in FR and ORI cultivars and the volatile concentration of 'Aissa' and 'Should' cultivars have agreed with those in 'NA', 'FR' and 'NO' cultivars. However, the results of this study did not agree with Rodríguez et al. (2015), who obtained hydrocarbons as the most abundant chemical family.

'NT' and 'FR' showed the highest total volatile content (14.83 and 12.06 mg $100g^{-1}$

respectively), making them attractive for consumers because, in general, the more volatile content, the higher consumer acceptance. By contrast, 'NO' and 'NE' presented the lowest concentration of volatile compounds (1.10 and 3. 33 mg 100 g $^{-1}$ respectively). Arena et al. (2001) obtained similar values to those found in NE cultivar in 'Yellow' and 'White' cultivars, and 'Red' cultivar presented lower total volatile content than all the cultivars studied in this research.

About the predominant compounds in each cultivar, nonanol and 2,6-nonadienal, which are related with vegetable, green, melon and fatty notes, were the predominant compound in 'FR', 'NA' and 'ORI' cultivars; 1 hexanol + 2-hexenal, which are associated with green, herbaceous, almond and fruity notes, were the main compounds in 'NO' cultivar, and D-limonene and γ-terpinene, which are linked with citrus, sweet and herbaceous notes, were the principal compounds in 'NT' and 'NE' cultivars.

Sensory evaluation is needed to complete the knowledge of the aroma of prickly pear fruit and the effect of the cultivar.

Objective 3: Evaluation of the quality parameters of prickly pear fruits during their conservation under different conditions

The results of this objective are reflected in the next publications:

- 6th publication: *Influence of storage on physiological properties, chemical composition, and bioactive compounds on cactus pear fruit (Opuntia ficus-indica (L.) Mill.).* In this publication, the changes in fruit quality parameters (weight loss, firmness, color, titratable acidiy, and total soluble solids), ethylene production, respiration rate, antioxidant activity and bioactive compounds (total phenols and carotenoids) on cactus pear fruit from 'Orito' cultivar during cold and shelf-life storage were analyzed.
- 7 th publication: *Phytochemical profile of Opuntia ficus-indica (L.) Mill fruits (cv. 'Orito') stored at different conditions.* In this publication were determined the phytochemical compounds of 'Orito' fruits and their changes during cold and shelf-life storage.

8.4 Ethylene production and respiration rate

Although prickly pear fruit was classified as non-climacteric fruit (Cantwell, 1995; Lakshminarayana & Estrella, 1978), 'Orito' cultivar showed a suppressed-climacteric pattern in ethylene production and respiration rate, similar to some cultivars of plum (Minas et al., 2015; Zuzunaga et al., 2001). In this case, there are not increase in respiration rate and ethylene production related to ripening.

Regarding respiration rate in 'Orito' fruit, cold storage decreased the CO_2 production in the first seven days of storage; then, the CO_2 production remained stable until the end of cold storage. However, under shelf-life conditions, CO_2 production increased slightly after 14 days and then decreased up to values below the initials at the end of storage. Increasing the temperature from 2ºC to room temperature resulted in a greater increase in CO_2 production rate, but after 14 days, the production of CO_2 began to decrease under both conditions. These increased in CO_2 production in response to temperature were observed in other cultivars of cactus pear fruit and cladodes (Cantwell et al., 1992; Schirra et al., 1999), and results obtained of the CO_2 production were in accordance with those obtained by other authors (Corrales-García et al., 1997; Lakshminarayana & Estrella, 1978).

Concerning the ethylene production, this compound increased until day 21 and decreased at the end of storage under cold and shelf-life conditions, but shelf-life storage showed higher levels of ethylene. Even so, 'Orito' fruit presented low ethylene emission rates under both cold and shelf-life conditions. These results agreed with those obtained by other authors who evaluated other prickly pear cultivars (D'Aquino et al., 2014; Schirra et al., 1999).

