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

Evaluación Agronómica de Compuestos Bioactivos y Perfil Genético de la Alcaparra (*Capparis spinosa* L.)

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Universidad Miguel Hernández de Elche

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Tesis para el Grado de Doctora de la

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Evaluación Agronómica, Análisis de Compuestos Bioactivos y Perfil Genético de la Alcaparra (*Capparis spinosa* L.)

Tesis presentada por Maria del Mar Grimalt Trò para optar al título de Doctora por la Universidad Miguel Hernández

Maria del Mar Grimalt Trò

Directoras:

Directora:

Co-directora:

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En estas páginas se cierra un capítulo de mi vida, unos de los mejores años han sido entre los pasillos de esta, mi universidad, la EPSO. Empecé siendo una niña de apenas 18 años recién cumplidos. Aquí me he formado, he crecido tanto personal como profesionalmente, y ha sido gracias a todas y cada una de las personas que se han cruzado en mi camino durante estos años. Me han ayudado a ser más fuerte y a creer en mi misma. Por ello, me gustaría dar las gracias a todas esas personas que han pasado por mi camino, aunque solo haya sido por un instante, pero que han influido en mi persona y han aportado su granito de arena, son tantas que no puedo citarlas a todas, pero vosotros sabéis quienes sois, GRACIAS.

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"Abandonar los sueños, aunque cuesten, no puede ser una opción"

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Categoría

Esta tesis doctoral está clasificada en la categoría de compendia de publicaciones para optar al título de Doctor por la Universidad Miguel Hernández de Elche.

Para ello, los 6 artículos de investigación seleccionados y su calidad, de acuerdo con la edición 2021 del Journal Citation Reports ® (JCR®), son los siguientes:

Publicación I

Grimalt, M., Hernández, F., Legua, P., Almansa, M.S., Amorós, A. (2018). Physicochemical composition and antioxidant activity of three Spanish caper (*Capparis spinosa* L.) fruit cultivars in three stages of development. Scientia Horticulturae 240: 509-515. Doi: https://doi.org/10.1016/j.scienta.2018.06.061

Publicación II

Grimalt, M., Legua, P., Hernández, F., Amorós, A., Almansa, M.S. (2019) Antioxidant Activity and Bioactive Compounds Contents in Different Stages of Flower Bud Development from Three Spanish Caper (*Capparis spinosa*) Cultivars. The Horticulture Journal 88 (3): 410-419. Doi: 10.2503/hortj.UTD-060

Publicación III

***Grimalt, M.**, Hernández, F., Legua, P., Amorós, A., Almansa, M.S. (2022). Antioxidant activity and the physicochemical composition of young caper shoots (Capparis spinosa L.) of different Spanish cultivars. Scientia Horticulturae 2022 293:110646. Doi: https://doi.org/10.1016/j.scienta.2021.110646*

Publicación IV

Wodyło, A., Nowicka, P., **Grimalt, M.,** Legua, P., Almansa, M.S., Amorós, A., Carbonell-Barrachina, A.A., Hernández, F., (2019). Polyphenol Compounds and Biological Activity of Caper (*Capparis spinosa* L.) Flowers Buds. Plants 8, 539. Doi; 10.3390/plants8120539

Publicación V

Grimalt, M., Sánchez-Rodríguez, L., Hernández, F., Legua, P., Carbonell-Barrachina, A.A., Almansa, M.S., Amorós, A. (2021). Volatile Profile in Different Aerial Parts of Two Caper Cultivars (*Capparis spinosa* L.). Journal of Food Quality 2021 (9). IA 6620776 Doi: https://doi.org/10.1155/2021/6620776

Publicación VI

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Physicochemical composition and antioxidant activity of three Spanish caper (*Capparis spinosa* L.) fruit cultivars in three stages of development

Autores

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Revista

Scientia Horticulturae

Publicación

2018

Editor

ELSEVIER

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Doi

10.1016/j.scienta.2018.06.061

ISSN

0304-4238

Quartil

Q1

Rango (2018)

5/36

Journal Impact Factor (JIF)

1.961

Impact Factor/5 years

1.650

Antioxidant Activity and Bioactive Compounds Contents in Different Stages of Flower Bud Development from Three Spanish Caper (*Capparis spinosa*) Cultivars

Autores

Grimalt, M., Legua, P., Hernández, F., Amorós, A., Almansa, M.S.

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Publicación

2019

Editor

The Japanese Society for Horticultural Science

JCR® Categoría

Horticulture – SCIE

Doi

10.2503/hortj.UTD-060

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Quartil

Q2

Rango (2019)

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Journal Impact Factor (JIF) (2016)

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Impact Factor/4 years

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Antioxidant activity and the physicochemical composition of young caper shoots (*Capparis spinosa* L.) of different Spanish cultivars

Autores

Grimalt, M., Hernández, F., Legua, P., Amorós, A., Almansa, M.S.

Revista

Scentia Horticulturae

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ELSEVIER

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Horticulture - SCIE

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Q1

Rango (2021)

4/36

Journal Impact Factor (JIF) (2021)

4.342

Impact Factor/5 years

2.859

Polyphenol Compounds and Biological Activity of Caper (*Capparis spinosa* L.) Flowers Buds

Autores

Wojdyło, A., Nowicka, P., **Grimalt, M.**, Legua, P., Almansa, M.S., Amorós, A., Carbonell-Barrachina, A.A., Hernández, F.

Revista

Plants

Publicación

2019

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Plant Sciences - SCIE

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Quartil

Q1

Rango (2019)

58/234

Journal Impact Factor (JIF) (2019)

2.762

Impact Factor/2 years

2.697

Volatile Profile in Different Aerial Parts of Two Caper Cultivars (*Capparis spinosa* L.)

Autores

Grimalt, M., Sánchez-Rodríguez, L., Hernández, F., Legua, P., Carbonell-Barrachina, A.A., Almansa, M.S., Amorós, A.

Revista

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Doi

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Quartil

Q3

Rango (2021)

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Journal Impact Factor (JIF) (2021)

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Impact Factor/5 years

1.923

Relationships between chemical composition, antioxidant activity and genetic analysis with ISSR markers in flower buds of caper plants (*Capparis spinosa* L.) of two subspecies *spinosa* and *rupestris* of Spanish cultivars

Autores

Grimalt, M., García-Martínez, S., Carbonell, P., Hernández, F., Legua, P., Almansa, M.S., Amorós, A.

Revista

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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 Dña. María del Mar Grimalt Trò ha realizado bajo la supervisión de nuestro Programa de Doctorado el Trabajo Titulado "Evaluación Agronómica de Compuestos Bioactivos y Perfil Genético de la Alcaparra (*Capparis spinosa* L.)" conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo con el Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo con los objetivos previstos de forma satisfactoria para su defensa como tesis doctoral.

Lo que firmo para los efectos oportunos, en Orihuela a 27 de septiembre de 2022

Dra. Dña. Juana Fernández López

Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias.

La Dra. Dña. *Maria Asunción Amorós Marco*, directora, y la Dra. Dña. *Maria Soledad Almansa Pascual de Riquelme*, codirectora de la tesis doctoral titulada "Evaluación Agronómica de Compuestos Bioactivos y Perfil Genético de la Alcaparra (*Capparis spinosa* L.)"

INFORMA/N:

Que D./Dña. *Maria del Mar Grimalt Trò* ha realizado bajo nuestra supervisión el trabajo titulado "Evaluación Agronómica de Compuestos Bioactivos y Perfil Genético de la Alcaparra (*Capparis spinosa* L.)" 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 firmamos para los efectos oportunos, en Orihuela a 24 de octubre de 2022.

Directora de la tesis

Dra. Dña. *Maria Asunción Amorós Marco*

Codirectora de la tesis

Dr. D./la Dra. Dña. *Maria Soledad Almansa Pascual de Riquelme*

Índice

ABREVIACIONES Y SÍMBOLOS

Abreviaciones y Símbolos

Abreviaciones

ABTS$^+$	2,2-azino-bis(3-Ethyl-benzothiazoline-6-sulfonic acid)	**ABTS$^+$**	Ácido 2,2-azinobis (3-etilbenzotazolina-6-sulfónico)
AChe	Acetylcholinesterase	**AChe**	Acetilcolinesterasa
ALB	Capers of Alberca (Murcia)	**ALB**	Alcaparras de la Alberca (Murcia)
ALCY	Capers of Alcayna (Murcia)	**ALCY**	Alcaparras de la Alcayna (Murcia)
ANOVA	Analysis of variance	**ANOVA**	Análisis de la varianza
BSA	Bovine Serum Albumin	**BSA**	Serum de Albumia bovina
BuChE	Butyrylcholinesterase	**BuChE**	Butyrylcholinesterasa
CA	Caffeoylquinic acids	**CA**	Ácido caffeoilquínico
dCA	Derivatives of caffeoylquinic acids	**dCA**	Derivados del ácido caffeoylquinic
dFQA	Derivatives of feruloylquinic acids	**dFQA**	Derivados del ácido feruloilquínico
dISO	Derivatives of isorhamnetin	**dISO**	Derivados de isorhamnetina
dK	Derivatives of kaempferol	**dK**	Derivados de kaempferol
dM	Derivatives of myricetin	**dM**	Derivados de miricetina
dpCA	Derivatives of *p*-coumaric acids	**dpCA**	Derivados del ácido *p*-coumárico
DPPH·	2,2-diphenyl 1-icrylhydrazyl	**DPPH·**	2,2-difenil-1-picrilhidracilo

dpQCA	Derivatives of *p*-coumaroylquinic acids	**dpQCA**	Derivados del ácido *p*-coumaroilquínico
dQ	Derivatives of quercetin	**dQ**	Derivados de quercetina
DW	Dry weight	**ps**	Peso seco
dFQA	Derivatives of feruloylquinic acids	**dFQA**	Derivados del ácido feruloilquínico
FQA	Feruloylquinic Acid	**FQA**	Ácido feruloilquínico
FRAP	Ferric-reducing antioxidant power	**FRAP**	Poder antioxidante reductor férrico
FW	Fresh weight	**pf**	Peso fresco
GAE	Gallic Acid Equivalents	**EAG**	Equivalentes de ácido gálico
HPLC	High Performance Liquid Chromatography	**HPLC**	Cromatografía líquida de alta resolución
H-TAA	Total Antioxidant Activity of Hydrophilic Fraction	**AAT-H**	Actividad Antioxidante total de la Fracción Hidrosoluble
LC-qTOF-MS/MS	Time-of-Flight-Mass Spectrofotometer/Mass Spectofotometer	**LC-qTOF-MS/MS**	Espectrofotómetro de masas de tiempo de vuelo/espectrofotómetro de masas
LSD	Least significant difference	**LSD**	Diferencia menos significativa
L-TAA	Total Antioxidant Activity of Lipophilic Fraction	**AAT-L**	Actividad Antioxidante Total de la Fracción Liposoluble
ORAC	Oxygen Radical Absorbance Capacity	**ORAC**	Capacidad de absorción de radicales de oxígeno
ORI	Capers of Orihuela (Alicante)	**ORI**	Alcaparras de Orihuela (Alicante)
pCA	Derivatives of *p*-coumaric acids	**pCA**	Derivados del ácido *p*-coumárico
RE	Rutin Equivalents	**ER**	Equivalentes de rutina
R_t	Retention Time	**R_t**	Tiempo de Retención

SA	Sinapic acid	**SA**	Ácido Sinapic
SPSS	Statistical Package for the Social Sciences	**SPSS**	Paquete estadístico para ciencias sociales
TAA	Total Antoxidant Activity	**AAT**	Actividad Antioxidante Total
TFC	Total Content of Flavonoids	**TFC**	Contenido Total Flavonoids
TFoC	Total Flavonol compouds	**TFoC**	Compuestos Flavonoles Totales
TIC	Total Ion Current	**TIC**	Corriente total de iones
TPC	Total Phenol Content	**TPC**	Contenido total de Fenoles
TROLOX	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid	**TROLOX**	Ácido 6-Hidroxi-2,5,7,8-tetrametilchroman-2-carboxílico
UPLC	Ultra Performance Liquid Chromatography	**UPLC**	Cromatografía líquida de ultra rendimiento
UPLC-PDA-FL	Ultra-Performance Liquid Chromatography-Photodiode Array-Fluorescence Detector	**UPLC-PDA-FL**	Cromatografía líquida de ultra rendimiento, matriz de fotodiodos, detector de fluorescencia

Símbolos

*b**	Blue-yellowness colour	*b**	Color azul-amarillento
$[M-H]^-$	Deprotonated molecular ion	$[M-H]^-$	Ion molecular desprotonado
Ø	Diameter	Ø	Diámetro
$\varepsilon^{1\%}_{cm}$	Extinction coefficient	$\varepsilon^{1\%}_{cm}$	Coeficiente de extinción
F-3-ols	Flavan-3-ols	F-3-ols	Flavan-3-ols
IC_{50}	Half maximal Inhibitory Concentration	IC_{50}	Concentración inhibitoria media máxima
Λ_{max}	Higher absorbance	Λ_{max}	Absorbancia máxima
*L**	Lightness	*L**	Luminosidad
MS $[M-H]^-$	Main ions and formulas for deprotonated molecules	MS $[M-H]^-$	Principales iones y fórmulas de moléculas desprotonadas
m/z	Mass-to-charge ratio	*m/z*	Relación masa-carga
*a**	Red-greenness colour	*a**	Color rojo-verdoso

ESTRUCTURA DE LA TESIS DOCTORAL

Estructura de la Tesis Doctoral

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

- **Resumen**: se ha descrito los resultados y conclusiones más relevantes (castellano, inglés y valenciano)
- **Introducción**: se han descrito las características generales más relevantes del cultivo de la alcaparra, además de sus propiedades agronómicas y funcionales.
- **Objetivos**: se ha expuesto el objetivo global y los objetivos parciales de la investigación.
- **Metodología**: se ha realizado una breve descripción de la metodología empleada para la consecución de los objetivos.
- **Publicaciones científicas**: se ha realizado la transcripción literal de las publicaciones científicas que componen esta tesis:

    I. **Propiedades fisicoquímicas de las distintas partes comestibles de la alcaparra.**

    Conformado por tres artículos científicos donde se determinan las propiedades fisicoquímicas de las distintas partes comestibles del cultivo: botones florales, frutos y tallos tiernos, en cultivares diferentes ubicados en zonas geográficas distintas. El primero de ellos se titula '*Physicochemical composition and antioxidant activity of three Spanish caper (Capparis spinosa L.) fruit cultivars in three stages of development*' y está publicado en Scientia Horticulturae en el año 2018. El segundo artículo se titula *'Antoxidant Activity and Bioactive Compounds Contents in Different Stages of Flower Bud Development from Three Spanish Caper (Capparis spinosa) Cultivars'* se encuentra publicado en la revista Horticulture Journal en el año 2019. Por último, el tercero se titula *'Antioxidant activity and the physicochemical composition of young caper shoots*

(Capparis spinosa L.) of different Spanish cultivars' y yse encuentra publicado en la revista Scientia Horticulturae en el año 2022.

II. **Estudio de los compuestos funcionales y actividad biológica de los botones florales de la alcaparra.**

Artículo científico donde se presentan los resultados obtenidos en el estudio de los compuestos polifenólicos y la actividad biológica de los botones florales. El artículo se titula '*Polyphenol Compounds and Biological Activity of Caper (Capparis spinosa L.) Flowers Buds'* publicado en la revista Plants en el año 2019.

III. **Estudio de los compuestos volátiles de la alcaparra.**

Artículo científico donde se determinan los compuestos volátiles que se encuentran en las diferentes partes comestibles de la alcaparra. El artículo se titula *'Volatile Profile in Different Aerial Parts of Two Caper Cultivars (Capparis spinosa L.)'* publicado en la revista Journal of Food Quality en el año 2021.

IV. **Estudio del perfil genético del cultivo de la alcaparra.**

Artículo científico donde se muestra la relación existente entre la actividad antioxidante y compuestos químicos con un análisis genético realizado con marcadores moleculares en las hojas de la planta de alcaparra entre dos subespecies diferentes *rupestris* y *spinosa*. El artículo científico se titula *'Relationships between chemical composition, antioxidant activity and genetic analysis with ISSR markers in flower buds of caper plants (Capparis spinosa L.) of two subspecies spinosa y rupestris of Spanish cultivars'* publicado en la revista Genetic Resources and Crop Evolution en el año 2022.