8.5 Fruit quality parameters under different storage conditions

'Orito' fruit showed a low weight loss during the 28 days of storage under cold (2.22%) and shelf life (3.71%) conditions. Due to the weight losses in this study did not reach 4%, 'Orito' fruit mainained their quality and marketability, since weight losses above 6-8% cause an irreversible alteration of sensory quality (Lamúa, 2000). 'Cristalina', 'Picochulo' and 'Burrona' cultivars showed a weight loss of less than 4% under the same conditions, but 'Copena-Torreoja' cultivar showed more than 10% of weight loss under shelf-life storage (Corrales-García et al., 1997; López-Castañedaet al. 2010).

Regarding firmness, changes in this parameter depend on the class of fruit and even of the cultivar (Díaz-Mula, 2011). In the case of 'Orito' fruit, at the end of storage, firmness increased 16.6% under cold conditions and decreased 3.58% under shelf-life conditions with respect to day zero. Thus, 'Orito' fruit showed an acceptable quality and marketability, since there was not loss of firmness during cold storage, and during shelf-life conditions firmness loss was very low comparted to that other fruit such as apricot (72%), tomato (55%) or lemon (26%) under similar conditions (Valero & Serrano, 2010). The results of this study agreed with other studies (D'Aquino et al., 2014; Schirra et al., 1999) which determined that cold storage prevented firmness loss in cactus pear fruit, and this rapidly declined when fruit was kept at 20 º C.

Values of TSS and TA remained stable during both shelf life and cold storage because 'Orito' fruit showed a non-climacteric fruit pattern in these parameters, in which the concentration of nutrients remains stable during storage. Values of TSS of > 12-13 % are required to ensure good quality of fruit, and cactus pear fruit from the 'Orito'

cultivar showed TSS content between 14-14.9%, so that values in 'Orito' fruit wereoptimal, similar to those obtained by Andreu et al. (2018). TA values showed slightly higher values than those obtained by Graça-Miguel et al. (2018) and similar that those obtained by Schirra in the 'Giallia' cultivar.

Concerning colour, Hue angle decreased after seven days under shelf life conditions and stayed constant until the rest of storage. However, this parameter did not show significant differences under cold conditions. Decreases in the Hue angle are related to peel darkening in fruit. The trend during both storage conditions was in accordance with other studies (Allegra et al., 2015; Ochoa-Velasco & Guerrero-Beldrán, 2016), in which analyzed color changes in *O. ficus-indica* and *O. albicarpa* fruit under cold and shelf-life conditions.

8.6 Changes in bioactive compounds and antioxidant activity under different storage conditions

Total phenol content remained stable during cold storage, which agreed with the results obtained by Coria-Cayupán et al. (2011) in *O. megacantha* fruit. However, under shelf-life storage, total phenol content increased after seven days and decreased at the end of storage. The concentration of these compounds was similar to those obtained by Moussa-Ayoub et al. (2014) but was lower than those obtained by Ramírez-Ramos et al. (2015). The variation of the concentration of these compounds may be due to agronomic practices, environmental conditions, the pre- and postharvest management of fruit, ant the reduction of these compounds during fruit ripening (Ramírez-Ramos et al., 2015). Anorve-Morga et al. (2006) obtained that, during storage, there was an increase in phenol content in cactus pear fruit that was directly influenced by temperature, which could explain the results in 'Orito' fruit.

Both cold and shelf-life storage increased H-TAA, reached the highest concentration after 21 days in both cases. This trend had been reported in non-climateric fruit during cold storage such as citrus and plum (Díaz-Mula, 2011; Rapisarda et al., 2008). However, L-TAA, which was significantly lower than H-TAA, remained stable during both storage periods. These results suggested that hydrophilic compound

contributed more than lipophilic compounds in the antioxidant capacity of cactuspear fruit. Carotenoids, which are lipophilic compounds, showed very low concentration in 'Orito' fruit and presented a similar trend to L-TAA. The concentration of carotenoids in 'Orito' fruit was lower than those obtained by Kuti (2004) in a green-skined cactus pear cultivar. Oranges, which are non-climacteric fruit, showed similar behaviour of 'Orito' fruit, whitout changes in carotenoid concentration during cold storage (Plaza et al., 2011).