- **Resultados, discusión y conclusiones**: se ha realizado un breve resumen de los principales resultados y discusiones obtenidos en cada uno de los artículos y sus conclusiones.
- **Conclusiones generales**: se han redactado las conclusiones obtenidas en la presente Tesis Doctoral.

- **Investigaciones futuras**: se determinan las posibles futuras investigaciones que se pueden desarrollar a partir de los resultados obtenidos.
- **Referencias bibliográficas**: se indican las referencias bibliográficas empleadas en las secciones complementarias a las publicaciones.

RESUMEN/ABSTRACT/RESUM

Resumen

La especie *Capparis spinosa* L. es una mata leñosa, rastrera y muy resistente a la sequía que se cultiva en la cuenca mediterránea. En España es muy común utilizar como alimento distintas partes aéreas como los caparrones, botones florales y tallos tiernos, que se pueden consumir en encurtido o salmuera. Se han realizado muy pocos estudios sobre los cambios en las propiedades fisicoquímicas y funcionales entre los distintos estados de desarrollo. Los caparrones tienen tres estados de desarrollo según su diámetro; 'Finos'; 'Medianos' y 'Gruesos' y los botones de alcaparra cuentan con seis estados de desarrollo; 'Nompareilles'; 'Surfines'; 'Capucines'; 'Capotes'; 'Finas' y 'Gruesas'. En esta Tesis Doctoral se realizó por primera vez un estudio comparativo sobre la evolución de parámetros físicos, químicos, nutritivos y compuestos bioactivos de caparrones y alcaparras de distintos cultivares de diferentes zonas geográficas de España. Uno de los objetivos del presente trabajo fue estudiar el desarrollo del contenido de fenoles, flavonoides y flavonoles totales, que fueron muy altos en todas las etapas del desarrollo, tanto de los frutos como de las alcaparras, aunque mayores en los botones florales. También se determinaron los valores de AAT obtenidos por los métodos DPPH·, $ABTS^{+}$ y FRAP, así como la AAT-H y AAT-L. También se determinó el contenido en proteínas y azúcares. Los botones florales tendían a disminuir los valores del contenido antioxidante a medida que se desarrollaban y aumentaban de tamaño. Los tallos tiernos mostraron valores muy elevados de AAT.

Asimismo, en la presente investigación se analizaron los componentes potenciales de promoción de la salud de los botones florales de la alcaparra en sus seis estados de desarrollo. Los compuestos polifenólicos (flavonoles, ácidos hidroxicinámicos, flavan-3-ols) se identificaron mediante cromatografía líquida-espectrofotómetro de masas/espectrofotómetro de masas de tiempo de vuelo cuádruplo (LC-qTOF-MS/MS) y se cuantificaron mediante cromatografía líquida de ultra rendimiento-detector de fluorescencia de matriz de fotodiodos (UPLC-PDA-FL). Además, se examinaron las propiedades antioxidantes ($ABTS^{+}$, FRAP y ORAC), el potencial antidiabético (α-amilasa y α-glucosidasa) y la actividad antienvejecimiento (acetilcolinesterasa y butirilcolinesterasa) de las yemas. Los compuestos fenólicos totales en la alcaparra investigada variaron y dependieron del genotipo y la etapa de crecimiento de las yemas florales de alcaparra. Entre las seis etapas de crecimiento diferentes, la denominada 'Nonpareilles' se caracterizó por un contenido significativamente más alto de polifenoles que las cinco etapas restantes. Los flavonoles en las yemas florales de alcaparras representaron una mezcla de diferentes derivados de quercetina, kaempferol, miricetina e isorhamnetina glicosilados, que representan el 38-67%, 15-36%, 4-7% y 0,8-3%, respectivamente, de flavonoles totales. De las seis etapas de crecimiento

investigadas, las 'Nonpareilles' acumularon las mayores cantidades de compuestos bioactivos que se correlacionaron con propiedades antioxidantes y antidiabéticas, y fueron BuChE más potentes que los inhibidores de AChE, por lo tanto, muestran que las propiedades antioxidantes y nutricionales de las alcaparras son mejores cuanto más pequeñas son.

La presente Tesis Doctoral presentó por primera vez perfiles volátiles completos de cuatro partes aéreas de alcaparras (*Capparis spinosa* L.) del sureste de España. Los compuestos volátiles en hojas y tallos de alcaparras (juntos), flores, yemas de flores y frutos de dos cultivares se identificaron y cuantificaron mediante microextracción en fase sólida de espacio de cabeza y cromatografía de gases, con un detector de espectrometría de masa. El compuesto predominante fue el isotiocianato de metilo, seguido de nerolidol, trans-2-hexenal y nonanal jugando papeles clave en las flores, hojas y yemas de las flores, respectivamente. Los dos cultivares estudiados tenían los mismos compuestos volátiles, pero a concentraciones muy diferentes, aunque los dos cultivares estudiados se cultivan en las mismas condiciones climáticas y agronómicas.

No hay trabajos previos publicados sobre las diferencias en compuestos bioquímicos y funcionales entre las subespecies *spinosa* y *rupestris*, y en esta Tesis Doctoral se han analizado los compuestos funcionales de tápenas de 24 cultivares pertenecientes a ambas subespecies, con la finalidad de estudiar si se puede sustituir el cultivo de la subespecie *spinosa* por la *rupestris* sin perder las propiedades funcionales de las tápenas, ya que la *rupestris* no presenta espinas.

Dado que la alcaparra es un arbusto xerofítico perenne común con una notable adaptabilidad a ambientes hostiles, y dadas las excelentes propiedades de estos frutos y tápenas, podría considerarse una especie de gran interés para su cultivo, tanto por su resiliencia frente al cambio climático, como por sus numerosos beneficios para la salud. Por estas razones debería fomentarse un mayor consumo de alcaparras en la dieta y, por tanto, su mayor cultivo.

Abstract

The *Capparis spinosa* L. species is a woody, creeping and highly drought-resistant plant grown in the Mediterranean basin. In Spain it is very common to use as food different aerial parts such as capers, flower buds and tender stems, it can be consumed in pickle or brine. Very few studies have been carried out on the changes in the physicochemical and functional properties between the different stages of development. Caper fruits have three stages of development according to their diameter: 'Fine', 'Medium' and 'Thick' and, the caper buttons have six stages of development; 'Nompareilles'; 'Surfines'; 'Capucines'; 'Capes'; 'Fine' and 'Thick'. In this Doctoral Thesis, a comparative study was carried out for the first time on the evolution of physical, chemical, nutritive and bioactive compounds in different cultivars from different geographical areas of Spain. One of the objectives of this work was to study the development of the content of total phenols, flavonoids and flavonols, which were very high in all stages of development of both fruits and capers, although higher in flower buds. The TAA values obtained by the DPPH·, $ABTS^{+}$ and FRAP method, as well as the TAA-H and TAA-L, were also determined. The protein and sugar content were also determined. Flower buds tended to decrease in content values as they developed and increased in size. The tender stems showed very high values of TAA.

Likewise, in the present investigation, the potential health-promoting components of caper flower buds in their six stages of development were analysed. Polyphenol compounds (flavanols, hydroxycinnamic acids, flavan-3-ols) were identified by liquid chromatography, quadrupole time of flight, liquid chromatography-quadrupole time – of – flight – mass spectrofotometer/ mass spectrophotometer (LC-qTOF-MS/MS) and quantified by ultra-performance liquid chromatography-photodiode array-fluorescence detector (UPLC-PDA-FL). Furthermore, the antioxidant properties ($ABTS^{+}$, FRAP and ORAC), the antidiabetic potential (α-amylase and α-glucosidase) and the anti-aging activity (acetylcholinesterase and butyrylcholinesterase) of the flower buds were examined. The total phenolic compounds in the investigated caper varied and depended on the genotype and growth stage of the caper flowers buds. Among the six different growth stages, the so-called 'Nompareilles' was characterized by a significantly higher content of polyphenols than the remaining five stages. The flavanols in caper flower buds represented a mixture of different glycosylated quercetin, kaempferol, myricetin and isorhamnetin derivatives, representing 38-67%, 15-36%, 4-7% and 0.8-3%, respectively, of total flavanols. Of the six growth stages investigated, 'Nompareilles' accumulated the highest amounts of bioactive compounds that were correlated with antioxidant and antidiabetic properties, and were BuChE more potent

than AChE inhibitors, therefore showing that antioxidant and nutritional properties of flower buds are better the smaller.

The Doctoral Thesis presented for the first time complete volatile profiles of four aerial parts of capers from south-eastern Spain. Volatile compounds in caper leaves and stems (together), flowers, flower buds, and fruits of two cultivars were identified and quantified by headspace solid-phase microextraction and gas chromatography with a mass spectrometric detector. The predominant compound was methyl isothiocyanate, followed by nerolidol, trans-2-hexenal and nonanal playing key roles in flowers, leaves and flower buds, respectively. The two cultivars studied had the same volatile compounds, but at very different concentrations, although the two cultivars studied were grown under the same climatic and agronomic conditions.

There are no previous works published on the differences in biochemical and functional compounds between the *spinosa* and *rupestris* subspecies, so in this Doctoral Thesis the functional compounds of 24 cultivars belonging to both subspecies have been analyzed, in order to study if it can be substituting the subspecies *spinosa* for the *rupestris* without losing the functional properties of capers, since the *rupestris* do not have spines.

Given that the caper is a common perennial xerophytic shrub with a remarkable adaptability to hostile environments, and given the excellent properties of these fruits, it could be considered a species of great interest for its cultivation, both for its resilience against climate change, and for its numerous health benefits. Therefore, increased consumption of capers in the diet and their cultivation should be encouraged.

Resum

L'espècie *Capparis spinosa* L., és una mata llenyosa, rèptil i molt resistent a la sequera que es cultiva en la conca mediterrània. A Espanya és molt comú utilitzar com a aliment diferents parts aèries com els caparrons, botons florals i tiges tendres, que es poden consumir en escabetx o salmorra. S'han realitzat molt pocs estudis sobre els canvis en les propietats fisicoquímiques i funcionals entre els diferents estats de desenvolupament. Els caparrons tenen tres estats de desenvolupament segons el seu diàmetre; 'Fins'; 'Mitjans' i 'Gruixuts' i, els botons de tàpera compten amb sis estats de desenvolupament; 'Nompareilles'; 'Surfins'; 'Capucins'; 'Capots'; 'Fines' i 'Gruixudes'. En aquesta Tesi Doctoral es va realitzar per primera vegada un estudi comparatiu sobre l'evolució de paràmetres físics, químics, nutritius i compostos bioactius en diferents cultivars de diferents zones geogràfiques d'Espanya. Un dels objectius del present treball va ser estudiar el desenvolupament del contingut de fenols, flavonoides i flavonols totals, que van ser molt alts en totes les etapes del desenvolupament, tant dels fruits com de les tàperes, encara que majors en els botons florals. També es van determinar els valors de AAT obtinguts pel mètode DPPH·, $ABTS^{+}$ i FRAP, així com la AAT-H i AAT-L. També es van determinar el contingut en proteïnes i sucres. Els botons florals tendien a disminuir els valors del contingut antioxidant a mesura que es desenvolupaven i augmentaven de grandària. Les tiges tendres van mostrar valors molt elevats de AAT.

Així mateix, en la present investigació es van analitzar els components potencials de promoció de la salut dels botons florals de la tàpera en els seus sis estats de desenvolupament. Els compostos polifenólics (flavanols, àcids hidroxicinámics, flavan-3-ols) es van identificar mitjançant cromatografia líquida-espectrofotòmetre de masses/espectrofotòmetre de masses de temps de vol quàdruple (LC-qTOF-MS/MS) i es van quantificar mitjançant cromatografia líquida d'ultra rendiment-detector de fluorescència de matriu de fotodíodes (UPLC-PDA-FL). A més, es van examinar les propietats antioxidants ($ABTS^{+}$, FRAP i ORAC), el potencial antidiabètic (α-amilasa i α-glucosidasa) i l'activitat antienvelliment (acetilcolinesterasa i butirilcolinesterasa) de les gemmes. Els compostos fenòlics totals en la tàpera investigada van variar i van dependre del genotip i l'etapa de creixement de les gemmes florals de tàpera. Entre les sis etapes de creixement diferents, la denominada 'Nonpareilles' es va caracteritzar per un contingut significativament més alt de polifenols que les cinc etapes restants. Els flavanols en les gemmes florals de tàperes van representar una mescla de diferents derivats de quercetina, *kaempferol, miricetina i isorhamnetina glicosilats, que representen el 38-67%, 15-36%, 4-7% i 0,8-3%,

respectivament, de flavanols totals. De les sis etapes de creixement investigades, els 'Nonpareilles' van acumular les majors quantitats de compostos bioactius que es van correlacionar amb propietats antioxidants i antidiabètiques, i van ser BuChE més potents que els inhibidors de AChE, per tant, mostren que les propietats antioxidants i nutricionals de les tàperes són millors com més xicotetes són.

La present Tesi Doctoral va presentar per primera vegada perfils volàtils complets de quatre parts aèries de tàperes (*Capparis spinosa* L.) del sud-est d'Espanya. Els compostos volàtils en fulles i tiges de tàperes (junts), flors, gemmes de flors i fruits de dues cultivars es van identificar i es quantificaren mitjançant microextracció en fase sòlida d'espai de cap i cromatografia de gasos, amb un detector d'espectrometria de massa. El compost predominant va ser el isotiocianat de metil, seguit de nerolidol, trans-2-hexenal i nonanal jugant papers clau en les flors, fulles i gemmes de les flors, respectivament. Les dues cultivars estudiats tenien els mateixos compostos volàtils, però a concentracions molt diferents, encara que les dues cultivars estudiats es cultiven en les mateixes condicions climàtiques i agronòmiques.

No hi ha treballs previs publicats sobre les diferències en compostos bioquímics i funcionals entre les subespècies *spinosa* i *rupestris*, i en aquesta Tesi Doctoral s'han analitzat els compostos funcionals de tàpenes de 24 cultivars pertanyents a totes dues subespècies, amb la finalitat d'estudiar si es pot substituir el cultiu de la subespècie *spinosa* per la *rupestris* sense perdre les propietats funcionals de les tàpenes, ja que la *rupestris* no presenta espines.

Atés que la tàpera és un arbust xeròfil perenne comú amb una notable adaptabilitat a ambients hostils, i donades les excel·lents propietats d'aquests fruits i tàpenes, podria considerar-se una espècie de gran interés per al seu cultiu, tant per la seua resiliència enfront del canvi climàtic, com pels seus nombrosos beneficis per a la salut. Per aquestes raons hauria de fomentar-se un major consum de tàperes en la dieta i, per tant, el seu major cultiu.

INTRODUCCIÓN

1. Introducción

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

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

El objetivo principal de la presente tesis doctoral ha sido estudiar los alcaparros de variedades españolas, realizar una caracterización físico-química y funcional de distintos cultivares de alcaparros cultivados en el sureste español, en los botones florales y caparrones en diferentes estados de desarrollo, en los brotes tiernos y conocer el perfil fenólico y propiedades para la salud humana de las alcaparras, así como, conocer el perfil de compuestos volátiles de las diferentes partes aéreas de la planta y determinar el perfil genético mediante el uso de marcadores moleculares ISSR de dos subespecies, *rupestris* y *spinosa*. La finalidad ha sido conocer en profundidad estos cultivares y así poder seleccionar, para transferir al sector agroalimentario, aquellos cultivares que mejores cualidades presenten para su producción.

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

I. La primera publicación recoge los resultados obtenidos de evaluar las propiedades físicas y bioquímicas, así como las propiedades funcionales de los alcaparrones de 3

cultivares diferentes de plantas en tres estados del desarrollo de estos frutos. Este es el primer estudio que analiza estos parámetros en diferentes estados del desarrollo de los alcaparrones. Este trabajo está publicado en la revista *Scientia Horticulturae*.

Grimalt, M., Hernández, F., Legua, P., Almansa, M.S., Amorós, A. (2018). Physicochemical composition and antioxidant activity of three Spanish caper (*Capparis spinosa* L.) fruit cultivars in three stages of development. Scientia Horticulturae 240: 509-515. Doi: https://doi.org/10.1016/j.scienta.2018.06.061

II. La segunda publicación consistió en un estudio comparativo de las tápenas en 6 estados del crecimiento, estudiando las propiedades bioquímicas y funcionales de las yemas florales de 3 cultivares de alcaparro. Este es el primer estudio que analiza las propiedades bioquímicas y funcionales de las alcaparras en diferentes estados del desarrollo. Se publicó en la revista *Horticulture Journal.*

Grimalt, M., Legua, P., Hernández, F., Amorós, A., Almansa, M.S. (2019) Antioxidant Activity and Bioactive Compounds Contents in Different Stages of Flower Bud Development from Three Spanish Caper (*Capparis spinosa*) Cultivars. Horticulture Journal 88 (3): 410-419. Doi: 10.2503/hortj.UTD-060

III. La tercera publicación consistió en realizar un estudio sobre la composición en actividad antioxidante y componentes fisicoquímicos de los brotes jóvenes de los alcaparros de la especie *Capparis spinosa* L. Es la primera vez que se estudian las propiedades fisicoquímicas de esta parte comestible del alcaparro. Se publicó en la revista *Scientia Horticulturae*.

Grimalt, M., Hernández, F., Legua, P., Amorós, A., Almansa, M.S. (2022). Antioxidant activity and the physicochemical composition of young caper shoots (*Capparis spinosa* L.) of different Spanish cultivars. Scientia Horticulturae 2022 293: 110646 Doi: https://doi.org/10.1016/j.scienta.2021.110646

IV. La cuarta publicación consistió en la realización del perfil fenólico de las alcaparras de dos cultivares y un estudio de las propiedades para la salud humana de las alcaparras en seis estados de crecimiento, siendo la primera vez que se publican dichos análisis. Se publicó en la revista *Plants*.

Wodyło, A. Nowicka, P., **Grimalt, M.,** Legua, P., Almansa, M.S., Amorós, A., Carbonell-Barrachina, A.A., Hernández, F. (2019). Polyphenol compounds and Biological Activitty of Caper (*Capparis spinosa* L.) Flower Buds. Plants 8, 539. Doi: 10.3390/plants8120539

V. La quinta publicación consistió en la realización del perfil de compuestos volátiles de todas las partes aéreas del alcaparro de dos cultivares. Es la primera vez que se ha hecho el perfil de compuestos volátiles de las flores del alcaparro. Se publicó en la revista *Journal of Food Quality*.

Grimalt, M., Sánchez-Rodríguez, L., Hernández, F., Legua, P., Carbonell-Barrachina, A.A., Almansa, M.S., Amorós, A. (2021). Volatile Profile in Different Aerial Parts of Two Caper Cultivars (*Capparis spinosa* L.). Journal of Food Quality 2021, ID 6620776 (9 pg). Doi: https://doi.org/10.1155/2021/6620776

VI. La sexta publicación se basa en relacionar los compuestos bioquímicos, actividad antioxidante y análisis genético con marcadores moleculares ISSR en botones florales de la planta de la alcaparra de la especie *Capparis spinosa* L. en dos subespecies, *spinosa* que presenta espinas y *rupestris* sin espinas. Es la primera vez que se realiza esta comparativa. Se publicó en la revista *Genetic Resources and Crop Evolution*.

Grimalt, M., García-Martínez, S., Carbonell, P., Hernández, F., Legua, P., Almansa, M.S., Amorós, A. (2022). Relationship between chemical composition, antioxidant activity and genetic analysis with ISSR markers in flower buds of caper plants (*Capparis spinosa* L.) of two subspecies *spinosa* and *rupestris* of Spanish cultivars. Genetic Resources and Crop Evolution 69:1451-1469. Doi: https://doi.org/10.1007/s10722-021-01312-3

Origen y expansión

La familia *Capparaceae* comprende aproximadamente 40-50 géneros en regiones tropicales, subtropicales y templadas, a la que pertenecen 700-900 especies (Hall et al., 2002: Gristina et al., 2011), entre ellas destacan tres: *Capparis spinosa, Capparis ovata y Capparis decidua,* por haber sido investigadas extensamente por sus propiedades nutricionales y terapéuticas (Gull et al., 2015). El género *Capparis* incluye aproximadamente 250 especies (Jacobs, 1965; Gristina, 2011). *Capparis spinosa* es un arbusto conocido como caper (English), alcaparra o alcaparro (español), cappero (italiano) y muziplik (Türkiye), entre otros nombres.

La especie *Capparis spinosa* L. se considera autóctona de Asia Occidental y se cree que su antiguo hábitat son las zonas secas de Asia Occidental o central (Romeo et al., 2007). Se han encontrado restos de alcaparras en yacimientos arqueológicos en el Mesolítico inferior (Hansen, 1991; Tlili, et al., 2011), donde aparecieron botones florales carbonizados y frutos inmaduros en una jarra en Telles-Sweyhat, Siria, por lo que se consideró que se almacenaba como condimento, con una fecha aproximada de 2400-1400 a.C. (Van Zeist et al., 1985; Tlili et al., 2011).

Desde hace un cierto tiempo, se está cultivando extensamente en las costas de los países mediterráneos (Gull et al., 2015). El cultivo se ha extendido desde las costas atlánticas de las Islas Canarias y Marruecos hasta el mar Negro, Crimea y Armenia, hacia el este, llegando hasta el Mar Caspio y en el este de Irán (Figura 1) (Aliyazicioglu et al., 2015). El cultivo de la alcaparra en España, se extiende en las zonas del sur-este peninsular, centrándose en la Región de Murcia, Comunidad Valenciana, Andalucía y las Islas Baleares.

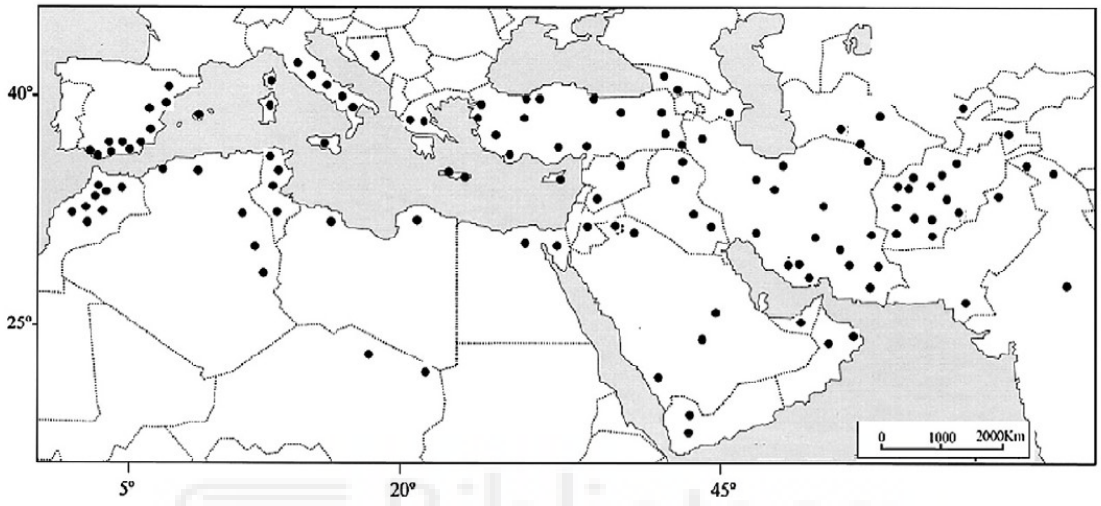


Figura 1. Distribución natural de las alcaparras según Inocencio et al. (2006)

Clasificación Taxonómica

Clasificación sistemática *Capparis spinosa* L.:

- División: Fanerógamas
- Subdivisión: Angiospermas
- Clase: Dicotiledóneas
- Subclase: Magnoliópsida
- Orden: Capparales
- Familia: Capparaceae
- Género: *Capparis*
- Especie: *Capparis spinosa* L.

Descripción botánica

La alcaparra es un arbusto perenne de hoja caduca y ciclo estival que presenta un crecimiento rastrero. Inicialmente puede considerarse una planta colonizadora, debido a que puede crecer en lugares muy complicados donde prácticamente no existe suelo, como en los márgenes de

piedras y en grietas de rocas, entre otros, aunque prefiere suelos sueltos y bien drenados. Es uno de los pocos arbustos perennes que crece y florece durante veranos secos y calurosos. Según varios estudios, el alcaparro es una planta xerófila (Rhizopoulou, 1990; Gan et al., 2013), es decir, se encuentra adaptada a vivir en lugares o ambientes secos, presentando raíces muy largas, que le permiten sobrevivir a periodos de extrema sequía. La especie *Capparis spinosa* L. se adapta a distintos tipos de suelos, incluidos alfisoles, regosoles y litosoles, y muestra una buena respuesta a suelos volcánicos o alcalinos. Tolera pHs del 6,1 al 8,5 y puede soportar temperaturas extremas desde -8 hasta 40 ºC (Mishra et al., 2009; Legua et al., 2013).

La planta del alcaparro crece ampliamente después de las lluvias de abril – mayo y comienza a desaparecer al comienzo del clima frío, en los meses de septiembre – octubre. Es una mata rastrera de unos 30 – 100 cm de alto. La planta del alcaparro suele recibir distintos nombres, según a que parte se refiere, como indica la tabla 1.

Tabla 1. Nomenclatura de las distintas partes aéreas de *Capparis spinosa* L

Nombres que recibe la planta	
Planta	Alcaparra Alcaparro Tapenera
Botón floral	Tápena Capota
Fruto	Caparrones Alcaparrones
Tallos y hojas	Brotes tiernos Tallos tiernos Brotes jóvenes

Raíces

Sus raíces suelen ser pivotantes y pueden llegar a alcanzar una profundidad de 6 a 10 metros (Legua et al., 2013), lo que les permite extraer agua del subsuelo en condiciones de sequía. Si las plantas se encuentran abastecidas de agua mediante el riego por goteo, su desarrollo es mucho menor. Presentan raíces secundarias de color blanquecinas, lisas y carnosas. Generalmente, la planta del alcaparro suele reproducirse por semilla, por lo que su raíz suele ser pivotante, pero en los casos en los que proceda de estaquillas leñosas o herbáceas no presentan este mismo tipo de raíz, sino que su sistema radicular es más superficial.

La zona de unión entre los tallos y el sistema radicular forma un muñón que, en el caso de plantas viejas, puede alcanzar los 25 cm de diámetro. En este muñón suelen encontrarse numerosas yemas que podrán brotar en primavera (Melgarejo y Salazar, 2000).

Tallos

La tapenera es una mata perenne cuyas ramas se renuevan todos los años, por lo que no presentan un verdadero tronco. Tiene un porte rastrero que puede alcanzar los 3 metros de longitud, esto es de gran interés ya que presenta una mayor eficacia para reducir la evaporación del agua y la erosión por el viento o el agua de escorrentía (Melgarejo y Salazar, 2000). Uno de los subproductos de la alcaparra son los tallos tiernos (Figura 2), que es muy típico encontrarlos en forma de encurtido en ensaladas en la zona del sur-este peninsular. Suelen recolectarse cuando todavía no presentan el color verde característico, sino que tienen más bien un color violáceo. Se consumen los 10 últimos centímetros, ya que son los más tiernos.

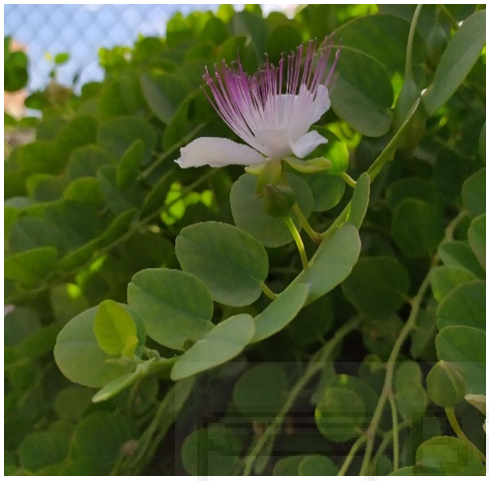


Figura 2. Tallo tierno de *Capparis spinosa* L.

Hojas

Presentan una forma ligeramente acorazonada, de consistencia crasa y algo gruesas (Figura 2). Tienen un color de verde a rojo, con estípulas precozmente caducas. Poseen un nervio principal y varios secundarios con estomas en ambos lados de la hoja, distribuidos de manera uniforme. En el periodo estival se mantienen abiertos durante el día (Domínguez, 2013).

Botones Florales

Las tápenas o alcaparras están formadas por todas las partes de la flor que todavía no se ha desarrollado. Son de color verdoso y su tamaño puede variar en función de su estado de desarrollo (Figura 3), según el calibre se pueden clasificar en seis estados de desarrollo (Tabla 2).

Tabla 2. Clasificación de las tápenas en función de su calibre

Denominación	Diámetro en milímetros (mm)
Nompareilles	Inferior a 7
Surfines	7-8
Capucines	8-9
Capotes	9-11
Finas	11-13
Gruesas	Superior a 13

Las tápenas suelen consumirse encurtidas como acompañantes en ensaladas, pasta o en forma de salsas. Los estados de desarrollo más comerciales suelen ser los surfines y capucines. La aparición de las tápenas es continua en todas las etapas de desarrollo, por lo que se puede observar simultáneamente las tápenas, flores y frutos.

Figura 3. Botones florales de *Capparis spinosa* L.

Flores

Las flores son hermafroditas, solitarias y se forman a partir de las yemas axilares, cada yema axilar forma una única flor. Cuando están completamente abiertas, pueden alcanzar unos 5-7 cm de diámetro. Presentan cuatro pétalos blancos o ligeramente rosados y cuatro sépalos verdes. Posee una gran cantidad de estambres con largos filamentos de color violáceo y anteras amarillas (Figura 4). El polen y el pistilo maduran simultáneamente, por lo que puede producirse tanto autofecundación como fecundación cruzada (Melgarejo y Salazar, 2000).

Figura 4. Detalle de la flor de *Capparis spinosa* L.

Frutos

Una vez fecundadas las flores, aparecen los frutos, denominados caparrones o alcaparrones. Se trata de una baya carnosa con un largo pedúnculo (Figura 5). Presenta un color verde cuando es inmaduro, con estrías o dibujos de color blanquecino y, a medida que va madurando, adquiere un color rojizo. Cuando llega a la madurez el fruto se abre y deja las semillas a disposición de los insectos, animales o fenómenos naturales para que las diseminen. Los frutos presentan tres estados de desarrollo, que se clasifican según su calibre en finos, medianos y gruesos (Tabla 3). El estado de desarrollo óptimo para su consumo suele ser el fino o mediano, antes de que las semillas se endurezcan.

Tabla 3. Clasificación de los frutos según su calibre.

Denominación	Diámetro en milímetros (mm)
Finos	Inferior a 13
Medianos	13-20
Gruesos	Superior a 20

Figura 5. Detalle de los frutos de *Capparis spinosa* L.

Semillas

Son de color marrón oscuro cuando llegan a la madurez. El número de semillas por fruto puede variar entre 400 a 500 (Legua et al., 2013). Son reniformes y miden en torno a 2 – 3 mm de longitud en su dimensión máxima. Presentan una capa lignificada de 4 a 10 células de espesor, con un endotegumento lignificado compuesto por células cúbicas y paredes radiales fuertemente engrosadas, por estos motivos, la testa es muy dura y difícilmente permeable al agua. Estas son unas de las causas del bajo porcentaje de germinación (Domínguez, 2013).

Importancia económica

Producción mundial

Según la base de datos de las Naciones Unidas (UN-COMTRADE), la producción anual para el periodo 1994-2003 se concentraba en Marruecos, Turquía, España, Siria e Irán, los cuales producían más del 70% de la oferta mundial. En la actualidad estos datos han variado significativamente, disminuyendo la producción de alcaparras a nivel mundial de forma significativa.

En el periodo comprendido entre 2006 – 2014 los principales países exportadores han sido Marruecos, Siria, Kirguistán, Turquía y España, en la Figura 6 se representa el porcentaje de intervención de cada uno.

Las exportaciones de alcaparra tenían una tendencia creciente hasta 2007, después sufrieron una caída importante y, posteriormente, fueron aumentando hasta el 2014 donde se observó un decrecimiento total, llegando hasta datos no significativos en los años posteriores.

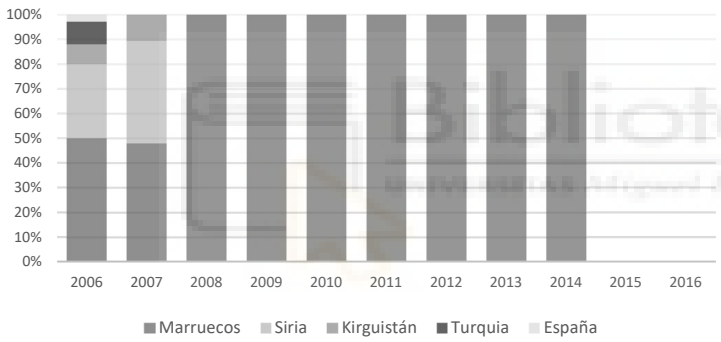


Figura 6. Principales países exportadores de alcaparras en el periodo 2006-2016.