8.7 Changes in phytochemical profile under storage

The analysis of phytochemical profile of prickly pear fuit pulp (cv. 'Orito) allowed the tentative identification of 18 compounds. The most relevant classes of phytochemicals were betalains, phenolic acids and lignans. In additions, organic acids, an amino acid and a prenylflavonoid were detected. The compound which showed the largest area was piscidic acid, which did not show significant changes during cold and shelf-life storage.

No critical changes were detected for most of the phytochemicals during the cold storage of 'Orito' fruit, while three-day shelf life after storage did not change the phytochemical profile of prickly pear fruits. The content of eucomic acid isomer/derivative and syringaresinol increased, and the content of citric acid decreased. Eucomic acid isomer/derivative and syringaresinol increased their content starting on day seven of cold storage, and from this moment they remained stable until the end of cold storage. However, in these compounds no significant differences were found in the comparison of cold and shelf life in the same day of storage. Ferulic acid derivative decreased its content after seven days of cold storage, increased by day 21, and then decreased slightly again at the end of cold storage.

Betalains, phenolic acid and lignans are related with the phenylalanine metabolism. Phenylalanine is an aromatic amino acid which is a precursor of secondary metabolites in plants related to plant structure, reproduction, defense and communication Phenylalanine ammonia-lyase (PAL) is an enzyme that regulating the synthesis and accumulation of phenolic compounds in plants. This enzyme is

stimulated by cold, so that the variation of phenolic content in 'Orito' fruits during cold storage might be influenced by its activation, as has been observed in other crops (Jiang & Joyce, 2003; Ramírez-Ramos et al., 2018; Tomás-Barberán et al., 2000). The decrease of citric acid during cold storage could be attributed to the effect of polyphenol oxidase enzyme (PPO), which may affect the content of organic acids and is stimulated by cold storage (Sogvar et al., 2020). The stability of identified compounds during shelf-life storage could be related to the non-stimulation of PAL and PPO enzymes under these conditions.

There were no detected chilling injuries in 'Orito' fruit as they were found in other cultivars such as 'Copena-Torreoja', studied by Corrales-García et al. (1997). The results of these authors showed 100% chilling injuries after the first month of cold storage. Other cultivars studied by Corrales-García et al. (1997) presented chilling injuries after two or three months of cold storage. However, PAL and PPO enzymes are related to physiological disorders during cold storage in some fruits (Sogvar et al., 2020; Tomás-Barberán et al., 2018), so that more studies are required to evaluate the chilling-injury sensitivity of 'Orito' fruits after longer cold storage.

Objective 4: Economic estimation of prickly pear production and its feasibility in Spain

The results of this objective are reflected in the next publication:

- 8th publication: *Economic estimation of cactus pear production and its feasibility in Spain*. In this publication, the economic evaluation of cactus pear production structure, the economic analysis of cactus pear bio-functional, medicinal, nutraceutical and cosmetic properties and the economic estimation of cactus pear production value considering environmental issues were carried out.
- 9th publication: *Valorization of prickly pear [Opuntia ficus-indica (L.) Mill]: nutritional composition, functional properties and economic aspects.* This publication is a book chapter which contains a review of the results of this section.