Fuente: UN-COMTRADE

Según Pérez-Pizarro (2008) a lo largo del periodo 1994-2003 las importaciones de alcaparra presentaron una tendencia creciente llegando a una cifra superior a las 28.000 toneladas en 2003. Entre los principales importadores se encontraba Europa con 18.153 toneladas en el año 2003, equivalentes al 64% de las importaciones mundiales de alcaparras. Los principales importadores mundiales fueron España, Italia, Turquía, Venezuela y EE.UU.

Entre 2006-2016, se ha experimentado un decrecimiento muy importante en la importación de alcaparras, pasando de 19.715 toneladas en el año 2006 a la escasa cantidad de 1.415 toneladas en el año 2016. En la actualidad, los principales países importadores a nivel mundial de alcaparra son Venezuela con un 28.02% de la importación total, seguida de Italia, España, Turquía y Marruecos (Figura 7).

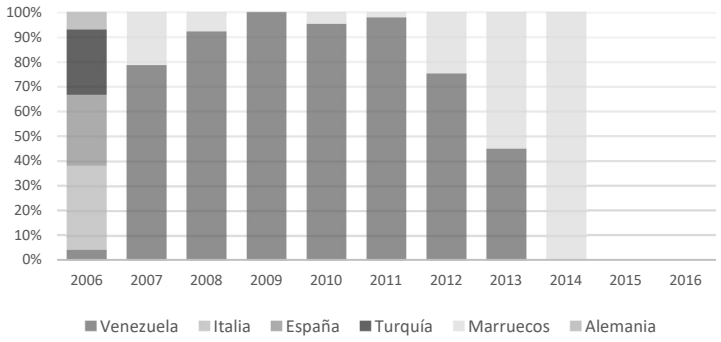


Figura 7. Principales países importadores del mundo en el periodo 2006-2016.

Fuente: UN-COMTRADE

Producción nacional

En el ámbito nacional, la superficie total de alcaparras ha experimentado un ligero descenso en los últimos 10 años. Se pasó de una superficie de plantación de 548 hectáreas en el año 2008 a 483 hectáreas en el año 2018. Sin embargo, la producción en toneladas ha sufrido un elevado incremento en relación a la superficie de plantación. En el año 2008 en España había una producción de 87 toneladas, la cual sufrió un elevado descenso pasando a 22 toneladas en el año 2011. Sin embargo, en el año siguiente (2012) hubo un notable incremento pasando a una producción de 344 toneladas, llegando al 2018 con un total de 424 toneladas (Figura 8).

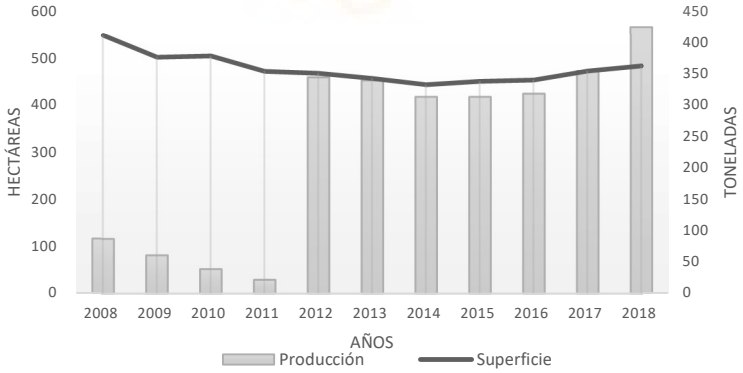


Figura 8. Superficie (Ha) y Producción (Tn) de alcaparras en el periodo 2008-2018 en España.

Fuente: MAPAMA, 2021

La Comunidad Autónoma con una mayor superficie de plantación es las Islas Baleares, con un total de 420 hectáreas, seguida de Andalucía con 51 hectáreas y, en tercer lugar, la Región de Murcia con un total de 12 hectáreas (Figura 9).

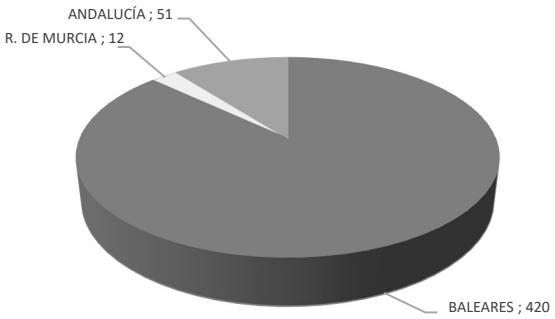


Figura 9. Superficie de plantación (ha) de alcaparra en el 2018 por Comunidades Autónomas.

Fuente: MAPAMA, 2021

A pesar de que la Comunidad Autónoma con una mayor superficie de plantación son las Islas Baleares, no es la que presenta una mayor producción, siendo Andalucía la que ha presentado en este año 2018 una mayor producción en toneladas, situándose en primer lugar con un total de 415 toneladas, seguido de la Región de Murcia con un total de 7 toneladas y, en tercer lugar, las Baleares, con un total de 2 toneladas (Figura 10). Estos datos se deben a que toda la superficie de plantación de las Baleares se encuentra en secano, mientras que en Andalucía la mayor parte es en regadío. A la vista de los datos obtenidos se puede observar el incremento de producción que se genera según si la producción es en secano o regadío.

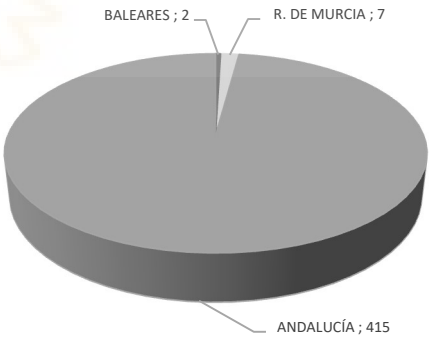


Figura 10. Producción de alcaparras (tn) en el año 2018 por Comunidades Autónomas.

Fuente: MAPAMA

Usos

Las alcaparras aportan un sabor único a muchos de los platos de la cocina mediterránea y, además de los numerosos beneficios que aportan a la salud humana, también potencian el sabor de los demás ingredientes en la cocina. Normalmente las alcaparras se suelen consumir en frío, aunque también se pueden consumir en platos calientes. El uso más común es como acompañante en algunos platos de carnes rojas o pescados blancos, en ensaladas o bien como salsas y, además sirve de condimento a las pizzas. Existen algunos platos más innovadores como es el humus de alcaparra que puede encontrarse en el libro digital '*De la ciencia al plato'*.

OBJETIVOS

2. Objetivos

El objetivo general de esta Tesis Doctoral es determinar las características agronómicas y propiedades fitoquímicas y funcionales de las diferentes partes comestibles de la tapenera: botones florales, flores, frutos y tallos jóvenes de diferentes ejemplares recolectados para poner en valor su uso, bien para la alimentación humana, animal y/o industrial. Además, se pretende realizar una comparativa de las propiedades químicas, actividad antioxidante y análisis genético mediante marcadores moleculares ISSR en dos subespecies de alcaparras, la subespecie *spinosa,* con presencia de espinas, y *rupestris* sin espinas.

Los objetivos específicos son:

Objetivo 1. Caracterización morfológica, química, bioquímica y funcional de los caparrones, mediante la determinación de polifenoles totales, proteínas, clorofilas, color y actividad antioxidante por diferentes métodos [$ABTS^+$, DPPH, FRAP, fracción hidro y liposoluble], así como el contenido en fenoles, flavonoides y flavonoles totales y la evolución de estos parámetros en los distintos estados de desarrollo (finos, medianos y gruesos) de los frutos en tres cultivares españoles (*Publicación I*).

Objetivo 2. Caracterización morfológica, química, bioquímica y funcional de los botones florales, mediante la determinación de polifenoles totales, proteínas, azúcares, clorofilas y actividad antioxidante por diferentes métodos [$ABTS^+$, DPPH, FRAP, fracción hidro y liposoluble] (*Publicación II*).

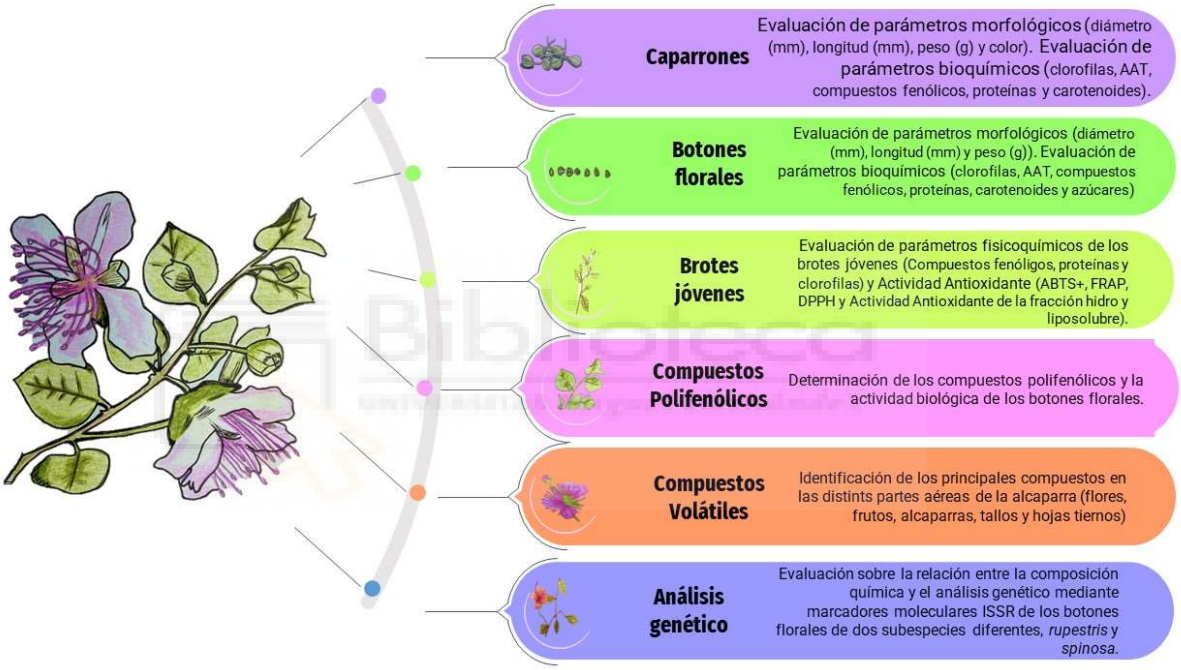
Objetivo 3. Caracterización fisicoquímica y actividad antioxidante de brotes jóvenes mediante la determinación del contenido en clorofilas, carotenoides, fenoles totales, flavonoides y flavonoles y actividad antioxidante mediante los métodos de [$ABTS^+$, $DPPH^{\cdot}$, FRAP y las fracciones hidro y liposolubles] (*Publicación III*).

Objetivo 4. Determinación de los compuestos polifenólicos y la actividad biológica de las tápenas de dos cultivares españoles. (*Publicación IV*).

Objetivo 5. Determinar el perfil de compuestos volátiles en las diferentes partes aéreas de la alcaparra (*Publicación V*).

Objetivo 6. Determinar la composición química y el contenido de actividad antioxidante y relacionarlo con el análisis genético con marcadores moleculares ISSR de botones florales de plantas de *Capparis spinosa* L. de dos subespecies, *spinosa* y *rupestris,* en distintos cultivares de España (*Publicación VI*).

Diagrama de la estructura de la Tesis Doctoral



MATERIALES Y MÉTODOS

3. Materiales y Métodos

En este apartado se incluyen las metodologías utilizadas para la determinación de los parámetros morfológicos, químicos y bioquímicos, así como la determinación de los compuestos volátiles y perfil genético determinados en las diferentes partes aéreas de la alcaparra.

I. Material vegetal

Para llevar a cabo la presente investigación se recolectaron a mano diferentes partes aéreas del cultivo de alcaparros. Se recogieron los frutos de alcaparras de tres cultivares 'Orihuela 4' ('ORI 4'), 'Alberca 2' ('ALB2') y 'Alcayna 2' ('ALC 2') (*Publicación I*), los botones florales de cuatro cultivares 'Orihuela 7' ('ORI 7'), 'Serón 3', 'Collados 5' y 'Orihuela 10' ('ORI 10') (*Publicación II y IV*), así como diferentes partes aéreas (tallos tiernos y hojas en conjunto, botones florales, frutos y flores) de los cultivares 'Orihuela 3' ('ORI 3') y 'Orihuela 7' ('ORI 7') (*Publicación V*). Todos los cultivos denominados 'ORI' se recolectaron en Orihuela (Alicante, España), 'ALB 2' en La Alberca (Murcia, España), 'ALC 2' en La Alcayna (Murcia, España), 'Serón 3' en Serón (Almería, España) y 'Collados 5' en Águilas (Murcia, España). Todos los cultivares se cultivaron en parcelas de secano y se cosecharon en junio, julio y septiembre de 2016 y 2017 (*Publicaciones I, II, IV y V*). Los tallos tiernos se recolectaron en 15 cultivares diferentes, denominados 'Orihuela 1' ('ORI 1'), 'Orihuela 2' ('ORI 2'), 'Orihuela 3' ('ORI 3'), 'Orihuela 4' ('ORI 4'), 'Orihuela 5' ('ORI 5'), 'Orihuela 6' ('ORI 6'), 'Orihuela 7' ('ORI 7'), 'Orihuela 8' ('ORI 8'), 'Orihuela 9' ('ORI 9'), 'Orihuela 10' ('ORI 10'), 'Orihuela 11' ('ORI 11'), 'Alcayna 1' ('ALC 1'), 'Alcayna 2' ('ALC 2') y 'Alberca 1' ('ALB 1'), 'Alberca 2' ('ALB 2') (*Publicación III*). Todos ellos se recolectaron entre los meses de abril y junio del 2018 en plantas adultas de vigor y tamaño similar (*Publicación III*). Para el estudio genético se recolectaron cuarenta hojas de cuatro plantas (diez por planta) de treinta y dos cultivares en cinco localidades del sureste de España, de las cuales 9 cultivares pertenecen a la subespecie *rupestris* y 22 a la subespecie *spinosa*; en Orihuela (Alicante, España) se recolectaron los cultivares denominados como 'TE1', 'TE2', 'TE3', 'TE4', 'TE5', 'TE6', 'TE7', 'TE8', 'TE9' y 'TE10' (subespecie *rupestris*). Los cultivares recolectados en Serón (Almería, España) se denominan 'TS1', 'TS2', 'TS3', 'TS4', 'TS5', 'TS6', 'TS7'y 'TS8' (subespecie *spinosa*). Los cultivares procedentes de Águilas (Región de Murcia, España) se denominan 'TA1', 'TA2', 'TA3', 'TA4', 'TA5', 'TA6' y 'TA7' (subespecie *spinosa*). Los cultivares de La Alberca (Murcia, España) se denominan 'TALB1', 'TALB2', 'TALB3', 'TALB4' y 'TALB5' (subespecie *spinosa*). Por último, los cultivares recolectados en La Alcayna (Murcia, España) se denominan 'TALCY1' y 'TALCY2' (subespecie *spinosa*) (*Publicación VI*).

Los frutos de alcaparra se clasificaron en tres estados de desarrollo: finos, con diámetros menores a 13 mm; medianos, entre 13-20 mm; y gruesos, con diámetros superiores a 20 mm (BOE, 1984). Los botones florales se clasificaron por tamaño como 'Nonpareilles' (Ø 0-7 mm), 'Surfines' (Ø 7-8 mm), 'Capucines' (Ø 8-9 mm), 'Capotas' (Ø 9-11 mm), 'Finas' (Ø 11-13 mm) y 'Gruesas' (Ø> 13 mm) (BOE, 1984) (Figura 11). Los frutos y botones florales, después de realizar las mediciones no destructivas, se almacenaron el mismo día a -80°C.

Para la recolección de las diferentes partes aéreas (Figura 12) (tallos y hojas, flores, frutos y botones florales) de cada cultivar, se recolectaron manualmente en el mismo estado de maduración y se transportaron inmediatamente al laboratorio para su posterior preparación y análisis. Para los análisis se utilizaron 30 unidades de muestreo por cultivar. Una vez en el laboratorio, las muestras se lavaron con agua corriente durante 2 min, se secaron y se congelaron con nitrógeno líquido, se molieron durante 10 s en un molinillo (Taurus Aromatic Ver II; Taurus Group, Barcelona, España), y se almacenaron a -80 °C hasta que se realizaron los análisis correspondientes.

Figura 11. Botones florales clasificados según BOE 1984.

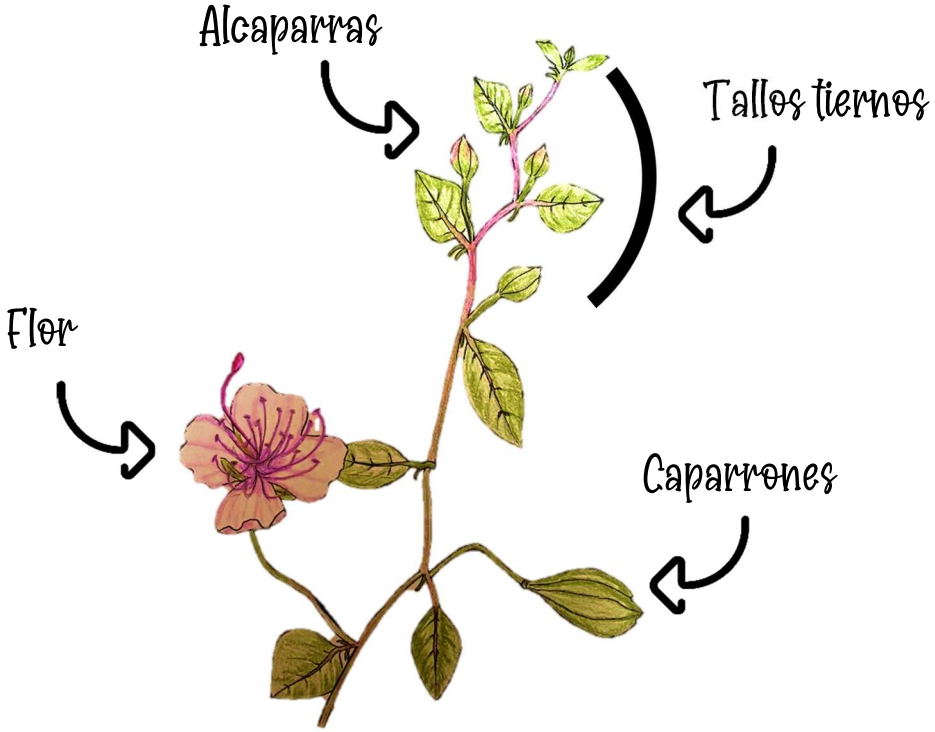


Figura 12. Esquema de las distintas partes del alcaparro.

Fuente: Elaboración propia

II. Parámetros físicos

En los alcaparros se midieron los siguientes parámetros físicos en 30 frutos por etapa y cultivo: diámetro ecuatorial (mm) y largo del fruto (mm) utilizando un calibrador digital (modelo CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK). El peso de los frutos (g) se midió con una balanza digital (modelo BL-600; Sartorius, Madrid, España). El color instrumental se midió en la superficie de los frutos en dos puntos opuestos en la zona ecuatorial. El color se evaluó según la Comisión Internacional de l'Eclairage (CIELab) y se expresó como L*, a*, b* y Chrome, con un espectrofotómetro Minolta C-300 Chroma Meter (Minolta Corp., Osaka, Japón) acoplado a un Procesador de datos Minolta DP-301.

En los botones florales se determinó el diámetro ecuatorial (mm) y la longitud (mm) de las alcaparras, las cuales se midieron con un pie de rey digital con precisión de 0,01 mm (modelo CD-15 DC; Mitutoyo (UK) Ltd, Telford, Reino Unido) y se calculó la relación longitud/diámetro. El peso de las alcaparras (g) se midió con una balanza digital con una precisión de 0,01 g (modelo BL-600; Sartorius, Madrid, España).

III. Parámetros bioquímicos y funcionales

Determinación de clorofilas, carotenoides y proteínas

Las clorofilas, carotenoides y proteínas se determinaron en frutos, tápenas y tallos jóvenes; en frutos y tápenas se midieron en peso fresco y en tallos jóvenes en peso seco.

Las clorofilas *a* y *b* se determinaron según los métodos oficiales (AOAC, 1990). Se leyó la absorbancia a 664 y 647 nm y los resultados se expresaron como mg x 100 g^{-1} peso fresco. Los carotenoides totales se extrajeron de acuerdo con Valero et al. (2011), con acetona y éter etílico para promover la separación de fases. La fase lipofílica se utilizó para estimar el contenido de carotenoides totales (Figura 13) mediante la lectura de la absorbancia a 450 nm, y los resultados se expresaron como mg de equivalentes de β-caroteno x 100 g^{-1} peso fresco, teniendo en cuenta el $\varepsilon_{cm}^{1\%}$ = 2560.

Figura 13. Determinación del contenido en clorofilas en el laboratorio.

El contenido de proteína se analizó por el método de Bradford (1976) utilizando el reactivo Bio-Rad. Para la cuantificación se utilizó una curva estándar de albúmina de suero bovino (BSA) pura según Almansa et al. (2016). Los resultados se expresaron como mg proteína x g^{-1} peso fresco.

El perfil de azúcares fue cuantificado en los botones florales de acuerdo con Hernández et al. (2016) y los resultados se expresaron en g x 100 g^{-1} de peso fresco.

Determinación de polifenoles totales, flavonoides y flavonoles

Tanto en frutos como en botones florales y brotes tiernos se cuantificaron los compuestos fenólicos totales (TFC) en fase hidrofílica según Singleton et al. (1999) con algunas modificaciones, utilizando el reactivo de Folin-Ciocalteu. Se midió la absorbancia a 765 nm, se realizó una curva de calibración con ácido gálico y los resultados en frutos y botones florales se expresaron como mg EAG x 100 g^{-1} peso fresco mientras que los brotes tiernos se expresaron en EAG x 100 g^{-1} peso seco.

En frutos se extrajeron los flavonoides totales (TFC) y flavonoles totales (TFoC) siguiendo el método de Zhuang (1992) con metanol al 80%. El análisis de los flavonoides totales se realizó

por espectrofotometría siguiendo el método de Zhuang (1992) con $NaNO_2$, 10% $AlCl_3$ y 1 M NaOH, y se midió la absorbancia a 512 nm en un espectrofotómetro Helio Gamma (modelo UVG 1002E; Helios, Cambridge, Reino Unido). Los resultados de flavonoides totales se expresaron en mg de equivalentes de rutina x 100 g^{-1} peso fresco. Para ello se realizó una línea de calibración de rutina cuya ecuación fue y = 4,479x + 0,06773 con una correlación del 99,88%. La cuantificación de flavonoles totales se realizó por espectrofotometría siguiendo el método de Kumaran et al. (2007) con $AlCl_3$ (2 mg mL^{-1}) y acetato de sodio, y se midió la absorbancia a 440 nm en un espectrofotómetro Helios Gamma (modelo UVG 1002E; Helios, Cambridge, UK). Los resultados se expresaron en mg de equivalentes de rutina × 100 g^{-1} peso fresco. Para ello se realizó una línea de calibración de rutina cuya ecuación fue y = 3,408x + 0,0297 con una correlación del 99,01%.

Para determinar el contenido total de flavonoides y flavonoles en los botones florales de alcaparra y brotes tiernos se usaron extractos metanólicos usando un método ligeramente modificado de Argentieri et al. (2012). Los extractos se prepararon con metanol al 80% y relación peso/volumen 1/50 y se agitaron durante 24 horas. Los flavonoides totales se cuantificaron mediante un método ligeramente modificado de Chang et al. (2002). La mezcla de reacción contenía 0,5 mL de extracto, 1,5 mL de etanol al 95%, 0,1 mL de $AlCl_3$, 0,1 mL de acetato de sodio y 2,8 mL de agua destilada. La reacción se mantuvo a temperatura ambiente durante 30 minutos y posteriormente se midió la absorbancia a 415 nm. Los flavonoles totales se cuantificaron utilizando el método de Kumaran et al. (2007) y la absorbancia se midió a 440 nm. El estándar de referencia fue la rutina y los resultados se expresaron en mg de equivalentes de rutina (RE) x 100 g^{-1} peso fresco.

Determinación de compuestos fenólicos

Para la separación, identificación y cuantificación de compuestos polifenólicos, se extrajeron tres réplicas de muestras individuales de alcaparras liofilizadas (máx. 0,5 g cada una) empleándose el método descrito previamente por Wojdyło et al. (2017). La identificación y cuantificación de compuestos fenólicos por LC-qTOF-MS/MS y UPLC-PDA-FL, respectivamente, se analizó como se describió previamente por Wojdyło et al. (2017). El análisis de las procianidinas poliméricas por el método del floroglucinol se realizó según el protocolo previamente descrito por Re et al. (1999). Los resultados se expresaron en miligramos por 100 g^{-1} de peso seco (ps).

Determinación de la actividad antioxidante total (AAT)

En los frutos frescos se prepararon extractos para el análisis de la actividad antioxidante hidrofílica total (AAT-H) y la actividad antioxidante lipofílica total (AAT-L) utilizando tampón Tris-acetato pH 6,0, $CaCl_2$ 20 mM y acetato de etilo para separar la solución acuosa y la fase orgánica, respectivamente (Arnao et al., 2001). Los resultados se expresaron como mg de equivalente de Trolox × 100 g^{-1} pf.

En los botones florales frescos se determinó la AAT-H y AAT-L por el método $ABTS^+$ según Cano et al. (1998). Se homogeneizó la muestra con el tampón fosfato 50 mM, pH 7,5 y 5 mL de acetato de etilo y se centrifugó a 15.000 *g* durante 20 minutos. Ambas fracciones se congelaron por separado a -80 ºC. Los resultados se expresaron como mg equivalente de Trolox x 100 g^{-1} pf.

En los brotes tiernos se prepararon los extractos para evaluar la actividad antioxidante total hidrofílica (AAT-H) y lipofílica (AAT-L). Se utilizó tampón Tris-acetato pH 6,6 y $CaCl_2$ 30 mM y acetato de etilo (1:1, v/v). La mezcla se sonicó durante 15 min a 20 ºC en un baño ultrasónico, se homogeneizó y se centrifugó a 15.000 *g* durante 20 min. Todos los resultados se expresaron como mg equivalentes de Trolox x 100 g^{-1} pf.

Los extractos metanólicos de las alcaparras fueron evaluados individualmente por sus posibles capacidades antioxidantes a través de cuatro pruebas complementarias. Brevemente, se mezcló 0,1 g de muestra con 10 mL de MeOH/agua (80:20, v/v) que contenía HCl al 1%, se sonicó a 20 °C durante 15 minutos y se dejó durante 24 horas a 4 °C. Luego el extracto se sonicó nuevamente durante 15 minutos y se centrifugó a 15.000 *g* durante 10 minutos.

La determinación de la actividad antioxidante en las distintas partes aéreas evaluadas se realizó mediante los métodos $ABTS^+$, DPPH·, FRAP y ORAC. Las capacidades de captación de radicales libres se determinaron por tres métodos. El método $ABTS^+$ (2,2'-azino-bis (ácido 3-etilbenzotiazolina-6-sulfónico) se llevó a cabo según el método Re et al. (1999). Se realizaron extractos de partes de alcaparras que reaccionaron con la solución $ABTS^+$ y se midió la disminución de la absorbancia a 734 nm. Para la eliminación de radicales libres DPPH· (2,2-difenil-1-picrilhidrazil) según el método de Brand-Williams et al. (1995) la absorbancia de la mezcla se midió espectrofotométricamente a 515 nm. Se realizó una prueba de FRAP (poder antioxidante reductor de hierro) según el método descrito por Benzie y Strain (1996). El aumento de absorbancia se midió con un espectrofotómetro a 593 nm de las curvas de calibración. Se prepararon, en el rango de 0,5 a 5,0 mM Trolox x L^{-1} para los tres métodos y,

mostraron buena linealidad (R2 = 0,998). Se realizaron análisis de capacidad antioxidante por triplicado, y los resultados se expresaron como mM Trolox pf en frutos, mg Trolox x 100 g^{-1} pf en los botones florales, y en los brotes tiernos y botones florales para el análisis genético se expresaron en mg de Trolox x 100 g^{-1} ps.

El análisis de la capacidad de absorción de radicales de oxígeno (ORAC) consiste en la medición espectrofluorométrica de la disminución de la fluorescencia provocada por la oxidación de una sustancia fluorescente bajo la influencia de los radicales libres, según Ou et al., 2002. Las extracciones se incubaron a 37 ºC. Durante el análisis se realizó una medición espectrofluorométrica cada 5 min a una longitud de onda de 493 nm y una longitud de emisión de 515 nm. Los resultados se expresaron en mM Trolox x 100 g^{-1} ps.

IV. Parámetros volátiles

Extracción de fracciones volátiles

La extracción de la fracción volátil se realizó mediante HS-SPME, y el análisis se realizó siguiendo el protocolo desarrollado por Cano-Lamadrid et al. (2018). Brevemente, se pesaron 5 g de la muestra triturada, se colocaron en un vial de 50 mL y se mezclaron con 15 mL de agua ultrapura, 1,5 g de NaCl y 15 µg de patrón interno (β-ionona 15 mg kg^{-1}). El vial se colocó en un baño a 36 ºC (para simular la temperatura de la boca y establecer las condiciones adecuadas para estudiar el olfato ortonasal), y después del equilibrio (15 min), se aplicó una mezcla de 50/30 µm de divinilbenceno/carboxino/polidimetilsiloxano (DV B / CAR / PDMS). La fibra de 2 cm se expuso al espacio de cabeza durante 30 min. Posteriormente, los compuestos volátiles se desorbieron del revestimiento de fibra en el puerto de inyección de GC-MS durante 3 min a 230 °C (Figura 14). Los experimentos de extracción se realizaron por triplicado.

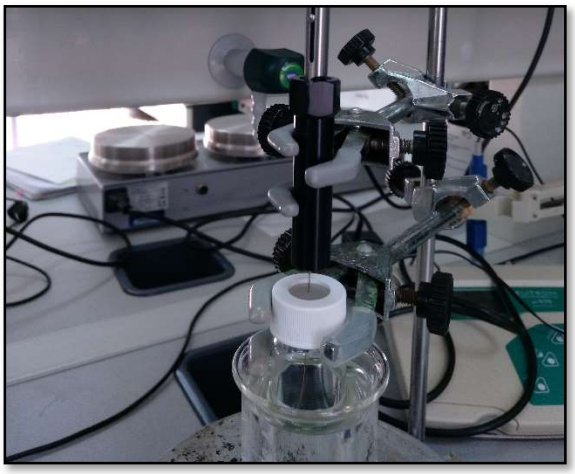


Figura 14. Extracción de la fracción volátil.

Análisis cromatográfico

La separación e identificación de compuestos volátiles se realizó utilizando un cromatógrafo de gases Shimadzu GC-17A acoplado con un detector de espectrómetro de masas Shimadzu QP-5050A (Shimadzu Corporation, Kyoto, Japón) (Figura 15). Los análisis se realizaron utilizando He como gas portador a un caudal de columna de 0,6 mL x min^{-1} y un caudal total de 13 mL x min^{-1} en una relación de split de 2:1. Las temperaturas del inyector y del detector fueron de 230 ºC y 300 ºC, respectivamente. Las muestras se analizaron utilizando el modo de escaneo en el rango de 40 a 350 m/z. La identificación se realizó utilizando 3 métodos: (i) tasas de retención para cada compuesto, (ii) tiempos de retención de GC-MS (estándar auténtico) y (iii) espectros de masas (biblioteca Wiley 09 MS (Wiley, Nueva York, NY, EE. UU.) y NIST14 (Gaithersburg, MD, EE. UU.)).

Figura 15. Análisis cromatográfico de los compuestos volátiles.

Finalmente, la cuantificación en mg x kg^{-1} peso fresco (pf) de los compuestos volátiles se realizó en un cromatógrafo de gases, Shimadzu 2010, con detector de ionización de llama, FID. Las condiciones cromatográficas y de columna fueron las reportadas previamente para el análisis de GC-MS. La temperatura del inyector fue de 200 ºC, y se utilizó N_2 como gas portador (1 mL x min^{-1}). Se usó β-ionona como estándar interno y las áreas de todos los compuestos se normalizaron usando su área; este compuesto fue elegido tras comprobar que no estaba presente en los perfiles de volátiles de las muestras en estudio. Este análisis se realizó por triplicado.