8.8 Characterization of fruit productive environment, cost analysis, and analysis of the gross economic profit margin

Production structure is different in México, Italy and Spain, although in Spain there is not a real productive sector, but there are isolated farms (FAO, 2018). Besides, there were clear differences among countries about cactus pear production cost. The main cost was harvest, pruning/*scozzolatura*/thinning and wages salaries in Mexico, Italy and Spain, respectively (Basile et al., 2002; Losada et al., 2017). The average production in México is 12.8 t ha^{-1} (400 crates), and the selling price is 3.2 € per crate (Losada et al., 2017). Thus, this gives a total of 1280 € per ha planted and a profit of approximately 340 € ha^{-1}. In the case of Italy, which presents a developed cactus pear producing sector, the average production is 11.1 t ha-1 (Basile et al., 2002). Timpanaro & Foti (2014) determined that the average income is 5714 € ha-1 and the average profit was 1659 € ha^{-1}. In Spain, the average production is 234 t ha^{-1} and the prices ranges between 1.05 and 1.8 € per kg. The average income is 555,255 € and the average profit 545,801 € per ha. The high price that the product reaches in Spain (and the profitability) is because the demand is much higher than the production. Spanish producers should look at Italy before growing to avoid problems derived of increasing production without real and effective distribution channels and mature demand.

8.9 Economic analysis of cactus pears production regarding bio-functional, medicinal, nutraceutical, and cosmetic properties

An exhaustive review was performed for determinate the average content of *O. ficus-indica* components with bio-functional, medicinal, nutraceutical and cosmetic content, and the results showed that prickly pear showed high quantities of these compounds (Albano et al., 2015; Cano et al., 2017; El-Mostafa et al., 2014; García-Cayuela et al., 2019; Mena et al., 2018). The content ranged from 8.4 μg g^{-1} dw in the case of luteolin to 18,865 μg g^{-1} dw in piscidic acid (El-Mostafa et al., 2014; García-Cayuela et al., 2019). The average market prices of these compounds, which were obtained through a questionnaire among main producers, ranged from 134.32 € perg in the case of coumaric and ferulic acids to to 265.58 € per 20 mg in the case of gossypetin. With these data, the value of 1 g dw of cactus pear was estimated in these terms, ranged from 0.36 € in the case of kaempferol to 141.04 € in the case of ferulic acid. The next step in the analysis is to determine the cost of obtaining these compounds in prickly pear, due to these processes and their cost depend on the type of plant material and compound to be extracted. These results showed an estimate of the viability of the cultivation of prickly pear for these purposes.

8.10 Economic estimation of cactus pear production value considering environmental issues

Unlike most plants, CO_2 intake and water loss occur mainly at night in Opuntias, when the humidity is higher and the temperature is lower, which is a mechanism to reduce water loss. CO_2 intake and Opuntia biomass accumulation depend on environmental conditions, Allegra et al. (2015) and Pimienta-Barrios et al. (2005) determined that in optimal environmental conditions (temperature, wet soil, and photosynthetic photon flow), *O. ficus-indica* takes 550 mol CO_2 m^2 daily.

Considering data from three Spanish crops of prickly pear, were determined that 1 ha of prickly pear plant can take 2,783,261 mol CO_2 per day (63.25 kg d^{-1}). Prickly pear plants are fully productive when they are 5 years old and can reach 20 years of full production, so 1 ha of *O. ficus-indica* can take approximately 462 t of CO_2 during its complete productive live. Besides, considering that a young plant (6-8 years old) shows 150 cladodes of 2.5 of average weight, and an adult plant (20 years old)

reaches, as average, 250 cladodes, reaching approximately 625 kg per adult plant. According to some authors (El-Mostafa et al., 2014; García-Cayuela et al., 2019; Mena et al., 2018) average water quantity of cactus pear is 80%, so an adult plant of prickly pear has 125 kg of dry mass. Considering that C content in a cladode is 36.2% (Gomez-Casanovas et al., 2007), an adult plant has 45.25 kg of C (approximately 166 kg of CO_2). In consequence, an adult cactus pear plant fixes 8.29 kg of CO2 per year through its cladodes.