V. Parámetros biológicos

El efecto inhibidor de la α-amilasa y la α-glucosidasa de los extractos de alcaparras se evaluó según el procedimiento descrito previamente por Wojdyło et al. (2016;2017). La absorbancia se

midió a 600 nm y 405 nm, respectivamente. Las muestras de referencia contenían tampón en lugar de enzima. Los resultados de α-amilasa y α-glucosidasa se expresaron como IC_{50} (mg/mL).

La inhibición de la colinesterasa se midió con los métodos de acetilcolinesterasa (AChE) y butirilcolinesterasa (BuChE) descritos por Wojdyło et al. (2017;2018). La absorbancia se midió después de 15 minutos a una longitud de onda de 412 nm. Los resultados se expresaron como porcentaje de inhibición. Todas las determinaciones de actividad biológica se analizaron por triplicado utilizando el espectrofotómetro PC UV-2401 (Shimadzu, Kyoto, Japón).

VI. Estudio genético

Extracción de ADN

El ADN genómico se extrajo de hojas jóvenes, siguiendo el método CTAB con ligeras modificaciones (Doyle y Doyle, 1990).

Optimización de PCR y selección de ISSR

Usamos 6 marcadores del juego de cebadores UBC #9 del Laboratorio de Biotecnología de la Universidad de British Columbia (Vancouver, Canadá), y 12 marcadores del trabajo de Al-Safadi et al. (2014). La amplificación con cada cebador arbitrario se repitió dos veces y solo se seleccionaron para la generación de datos aquellos cebadores que produjeron bandas consistentes y reproducibles.

Amplificaciones por PCR

Las reacciones se llevaron a cabo en un volumen de 25 L que contenía 30 ng de ADN molde, 0,5 U de TaqDNA polimerasa, dNTP 10 mM, cebador 10 µM en 1 x tampón de reacción que contenía Tris-HCl 10 mM (pH 8,3), KCl 50 mM y $MgCl_2$ 2,5 mM.

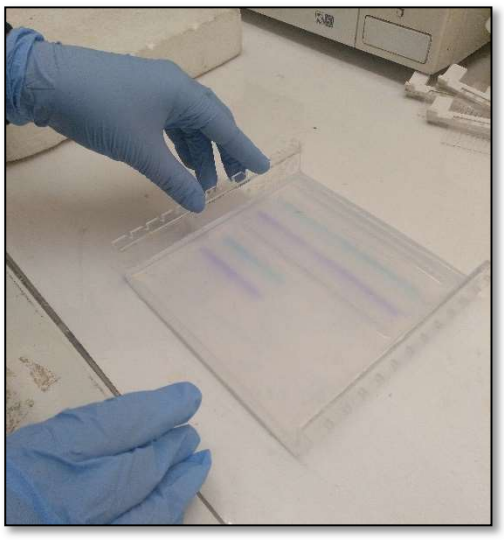


Figura 16. Electroforesis en gel de agarosa.

Condiciones de electroforesis

Los productos amplificados se cargaron en gel de agarosa al 1,5 % y se separaron en tampón TAE 19 a 100 V (Figura 16). Los geles se visualizaron bajo luz UV después de teñirlos con bromuro de etidio y se documentaron utilizando un sistema de análisis de imagen y documentación de gel (Vilber Lourmat, College-gien, Francia).

VII. Análisis de datos

Los análisis estadísticos se realizaron utilizando el paquete de software SPSS 18.0 para Windows (SPSS Science, Chicago, IL, EE. UU.). Un análisis estadístico descriptivo básico fue seguido por una prueba de análisis de varianza (ANOVA) para comparaciones de medias. El método utilizado para discriminar entre las medias (prueba de rangos múltiples) fue el procedimiento LSD de Fisher (diferencia mínima significativa) con un nivel de confianza del 95,0%. Las correlaciones se obtuvieron usando el coeficiente de correlación de Pearson (Publicación I y II). Además, para estudiar la relación estadística entre cultivares se realizó un análisis de componentes principales (ACP) mediante el coeficiente de correlación de Pearson y se realizó mediante el software XLSTAT Premium 2016 (Addinsoft, Nueva York, EE.UU.) (Publicación VI).

Se obtuvieron valores medios ± desviación estándar para compuestos polifenólicos en muestras de alcaparras. Los valores medios se sometieron a análisis de varianza y prueba de rango múltiple de Duncan para comparación de medias (p = 0,05) y correlación de Pearson utilizando Statistica versión 13.0 (Stat-Soft, Cracovia, Polonia) (Publicación IV).

Se realizaron análisis de varianza de una vía (ANOVA) y pruebas de rango múltiple de Tukey para comparar datos experimentales y determinar diferencias significativas entre cultivares ($p < 0,05$). También se llevó a cabo un análisis de componentes principales (ACP) utilizando la correlación de Pearson. Se utilizó el software XLSTAT Premium 2016 (Addinsoft, Nueva York, EE. UU.) y Statgraphics Plus (Versión 3.1, Statistical Graphics Corp., Rockville, MA, EE. UU.) (Publicación V).

Los patrones de bandas se puntuaron como presentes (1) o ausentes (0). Sólo se consideraron los fragmentos claros y repetibles en el análisis genético. La determinación del tamaño de la banda se llevó a cabo utilizando el marcador de peso molecular GeneRuler100 bp Plus DNA Ladder (ThermoFisher Scientific, Waltham, EE. UU.). Se calcularon tres índices: MR (Relación Multiplex), PIC y RP (Poder de Resolución). Las relaciones filogenéticas entre accesiones se estimaron a partir de los datos de caracterización molecular, utilizando el paquete NTSYSpc 2.0 (Adams et al., 1998). El dendrograma se construyó utilizando el método de grupo de pares no

ponderados con análisis de agrupamiento de promedio aritmético (UPGMA) basado en las matrices de coeficiente de similitud genética (Nei y Li, 1979) (*Publicación VI*).

PUBLICACIONES

4. Publicaciones

4.1. Publicación I

PUBLICACIÓN I
Physicochemical composition and antioxidant activity of three Spanish caper (*Capparis spinosa* L.) fruit cultivars in three stages of development
M. Grimalt[1], F. Hernández[2], P. Legua[2], M. S. Almansa[1] and A. Amorós[1]* Scientia Horticulturae 2018, 240, 509-515 Doi: https://doi.org/10.1016/j.scienta.2018.06.061

PUBLICACIÓN I: TRANSCRIPCIÓN LITERAL

Physicochemical composition and antioxidant activity of three Spanish caper (*Capparis spinosa* L.) fruit cultivars in three stages of development

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Abstract

Few studies have been performed on changes to physicochemical and functional properties during caper fruit development. In this work, a comparative study on the evolution of physical, chemical, and nutritive parameters and bioactive compounds of three Spanish caper cultivars was performed for the first time. These fruits were characterized by presenting the exocarp green in all stages of development, with very slight changes, which were not produced by an increase in the synthesis of carotenoids, but by partial degradation of chlorophylls a and b. There was a decrease in the protein content of the caper fruits. The H-TAA was approximately twice as much as the L-TAA. The amounts of total phenols (TPC), total flavonoids (TFC), and total flavonol compounds (TFoC) were very high in all stages of fruit development, as approximately 60% of the total phenols (TPC) were total flavonoids (TFC) and 60% of the TFC were TFoC. Given that the caper is a common perennial xerophytic shrub with a remarkable adaptability to harsh environments, and given the excellent properties of these fruits, it could be considered a species of great interest for its cultivation due to its resilience against climate change.

Keywords: ABTS; DPPH; Flavonoids; Flavonols; Phenols

1. Introduction

The weather is changing due to climate change and global warming. There is already evidence of this process in agriculture, and higher temperatures and more drought mean that agricultural production is being lost (Ray et al., 2015). High temperatures can cause damage to plant tissues as well as their metabolism and physiology, which can cause a decrease in the growth of plants (Ohama et al., 2017), and if the increase in temperature is excessive, it can lead to the death of plants and crop losses (Yamori et al., 2014). Given this situation, xerophilous plants can have great value because they can adapt to global warming. The caper (*Capparis spinosa* L.) is a common perennial xerophytic shrub with a remarkable adaptability to harsh environments (Chedraoui et al., 2017). This species is a plant that grows in dry and arid environments and it is found in Mediterranean regions in both cultivated and non-cultivated wildness. *C. spinosa* can change its leaf, stem, and root structures when adapting to drought conditions (Gan et al., 2013). The leaf, stem, and root of *C. spinosa* under drought conditions were better developed than those under normal conditions (Gan et al., 2013). These characteristics make the caper be considered as a colonizing plant, which in fact, can be found not only cultivated, but also wild among abandoned terraces and growing between rocks. Its distribution stretches from the Atlantic coast of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, in the Mediterranean basin and eastward to the Caspian Sea, and into Iran. It grows in North Africa, Europe, West Asia, Afghanistan, and Australia (Inocencio et al., 2006; Fici, 2014). This plant species is of great interest for its medicinal/pharmacological properties and its culinary uses. Its phytochemical importance relies on many bioactive components present in different organs and its cultivation can be of considerable economic value (Chedraoui et al., 2017). Its curative and medicinal properties have been known since ancient times and are linked to the presence of bioactive compounds of an antioxidant nature (flavonoids, flavonols), sugars, alkaloids, vitamins, etc. (Tlili et al., 2011). The plant has been used

traditionally to prevent and/or treat a number of health disorders such as diabetes, hepatitis, obesity, and kidney problems (Anwar et al., 2016). Its anticarcinogenic potential (Kulisic-Bilusic et al., 2012), anti-arthritic, anti-inflammatory (Talat et al., 2015), and antibacterial effects (Nabavi et al., 2016) have also been proven. In addition, different parts of capers, like the fruits and caper buds, are usually pickled and added to salads, sauces, and jams (Anwar et al., 2016). Therefore, the caper is not only able to reduce erosion and slow down the desertification process, but can also do so productively, as a cost-effective alternative to other species.

This species has been cultivated since ancient times; however, in recent years its production has decreased significantly worldwide, with a decline in caper exports to testimonial values as of 2015 (UN Comtrade, 2017). In Spain, the production of capers buds decreased from 765 tons in 1999 to a production of 61 tons in 2009 (MAPAMA, 2017), mainly due to the cost of labor.

Therefore, the great potential of *C. spinosa* must be valued and its cultivation increased again, especially in areas such as the Mediterranean basin where soils are increasingly more arid, with unique crops of this species or together with other fruit species (Chedraoui et al., 2017), for which it is necessary to thoroughly understand this species. Many investigations have been conducted with caper buds, and several reviews have been carried out on the beneficial properties of this species for health; however, much less attention has been paid to the study of fruits that can be called caper fruits (Arrar et al., 2013), caper berries (Allaith, 2016; Jiménez-López et al., 2018), or caberberry (Legua et al., 2013). Therefore, the objective of this work was to study the physicochemical composition and antioxidant activity of caper fruits. As far as can be ascertained, no comprehensive studies on the development of caper fruits have been done. This is the first paper that studies the evolution of physicochemical parameters, antioxidant activity, and total phenols during three stages of caper fruit development.

2. Materials and methods

2.1. ***Plant material***

Caper fruits of three cultivars, 'Orihuela 4' ('ORI 4'), 'Alberca 2' ('ALB 2'), and 'Alcayna 2' ('ALC 2') were hand-collected during two consecutive seasons, 2016 and 2017. 'ORI 4' was collected in Orihuela (Alicante, Spain), 'ALB 2' in La Alberca (Murcia, Spain) and 'ALC 2' in La Alcayna (Murcia, Spain). All cultivars were grown in rainfed plots and harvested in June, July, and September 2016 and 2017. The caper fruits were classified into three stages of development (**Figure 1**): *finos*, with diameters less than 13 mm (thin); *medianos*, between 13 – 20 mm (medium); and *gruesos*, with diameters larger than 20 mm (thick) (BOE, 1984). These three stages of fruit development correspond to phenological stages 73, 75, and 79, respectively (Legua et al., 2013). One hundred fruits were harvested per stage and cultivar, and after performing non-destructive measures, they were stored the same day at -80ºC.

Fig. 1. Different sizes of caper fruits 'ALB 2' cultivar.

2.2. ***Physical parameters***

The following physical parameters were measured in 30 fruits per stage and cultivar: equatorial diameter (mm) and fruit length (mm) using a digital caliper (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK); fruit weight (g) was measured using a digital balance (model BL-600; Sartorius, Madrid, Spain). Instrumental color was on the surface of the caper fruits at two opposite points

of the equatorial zone. Color was assessed according to the *Commission Internationale de l'Eclairage* (CIE*Lab*) and expressed as *L**, *a**, *b**, and Chrome, with a Minolta C-300 Chroma Meter spectrophotometer (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor.

2.3. ***Biochemical parameters***

The following parameters were measured in triplicate, with 70 fruits destined to the analysis of biochemical parameters: (i) Chlorophylls a and b were extracted from each sample using 85% acetone (AOAC, 1990). Absorbance was read at 664 and 647 nm, using a Helios Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). Results were expressed as mg x 100 g^{-1} fresh weight (fw); (ii) Total carotenoids were extracted according to Valero et al. (2011) with acetone and diethyl ether to promote phase separation. The lipophilic phase was used to estimate the total carotenoid content by reading the absorbance at 450 nm, and the results were expressed as mg of carotenoids x 100 g^{-1} fw, taking into account $\varepsilon\ ^{1\%}_{cm}$ = 2560; (iii). The protein content was analyzed by the Bradford (1976) method, using the Bio-Rad reactive and quantified according to Almansa et al. (2016). Results were expressed as mg x g^{-1} fw.

Extracts of caper fruits for the analysis of hydrophilic-total antioxidant activity (H-TAA) and lipophilic-total antioxidant activity (L-TAA) were prepared using Tris-acetate buffer pH 6.0, 20 mM $CaCl_2$, and ethyl acetate to separate the aqueous and organic phases, respectively (Arnao et al., 2001). The results were expressed as mg Trolox equivalent x 100 g^{-1} fw.

For the antioxidant activity determination by the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power) methods, a methanol extract was prepared as described by Wojdyło et al. (2013). The free radical scavenging capacities were determined by three methods, ABTS (Re et al., 1999), $DPPH^{\bullet}$ radical (Brand-Williams et al., 1995), and FRAP (Benzie and Strain,

1996). Calibration curves, in the range of 0.5–5.0 mmol Trolox x L^{-1}, were prepared for the three methods and showed good linearity (R^2= 0.998). Antioxidant capacity analyses were run in triplicate, and the results were expressed as mM Trolox fw.

Total phenolic compounds (TFC) were quantified in the hydrophilic phase according to Singleton et al. (1999) using the Folin–Ciocalteu reagent. A calibration curve was performed with gallic acid and the results were expressed as mg GAE x 100 g^{-1} fw.

Total flavonoid (TFC) and total flavonol (TFoC) compounds were extracted following the Zhuang method (1992) with 80% methanol. The analysis of total flavonoids was performed by spectrophotometry following the Zhuang method (1992) with $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH, and absorbance at 512 nm was measured on a Helios Gamma spectrophotometer (model UVG 1002E; Helios, Cambridge, UK). The results of total flavonoids were expressed in mg rutin equivalents x 100 g^{-1} fw. For this, a rutin calibration line was performed whose equation was y = 4.479x + 0.06773 with a correlation of 99.88%. Quantification of total flavonols was performed by spectrophotometry following the method of Kumaran et al. (2007) with $AlCl_3$ (2 mg mL^{-1}) and sodium acetate, and the absorbance at 440 nm was measured on a Helios Gamma spectrophotometer (model UVG 1002E; Helios, Cambridge, UK). Results of total flavonoids were expressed in mg rutin equivalents x 100 g^{-1} fw. For this, a rutin calibration line was performed whose equation was y = 3.408x + 0.0297 with a correlation of 99.01%.

2.4. ***Statistical analysis***

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

3. Results and discussion

3.1. Dimensions of caper fruit

Data showed that there was a significant difference in size among the three stages of development in each cultivar (**Table 1**). The caper fruit weight of cultivar 'ORI 4' varied between 2.3 and 12.4 g, those of 'ALB 2' varied between 0.9 and 8.0 g, and those of 'ALC 2' varied between 1.4 and 10.2 g. This was expected since the three stages of development were classified by their diameter (BOE, 1984). However, it was observed that the caper fruits of 'ORI 4' were significantly larger than both 'ALB 2' and 'ALC 2', which were significantly light, in the three stages of development. These differences in the weight led to differences in the diameter and length, with cultivar 'ORI 4' (heavier fruit) having the longest length among than the cultivars. However, the diameter of 'ORI 4' was only greater than the other two cultivars in the thin stage, but not the medium or thick stages. This was due to the shape of the caper fruits of these three cultivars, since 'ORI 4' in its fully developed stage was elongated (50.4 mm), while 'ALB 2' and 'ALC 2' were almost rounded, only slightly elongated. This was visualized in the length/diameter ratio, which reached values higher than 2 in the caper fruits of 'ORI 4' in the thick stage (2.4), while it was significantly lower in the other cultivars with a value of 1.7 in 'ALB 2' and 'ALC 2' cultivars.

The fruit weight and dimensions might be influenced by several factors, such as cultivar genotype, weather conditions, ecology, and crop load (Gao et al., 2012).

3.2. Biochemical properties of caper fruit

Caper fruits were characterized by having an external exocarp green color in all stages of development, even its maturity and natural fruits opening. Only the mesocarp and endocarp can change color, from white to reddish or yellowish, when the fruit ripens. In this paper, we have analyzed the color of the fruit exocarp, so the caper fruits were green, with slight intensities of this color in the three stages of development studied (**Table 2**). Parameter a* quantifies the green color with negative values and parameter a* was negative in all stages in the three

cultivars, between -14.0 to -8.4. The L* parameter did not show significant differences between the three stages of development studied in either in 'ORI 4' or 'ALB 2' (between 45.7 to 51.7), whereas 'ALC 2' showed greater luminosity when the stage of development increased (between 46.1 to 56.9). This increase in brightness was due to a more yellowish color when increasing development, as it was verified since parameter b*, which quantifies the yellow color, increased in 'ALC 2' (between 14.8 to 22.1) but not in 'ORI 4' or 'ALB 2' (between 18.4 to 30.7). Thus, the color intensity (Chrome index) increased in 'ALC 2' by increasing the stage of development (between 18.6 and 26.2) and 'ORI 4' (between 20.4 and 24.3), but not 'ALB 2'. The fruits modify their coloration due to synthesis and degradation of pigments throughout their development and maturation (Pék et al., 2010). In this case, the slight color changes were not produced by an increase in the synthesis of carotenoids (**Table 3**), since the carotenoid content was maintained in the caper fruits (between 1.1 to 1.7 mg carotenoids x 100^{-1} g fw), while there was a degradation of chlorophylls (a, b, and totals) by increasing the development of the caper fruits (between 15.4 and 5.8 mg chlorophyll a x 100^{-1} g fw). There were no significant differences between cultivars in either the carotenoid content or in total chlorophylls. The carotenoid contents were low compared to those obtained by Allaith (2016) with ripened caper fruits in Bahrain (3.9 mg x 100 g^{-1} fw).

The sugar and organic acid contents were analyzed by HPLC (data not shown); however, in all cases, the contents were below the detection limit of the device. However, Sher and Alyemeni (2010) found 0.4 g x 100 g^{-1} of sugar in caper fruits. Ren et al. (2012) found 6 organic acids in caper fruits, although they did not say at what concentration.

In the three cultivars, there was a decrease in the protein content of the caper fruits, with significant differences between the initial and final stages in all cases (**Table 4**). The protein content was higher in cultivars 'ALB 2' (between 1.1 to 0.7 mg protein x g^{-1} fw) with significant differences with cultivar 'ORI 4' (between 0.7 to 0.4 mg protein x g^{-1} fw). These protein contents

were low compared to those obtained in mature caper fruits (2 g x 100 g^{-1} fw) by Sher and Alyemeni (2010).

3.3. Total antioxidant activity (TAA) of caper fruit

The total antioxidant activity was quantified in caper fruits by three methods: $ABTS^{+}$, $DPPH^{\bullet}$, and FRAP (**Table 5**). In addition, the total antioxidant activity was quantified by the $ABTS^{+}$ method in the hydrophilic (H-TAA) and lipophilic (L-TAA) fractions of the caper fruits (**Table 4**). In this table, it was observed that the H-TAA was maintained or increased parallel to the stage of development of the caper fruits, with values between 24.0 and 102.6 mg Trolox eq x 100^{-1} g fw. Cultivar 'ORI 4' maintained the H-TAA, with a final value of 53.1 mg Trolox eq x 100^{-1} g fw, but the H-TAA significantly increased in 'ALB 2' and 'ALC 2' with the stage of development of the fruit, with final values of 102.6 and 67.4 mg Trolox eq x 100^{-1} g fw, so 'ALB 2' was the cultivar that reached the highest H-TAA content, although it was only significant with respect to 'ORI 4'. L-TAA was approximately half that of H-TAA in the 'ORI 4' and 'ALC 2' cultivars in the three stages of fruit growth, with values ranging from 23.2 to 31.8 mg Trolox eq x 100^{-1} g fw. However, the L-TAA behavior was the same as H-TAA, that is, maintaining itself, as in the case of 'ORI 4' and 'ALC 2', or increasing parallel to the increase in the stage of development of the fruit ('ALB 2'). Therefore, the L-TAA was also higher in the cultivar 'ALB 2' and significantly lower in the other two cultivars, which did not show significant differences between them.

Antioxidant activity by the ABTS method (**Table 5**) was maintained ('ALB 2') or decreased ('ORI 4' and 'ALC 2') by increasing the stage of fruit development between 8.6 and 1.2 mM Trolox fw in the three cultivars. 'ALB 2' was the cultivar that had the highest antioxidant activity by the ABTS method in the most developed stage of the fruit. This antioxidant activity by the ABTS method was lower than those obtained by Allaith (2016) in mature caper fruits. Practically the same tendency was found when analyzing the antioxidant activity by the FRAP method, since antioxidant activity in 'ORI 4' and 'ALC 2' decreased significantly by increasing the stage of fruit

development, while the behavior of the cultivar 'ALB 2' was the opposite, increasing the antioxidant activity with the development of the fruit. The values of the FRAP method oscillated between 0.3 and 12.5 mM Trolox fw. Allaith (2016) also found higher values than those found in this work in caper fruits from Bahrain. However, the antioxidant activity quantified by the DPPH method showed a different trend, with significant increases in the antioxidant activity of the caper fruits parallel to the increase in the fruit development with values ranging between 5.2 and 16.1 mM Trolox fw, being maximum in the 'ALB 2' cultivar and minimum in 'ALC 2' among which there were significant differences. These values were superior to those found by Allaith (2016) in caper fruits from Bahrain. Factors such as geographical source and genotype, but mainly maturity stage at harvest, may account for the observed divergence. The variation of the antioxidant capacity between cultivars could be due to differences in antioxidant compounds and their effectiveness (Robards et al., 1999).

*3.4. **Totals phenol, flavonoids, and flavonols of caper fruit***

The cultivar factor significantly affected TPC in caper fruits (**Table 6**). 'ALB 2' was the cultivar that showed a significantly higher content than the other two cultivars studied. This cultivar showed a significant increase in TPC parallel to the development of the fruit, while the other two cultivars showed the same TPC content in the three stages of the fruit development studied. The TPC values ranged between 61.5 and 119.2 mg GAE x 100 g^{-1} fw. This maximum value was equal to that obtained for mature caper fruit from Bahrain by Allaith (2016) and from Spain by Jiménez-López et al. (2018), and higher than those found by Arrar et al. (2013). This study showed higher TPC contents in caper fruits as compared to other fruits, such as avocado (21.86 mg GAE x 100 g^{-1} fw), mango (3.037 mg GAE x 100 g^{-1} fw), and apple (58.12 GAE x 100 g^{-1} fw) (Fu et al., 2011). It is known that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested in the form of a diet rich in fruits and vegetables (Weisburger, 1992). Therefore, our results have demonstrated that caper fruits could be

considered a good source of phenolic compounds and it would be recommended to consume caper fruits to ensure a maximum intake of phenolic compounds.

Arrar et al. (2013) found that 71.2% of the TPC in caper fruits were TFC, while Allaith (2016) found that it was 33%. We have also found that most TPC were TFC, about 60%, according to stage and cultivar (**Table 6**). The TFC values ranged between 39.5 and 81.6 mg eq rutin x 100 g^{-1} fw. The TFC content practically did not vary between stages of development or cultivars. Similar values were found by Jiménez-López et al. (2018) in ripened caper berries. The TFC content in caper fruits was very high and many phenolic compounds were found in this fruit, such as kaempferol-3 rutinoside, quercetin-3-O rutinoside (rutin), sakuranetin, isoginkgetin, ginkgetin (Musallam et al., 2012; Zhou et al., 2011), epicatechin, and myricetin (Francesca et al., 2016), among others. The flavonoids found in caper fruits were their main pharmacologically active compounds, and were linked with antioxidant and anticarcinogenic effects (Anwar et al., 2016). In addition, caper fruits have traditional medicinal applications, including diuretic, expectorant, and astringent activities and treatment of tuberculosis, atherosclerosis, hepatitis, and kidney diseases. The fruit also acts as a tonic, and is used to expel worms from the intestine and gas from the stomach (Anwar et al., 2016).

Arrar et al. (2013) found that 65% of the TFC in caper fruits are TFoC. We have also found that most TFCs are TFoC, with percentages of about 60%, according to stage and cultivar (**Table 6**). The TFoC values ranged between 22.2 and 39.9 mg eq rutin x 100 g^{-1} fw. The TFoC content practically did not vary between stages or cultivars. Most TFoC in caper fruits were rutin, quercetin, and myricetin (Francesca et al., 2016) and were responsible, among other compounds, for the healthy effects of the caper fruits (Anwar et al., 2016).

4. Conclusion

Here, for the first time, we have presented a study including the changes that occur during the development of caper fruits. The study was carried out in three Spanish caper cultivars from three different geographical zones. The present data indicated that these fruits were characterized by presenting the exocarp green in all stages of development, with very slight changes that were not produced by an increase in the synthesis of carotenoids, but rather by partial degradation of chlorophylls a and b. We have also demonstrated that caper fruits have good antioxidant potential capacity. This effect may be attributed to the contents of total phenols, flavonoids, and flavanols. These effects could justify the wide use of this plant as food and in traditional medicine, and as a plant to obtain bioactive compounds for the food industry, while it can be planted in increasingly dry places such as the Mediterranean basin given the threat of global warming. The cultivar 'ALB 2' was the best of the three studied, since it was the one with the highest TAA content and total phenols and flavonoids. The thick stage of development is better to be eaten since it presented a higher content of bioactive compounds.

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Table 1. Morphology of caper fruits as affected by cultivar and stage of development.

	Weight (g)			Diameter (mm)			Length (mm)			Length /Diameter		
Stage	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**
Thin	2.3±0.2 Ab†	0.9±0.1 Aa	1.4±0.2 Aa	11.4±0.3 Ab	8.3±0.6 Aa	10.5±0.4 Ab	30.2±1.2 Ab	19.1±0.8 Aa	17.4±0.8 Aa	2.6±0.1 Ac	2.4±0.1 Cb	1.7±0.1 Aa
Medium	5.8±0.4 Bb	4.3±0.3 Ba	4.6±0.5 Bab	16.4±1.0 Ba	16.6±0.5 Ba	16.7±0.7 Ba	42.6±0.8 Bc	30.8±0.5 Bb	27.3±1.0 Ba	2.7±0.1 Ab	1.9±0.0 Ba	1.6±0.0 Aa
Thick	12.4±1.1 Cb	8.0±0.5 Ca	10.2±0.9 Cab	20.8±0.3 Ca	21.6±0.5 Ca	21.9±0.5 Ca	50.4±2.2 Cb	36.0±0.6 Ca	37.9±1.3 Ca	2.4±0.1 Ab	1.7±0.0 Aa	1.7±0.0 Aa

† Values (means ± standard error) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n=30). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 2. Colour of caper fruits as affected by cultivar and stage of development.

	L*			a*			b*			Chrome		
Stage	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**
Thin	46.7±0.6 Aa†	53.1±0.8 Ab	46.1±1.0 Aa	-9.2 ± 0.2 Bb	-10.9 ± 0.1 Aa	-11.4 ± 0.8 Ba	18.4±0.8 Ab	2.3±1.1 Ac	14.8±1.0 Aa	20.4±0.8 Ab	27.19±1.1 Ac	18.6±1.2 Aa
Medium	45.7±1.2 Aa	52.3±0.9 Ab	53.3±1.1 Bb	-8.4 ± 0.2 Cc	-10.4 ± 0.2 ABb	-13.2 ± 0.5 Aa	18.6±0.4 Aa	25.1± 0.6 Ab	19.1±1.0 Ba	20.3±1.2 Aa	27.2±0.9 Ac	23.2±1.1 Bb
Thick	47.2±1.2 Aa	51.7±0.9 Ab	56.9±1.2 Cc	-13.5 ± 0.6 Aa	-10.1 ± 0.2 Bb	-14.0 ± 0.4 Aa	20.2±1.1 Aa	24.0± 0.6 Ab	22.1±0.9 Cab	24.3±1.2 Ba	26.0±0.6 Aa	26.2±0.9 Ba

† Values (means ± standard error) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n=30). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 3. Photosynthetic pigments (mg x 100^{-1} g fw) of caper fruits as affected by cultivar and stage of development.

	Chlorophyll a			Chlorophyll b			Total chlorophyll			Carotenoids		
Stage	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**
Thin	11.0±0.6 Ba†	15.2±1.5 Aa	15.0±2.0 Ba	5.2±0.5 Ba	7.2±1.0 Ba	6.7±0.3 Ba	16.3±1.1 Ba	22.5±2.5 Aa	21.7±2.3 Ba	1.1±0.4 Aa	1.5±0.0 Ba	1.7±0.2 Aa
Medium	11.7±1.1 Ba	15.4±0.8 Aa	15.1±1.9 Ba	5.6±0.3 Ba	9.6±0.0 Aba	7.4±0.9 Bab	17.3±1.4 Ba	25.0±0.8 Aa	22.5±2.1 Ba	1.5±0.2 Aa	1.1±0.0 Aa	1.3±0.1 Aa
Thick	5.8±1.0 Aa	11.6±1.5 Ab	6.8±1.0 Aa	3.8±0.8 Aa	5.4±0.7 Aab	2.8±0.3 Aa	9.7±1.7 Aa	17.0±2.2 Ab	9.6±1.3 Aa	1.2±1.3 Aa	1.0±0.0 Aa	1.2±0.2 Aa

† Values (means ± standard error) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n=3). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 4: Protein (mg x g^{-1} fw) and TAA in both hidro and liposoluble (mg Trolox x 100 g^{-1} fw) fractions of caper fruits as affected by cultivar and stage of development.

	Protein			H-TAA			L-TAA		
Stage	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**
Thin	0.7±0.1 Ba	1.1±0.0 Cb	1.1±0.1 Bb	68.3±4.1 Ac	24.0±3.8 Aa	40.3±6.0 Ab	23.9±4.2 Aa	44.7±3.1 Ab	23.2±4.3 Aa
Medium	0.5±0.6 ABa	0.8±0.0 Ba	1.0±0.0 Bb	54.1±13.2 Ab	76.7±9.3 Bb	43.4±6.4 Aa	23.9±2.4 Aa	67.2±6.5 Bb	22.1±2.3 Aa
Thick	0.4±0.0 Aa	0.7±0.0 Ab	0.7±0.0 Ab	53.1±2.2 Aa	102.6±17.8 Bb	67.4±0.5 Bab	27.5±2.8 Aa	75.3±2.6 Bb	31.8±3.3 Aa

† Values (means ± standard error) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n=3). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 5: Antioxidant activity (mM Trolox fw) of caper fruits as affected by cultivar and stage of development.

	ABTS			DPPH			FRAP		
Stage	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**
Thin	8.6±0.5 Ba	7.6±1.1 Aa	8.1±0.5 Ba	5.2±0.2 Aa	5.5±0.4 Aa	6.0±0.1 Aa	6.7±0.9 Ba	6.1±0.8 Aa	7.4±0.2 Ba
Medium	1.1±0.1 Aa	7.6±0.1 Ac	3.3±0.6 Ab	14.9±0.6 Bab	15.5±0.9 Bb	12.5±0.9 Ba	0.3±0.0 Aa	11.8±0.2 Bc	3.0±0.4 Ab
Thick	1.2±0.0 Aa	8.5±0.6 Ab	1.9±0.2 Aa	14.5±0.4 Bab	16.1±1.3 Bb	13.3±0.2 Ba	0.3±0.9 Aa	12.5±0.7 Bc	3.0±0.4 Ab

† Values (means ± standard error) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n=3). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

Table 6: Total phenols, flavonoids and flavonols contents of caper fruits as affected by cultivar and stage of development.