O. ficus-indica cultivation can be a successful way to mitigate climate change in arid and semiarid regions because can contribute efficiently to soil accumulation of organic carbon (Bautista-Cruz et al., 2018). ROAECS (Results Orientated Agro-Environment Climate Scheme) is a type of agro-environmental scheme based on the idea of paying farmers for achieving specific environmental goals (Burton & Scharz, 2013), which could be designed to adapt cactus pear production and management practices defining measurable and objective indicators consistent with ecological goals. Sequestration of carbon in cactus pear farms can be an effective indicator as it totally fulfils the stated requirements. Thus, cactus pear crop can function as a water reserve and as carbon reservoir in arid and semiarid regions, contributing to climate change mitigation, as well as it will reduce soil erosion and water pollution. Regarding carbon price, there are not guarantee for future carbon prices, so various scenarios should be considered. A 20 € per t of CO_2 could represent and average estimate during 2020-2030 (UK-Department of Energy and Climate Change, 2013).

9. CONCLUSIONS/*CONCLUSIONES*

Conclusions

The aim of this work was the analysis of the phytochemical, nutraceutical, and functional properties of the prickly pear, as well as the economic estimation of its production and feasibility in Spain. With the results obtained, it is intended to value the cultivation of the prickly pear due to its multiple applications, mainly in human food, animal feeding, pharmaceutical and cosmetic industries, as well as a tool for rural development and mitigation of the effects of climate change in arid and semi-arid zones. The main conclusions obtained in this study were:

1- Due to their high content of bioactive compounds, the fruits and cladodes of the studied cultivars of prickly pear were interesting for both fresh consumption and for the elaboration of processed foods, as well as for animal feed. Regarding the antioxidant activity and polyphenolic compounds, stand out the young cladodes of the cultivar 'FR', the peel of the fruits of the 'FR', 'NE' and 'NT' cultivars and the seeds of the 'NE' cultivar. Regarding the fatty acid profile, the pulp of the 'NJ' cultivar, the peel and seeds of 'NE' cultivar and the old cladodes of 'FR' cultivar highlighted for their percentage of polyunsaturated fatty acids, being linolenic acid the most abundant fatty acid. For all that the studied parts of prickly pear were optimal for fresh consumption, elaboration of processed foods, animal feeding and pharmaceutical and cosmetic uses. In addition, the seeds and the peel of the fruits are obtained as a waste product after the extraction of the pulp for the preparation of processed foods, so their use would reduce the production of waste in this process.

2- The cultivars 'NT' and 'FR' showed the highest concentration of volatile compounds, which affect to sensory quality of fruits and are related to acceptance by consumers. In this sense, it is necessary to delve into its organoleptic attributes to complete the knowledge in this area.

3- The storage of the prickly pear fruits of a commercial cultivar called 'Orito' maintained the quality parameters and the phytochemical profile at optimal values for a month after harvesting, in cold storage and for three days after this at room temperature, so that its marketability would be possible during all this time.

4- Prickly pear crop can be an effective tool for rural development in arid and semi-arid areas regarding production, job creation and environmental issues. In

addition to its simple environmental adaptation and economic maintenance, its high concentration in bioactive compounds, higher than that of other cultivable species in these areas, and its capacity for carbon sequestration, could increase the profitability of prickly pear production and thus promote an integral development of the rural areas in which the production of this crop takes place.

Conclusiones

El objetivo de este trabajo fue el análisis de las propiedades fitoquímicas, nutracéuticas y funcionales de la chumbera, así como la estimación económica de su producción y viabilidad en España. Con los resultados obtenidos se pretende poner en valor el cultivo de la chumbera debido a sus múltiples aplicaciones, principalmente en la alimentación humana, alimentación animal, industrias farmacéutica y cosmética, así como herramienta para el desarrollo rural y mitigación de los efectos del cambio climático en zonas áridas y semiáridas. Las conclusiones principales obtenidas en este estudio fueron las siguientes:

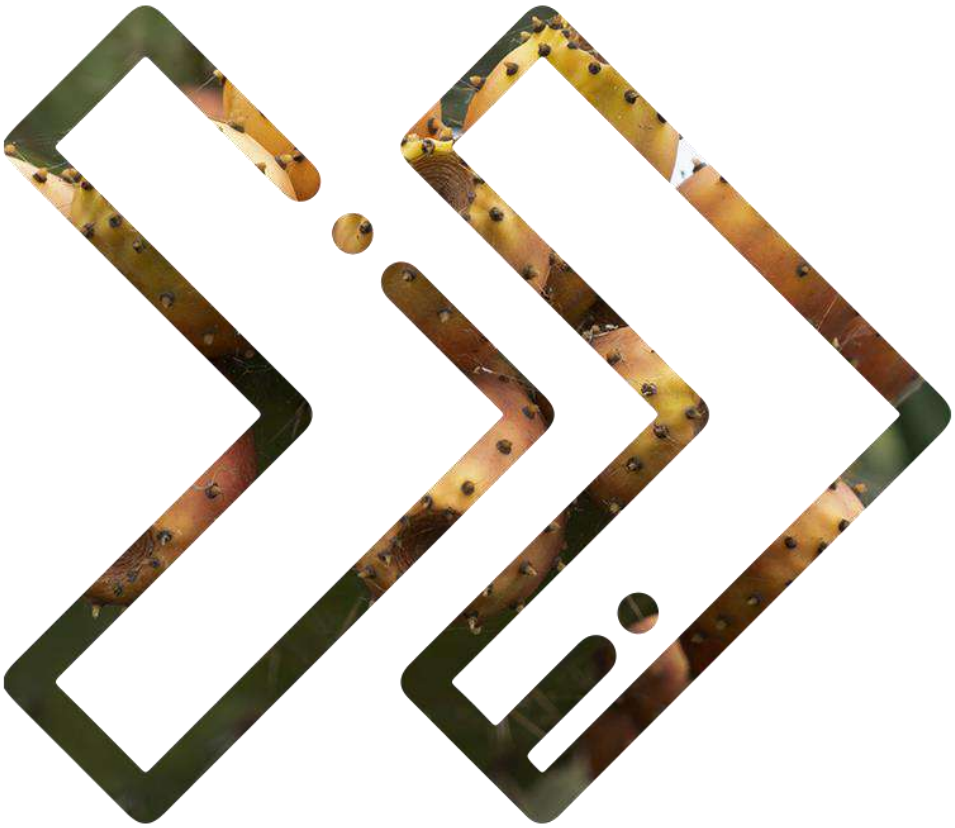
1- Debido a su alto contenido en compuestos bioactivos, los frutos y cladodios de los cultivares de chumbera estudiados fueron interesantes para la alimentación tanto en fresco como para la elaboración de productos alimenticios, así como para la alimentación animal. En lo referente a la actividad antioxidante y compuestos polifenólicos, destacan los cladodios jóvenes del cultivar 'FR', la piel de los frutos delos cultivares 'FR', 'NE' y 'NT' y las semillas del cultivar 'NE'. En cuanto al perfil de ácidos grasos, la pulpa del cultivar 'NJ', la piel y las semillas del cultivar 'NE' y los cladodios de más de un año del cultivar 'FR' destacaron por su porcentaje de ácidos grasos poliinsaturados, siendo el más abundante el ácido linoleico. Todo esto hace de las partes estudiadas de la chumbera óptimas para su consumo en fresco, en alimentos procesados, alimentación animal y sus usos en las industrias farmacéutica y cosmética. Además, las semillas y la piel de los frutos se obtienen como producto de deshecho tras la extracción de la pulpa para la elaboración de alimentos procesados, por lo que su aprovechamiento reduciría la producción de deshechos en este proceso.

2- Los cultivares 'NT' y 'FR' mostraron una concentración más elevada de compuestosvolátiles, los cuales afectan a la calidad sensorial de los frutos y están relacionados con la aceptación por parte de los consumidores. En este sentido, es necesario profundizar en sus atributos organolépticos para completar el conocimiento en este ámbito.

3- El almacenamiento de los frutos de chumbera, los higos chumbos, de un cultivar comercial llamado 'Orito' mantuvieron los parámetros de calidad y su perfil fitoquímico en valores óptimos durante un mes después de su

recolección, en almacenamiento en frío y durante tres días después de éste a temperatura ambiente, por lo que su comercialización sería posible durante todo este tiempo.

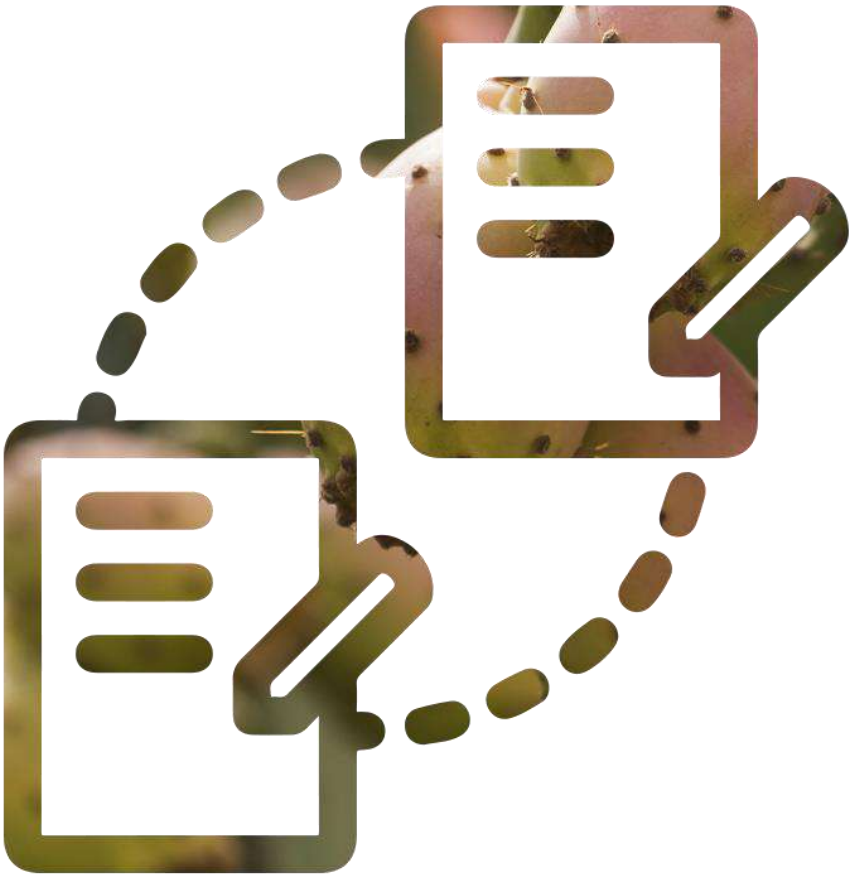
4- El cultivo de la chumbera puede ser una herramienta eficaz para el desarrollo rural en zonas áridas y semiáridas en lo que respecta a la producción, la creación de empleo y las cuestiones medioambientales. Además de su fácil adaptación ambiental y mantenimiento económico, su alta concentración en compuestos bioactivos, superior a la de otras especies cultivables en estas zonas, y su capacidad para el secuestro de carbono, podría incrementar la rentabilidad de la producción de la chumbera y así promover un desarrollo integral de las zonas rurales en las que se realiza la producción de este cultivo.



10. FUTURE RESEARCH

Once this research has finalized and based on the obtained results, further work must be done to complete the knowledge of prickly pear botanical parts and the valorization of this crop.

- Sensory evaluation of organoleptic attributes must be performed to complete the knowledge of the aroma of prickly pear fruit and the effect of the cultivar.
- Evaluation of the marketability of prickly pear fruits under different conditions, such as modified atmosphere packaging, must be carried out.
- The elaboration of prickly pear processed products derived of fruits, cladodes, and seeds of these cultivars, such as gummies, jams, syrups, drinks, and flours, might be done for evaluate their potential as new food products and the acceptance of these by consumers.
- More cultivars might be evaluated to amplify the knowledge of prickly pear Spanish cultivars and those of other countries.



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