	Total phenols (mg GAE x 100 g^{-1} fw)			Total flavonoids (mg eq. rutin x 100 g^{-1} fw)			Total flavonols (mg eq. rutin x 100 g^{-1} fw)		
Stage	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**	**'ORI 4'**	**'ALB 2'**	**'ALC 2'**
Thin	61.5±2.5 Aa†	74.6±3.5 Aab	86.2±9.6 Ab	44.5±3.6 Aa	51.2±3.3 Aa	39.5±0.4 Aa	31.3±2.5 Aa	39.9±0.8 Aa	39.7±4.1 Ba
Medium	67.8±3.3 Aa	101.3±3.4 Bb	77.8±5.5 Aa	52.9±1.7 Aa	81.6±5.7 Bb	41.2±4.7 Aa	30.9±3.1 Aa	36.0±3.7 Aa	28.4±3.1 Aa
Thick	66.8±4.9 Aa	119.2±0.6 Cb	73.4±7.0 Aa	53.1±7.1 Aa	66.2±10.7 ABa	47.6±9.8 Aa	30.9±4.4 Aab	36.1±2.2 Ab	22.2±1.2 Aa

† Values (means ± standard error) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n=3). Capital letters indicate differences between developmental stages and lowercase letters differences between cultivars.

4.2. Publicación II

PUBLICACIÓN II
Antioxidant Activity and Bioactive Compounds Contents in Different Stages of Flower Bud Development from Three Spanish Caper (*Capparis spinosa*) Cultivars
Mar Grimalt[1], Pilar Legua[2], Francisca Hernández[2], Asunción Amorós[1] and María Soledad Almansa[1]* The Horticulture Journal 88 (3): 410–419. 2019. Doi: 10.2503/hortj.UTD-060

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Antioxidant Activity and Bioactive Compounds Contents in Different Stages of Flower Bud Development from Three Spanish Caper (*Capparis spinosa*) Cultivars

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***Capparis spinosa* L. is cultivated in the Mediterranean basin. In Spain, flower buds, unripe fruits, and tender shoots, either pickled or brined, are used as food. The main objective of the present work was to study the development of total polyphenols, flavonoids, and flavonols contents, total antioxidant activity (TAA) and physicochemical parameters of the flower buds (capers) in six development stages, for the first time, from three cultivars harvested in Spain. Total polyphenols, flavonoids and flavonols contents were very high in all stages of caper development, and were very similar in the three cultivars. The TAA values obtained by the DPPH method, hydrophilic TAA (H-TAA) and total polyphenols, as well as protein and sugar contents, tended to decrease as capers developed and increased in size. Also, H-TAA was significantly higher than lipophilic TAA (L-TAA) in the three cultivars. The results obtained from the current report draw attention to the antioxidant potential of capers, especially those of the 'Collados 5' cultivar, and showed that the antioxidant and nutritional properties of capers were better the smaller they were. Increased consumption of capers in the diet and their cultivation should be encouraged.**

Key Words: flavonoid, flavonol, polyphenol, pigment, sugar.

Introduction

The caper bush (*Capparis spinosa* L.) is a common perennial winter-deciduous shrub largely cultivated in the Mediterranean basin. It grows in North Africa, Europe, West Asia, Afghanistan and Australia.

In recent years, interest in consuming foods with health benefits has increased (Wu et al., 2004). Plants have been valued as a rich source of medicinal and nutraceutical compounds for centuries. Among valuable flora, wild plants have gained a lot of attention as a food source and for their potential health benefits. Several members of the *Capparis* genus, especially *Capparis spinosa*, have been recognized as a beneficial food because of their high nutritional value and medicinal and pharmacological attributes linked to the presence of antioxidant bioactive compounds (polyphenols, flavonoids), sugars, alkaloids, vitamins, etc. (Anwar et al., 2016). The caper is a xerophytic Mediterranean shrub with a remarkable adaptability to harsh environments. Mediterranean countries are in a region of the world threatened by global warming and *C. spinosa* is a promising crop for arid or semi-arid regions within the climate change context (Chedraoui et al., 2017), since the caper plant is highly tolerant to drought and heat stress. Therefore, the caper plant's remarkable ability to adapt to hostile environments, along with its phytochemical importance, suggest that its cultivation could be of considerable economic value.

In Spain, the most valuable parts of *Capparis spinosa* used as food are the fresh aerial parts, especially the flower buds (capers), unripe fruits and tender shoots. These are pickled or kept in brine and used as an appetizer with olives, cheese, and nuts or as a complement to meat, salads, pasta, and other foods. Spain, Morocco, Italy, Turkey, and Greece are big exporters of caper

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buds, while the UK and the USA are big importers (Anwar et al., 2016). Few studies have reviewed *C. spinosa* focusing on the plant's nutritional qualities, food and medicinal uses, phytochemical properties, biological activities, ethnopharmacology, and crop management (Inocencio et al., 2000). The main objective of this study was to increase knowledge about capers from Spain by analysing the content of total polyphenols, flavonoids and flavonols, and the total (TAA) antioxidant capacities of the caper buds. In addition, the hydrophilic (H-TAA) and lipophilic (L-TAA) antioxidant activities, carotenoids, chlorophylls, proteins, and sugar contents of the unopened flower buds in six development stages of three cultivars ('Orihuela 7', 'Serón 3', and 'Collados 5') from the Spanish southeast were also quantified. A better understanding of Spanish cultivars is essential for the selection of high-quality caper genotypes that may be of interest for further nutraceutical studies.

Materials and Methods

Plant materials

Flower buds (capers) of *C. spinosa* from three cultivars, 'Orihuela 7', 'Serón 3', and 'Collados 5', were collected from Orihuela (Alicante) (38°5' N, 0°56' W, 23.6 m asl), Serón (Almería) (37°21' N, 2°32' W, 822 m asl), and Águilas (Murcia) (37°24' N, 1°34' W, 21 m asl), respectively. The cultivars were grown in rainfed areas in Spain and hand harvested weekly from May to June in 2016 and 2017 from adult plants. Five plants, similar in vigour and size, were selected from each cultivar.

Determination of pomological properties

According to the quality standards for foreign trade of capers and caper berries (BOE, 1984), the capers collected were classified by size in six stages of development in the laboratory: Nonpareilles (diameter less than 7 mm); Surfines (between 7–8 mm); Capucines (between 8–9 mm); Capotes (between 9–11 mm); Fines (between 11–13 mm) and Gruesas (over 13 mm) (Fig. 1). These stages of caper development correspond to phenological stage 55 (*Beginning of flower bud swelling*) according to Legua et al. (2013). Equatorial diameter (mm) and length (mm) of capers were measured using a digital caliper with 0.01 mm accuracy, and the length/diameter ratio was calculated. Caper weight (g) was measured using a digital balance with an accuracy of 0.01 g. Three samples of 50 capers were taken per stage and cultivar and after performing non-destructive measures, they were stored the same day at −80°C.

Total antioxidant activity determination

Methanolic extracts were individually assessed for their possible antioxidative capacities using three complementary tests. Briefly, 0.1 g of sample was mixed with 10 mL of MeOH/water (80:20, v/v) containing 1% HCl, sonicated at 20°C for 15 minutes and left for 24 hours at 4°C. Then, the extract was again sonicated for 15 minutes and centrifuged at 15,000 g for 10 minutes.

A DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging assay was carried out according to Brand-Williams et al. (1995). The decrease in absorbance of the mixture was measured spectrophotometrically at 515 nm. An ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay was performed according to method of Re et al. (1999). Plant

Fig. 1. Capers of 'Orihuela 7', 'Serón 3', and 'Collados 5' cultivars classified by size in six stages of development.

extracts were allowed to react with $ABTS^{\bullet+}$ solution and the decrease in absorbance was measured at 734 nm. A FRAP (ferric reducing antioxidant power) assay was conducted using the method of Benzie and Strain (1996). The increase in absorbance was measured using a spectrophotometer at 593 nm. Trolox was used as a reference standard and results were expressed as mg Trolox/100 g of fresh weight (FW).

H-TAA and L-TAA were determined by the $ABTS^{\bullet+}$ method according to Cano et al. (1998). Briefly, 0.5 g of sample was homogenized with 5 mL of phosphate buffer 50 mM, pH 7.5 and 5 mL of ethyl acetate and centrifuged to 15,000 g for 20 minutes. Both fractions were frozen separately at −80°C. Results were expressed as mg Trolox/100 g FW.

Determination of total polyphenols, flavonoids and flavonols

Total polyphenols content was analysed by Folin-Ciocalteu's phenol reagent method, using gallic acid as the standard according to Singleton et al. (1999). The absorbance was measured at 765 nm and the concentration was calculated as mg of gallic acid equivalents (GAE)/100 g FW.

Methanolic caper extracts were used to estimate the total flavonoids and flavonols using a slightly modified method of Argentieri et al. (2012). The extracts were made with 80% methanol and a weight/volume ratio of 1/50 and shaken for 24 hours. Total flavonoids were quantified by a slightly modified method of Chang et al. (2002). The reaction mixture contained 0.5 mL extract, 1.5 mL 95% ethanol, 0.1 mL $AlCl_3$, 0.1 mL sodium acetate and 2.8 mL distilled water. The reaction was maintained at room temperature for 30 minutes and the absorbance was subsequently measured at 415 nm. Total flavonols were quantified by the method of Kumaran et al. (2007) and the absorbance was measured at 440 nm. The reference standard was rutin and results were expressed as mg of rutin equivalents (RE)/100 g FW.

Determination of chlorophylls and carotenoids

Chlorophylls *a* and *b* were determined according to Official Methods (AOAC, 1990). Absorbance was read at 664 and 647 nm and the results were expressed as mg/100 g FW. Total carotenoids were extracted according to Valero et al. (2011), with acetone and diethyl ether to promote phase separation. The lipophilic phase was used to estimate the total carotenoids content by reading the absorbance at 450 nm, and the results were expressed as mg of β-carotene equivalents/100 g FW, taking into account the $\varepsilon^{cm}_{1\%} = 2560$.

Determination of proteins and sugars

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

For all assays, three extracts were prepared by stage, cultivar and year and two determinations were made for each of them (n = 12).

Statistical analyses

Statistical analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance test for mean comparisons. The method used to discriminate among the means (Multiple Range Test) was Fisher's LSD (Least Significant Difference) procedure at a 95% confidence level. Correlations were obtained by Pearson correlation coefficient.

Results

Pomological properties

The weights of the capers were 0.131–0.79 g, 0.09–0.952 g, and 0.103–0.649 g for 'Orihuela 7', 'Serón 3', and 'Collados 5' cultivars, respectively (Table 1). The caper weights showed significant differences between

Table 1. Average weight and ratio between the length and diameter in the different development stages of 'Orihuela 7', 'Serón 3', and 'Collados 5' caper cultivars.

Stage	Weight (g)			Length/Diameter Ratio		
	ORIHUELA 7	SERÓN 3	COLLADOS 5	ORIHUELA 7	SERÓN 3	COLLADOS 5
Nonpareilles	$0.131 \pm 0.006^{b}_{A}$	$0.090 \pm 0.004^{a}_{A}$	$0.103 \pm 0.005^{a}_{A}$	$1.064 \pm 0.027^{b}_{D}$	$0.948 \pm 0.023^{a}_{B}$	$0.989 \pm 0.023^{ab}_{AB}$
Surfines	$0.170 \pm 0.004^{b}_{A}$	$0.150 \pm 0.005^{a}_{AB}$	$0.150 \pm 0.004^{a}_{B}$	$1.048 \pm 0.013^{c}_{CD}$	$0.920 \pm 0.017^{a}_{AB}$	$0.993 \pm 0.011^{b}_{B}$
Capucines	$0.248 \pm 0.013^{b}_{B}$	$0.194 \pm 0.007^{a}_{B}$	$0.219 \pm 0.007^{a}_{C}$	$0.999 \pm 0.018^{b}_{BC}$	$0.905 \pm 0.013^{a}_{AB}$	$1.004 \pm 0.012^{b}_{B}$
Capotes	$0.357 \pm 0.012^{b}_{C}$	$0.328 \pm 0.011^{b}_{C}$	$0.292 \pm 0.010^{a}_{D}$	$0.991 \pm 0.011^{b}_{B}$	$0.879 \pm 0.018^{a}_{A}$	$1.003 \pm 0.009^{b}_{B}$
Fines	$0.507 \pm 0.015^{b}_{D}$	$0.465 \pm 0.011^{a}_{D}$	$0.474 \pm 0.010^{ab}_{E}$	$0.929 \pm 0.023^{a}_{A}$	$0.929 \pm 0.010^{a}_{B}$	$0.992 \pm 0.010^{b}_{B}$
Gruesas	$0.790 \pm 0.026^{b}_{E}$	$0.952 \pm 0.054^{c}_{E}$	$0.649 \pm 0.026^{a}_{F}$	$0.930 \pm 0.016^{ab}_{A}$	$0.917 \pm 0.09^{a}_{B}$	$0.956 \pm 0.013^{b}_{A}$

[z] Values (means ± SE) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) test at a 95% confidence level ($P < 0.05$) (n = 150). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.

the six stages of development, mainly in the Capucines stage; capers of 'Orihuela 7' were the heaviest, except for those in the Gruesas stage from the 'Serón 3' cultivar, which were significantly heavier.

On the other hand, 'Orihuela 7' capers had a length/diameter ratio that decreased as the capers grew (Table 1), going from being more elongated to more rounded. Capers of 'Collados 5' showed a length/diameter ratio very close to one in all development stages, and were more rounded from the beginning. Capers of 'Serón 3' had a lower ratio in all the stages.

Total antioxidant activity

The TAA of capers was evaluated using three different analytical methods: $ABTS^{\bullet+}$, DPPH and FRAP (Table 2). TAA by the $ABTS^{\bullet+}$ method ranged from 192.7–95.1 mg Trolox/100 g FW for the 'Collados 5' cultivar and showed only significant differences with 'Serón 3' and 'Orihuela 7' in the Nonpareilles stage and with 'Serón 3' in the Surfines stage. TAA tended to decrease as the capers developed in 'Orihuela 7' and 'Collados 5', but not in 'Serón 3', which increased up to the Capotes stage and then decreased. TAA content by the FRAP method ranged between 45.1–242.8 mg Trolox/100 g FW without statistical differences between the cultivars. TAA did not show significant changes between the stages of development, except between the Nonpareilles and Fines stages in the 'Serón 3' cultivar. The values obtained by the DPPH method were higher (433–1542.5 mg Trolox/100 g FW), and the only significant differences were between cultivars in the Nonpareilles and Capucines stages. TAA tended to decrease as the capers developed in the three cultivars.

In addition, the H-TAA was between 8–24, 12–38 and 32–68 times greater than the L-TAA in the different stages of development in the 'Orihuela 7', 'Serón 3', and 'Collados 5' cultivars, respectively (Fig. 2). The H-TAA was significantly higher in 'Collados 5' than in 'Serón 3' and 'Orihuela 7', but the L-TAA did not show any significant differences between cultivars in any de-

Table 2. Antioxidant activity in the different development stages of 'Orihuela 7', 'Serón 3', and 'Collados 5' caper cultivars.

Stage	$ABTS^{\bullet+}$ (mg Trolox/100 g FW)			FRAP (mg Trolox/100 g FW)			DPPH (mg Trolox/100 g FW)		
	ORIHUELA 7	SERÓN 3	COLLADOS 5	ORIHUELA 7	SERÓN 3	COLLADOS 5	ORIHUELA 7	SERÓN 3	COLLADOS 5
Nonpareilles	$132.7 \pm 19.3^{b}_{B}$	$80.1 \pm 0.5^{a}_{A}$	$192.7 \pm 0.8^{c}_{B}$	$132.7 \pm 39.5^{a}_{A}$	$45.1 \pm 17.0^{a}_{A}$	$122.6 \pm 28.3^{a}_{A}$	$805.9 \pm 11.5^{a}_{D}$	$943.6 \pm 39.3^{a}_{C}$	$1542.5 \pm 180.4^{b}_{B}$
Surfines	$102.6 \pm 5.5^{ab}_{AB}$	$82.6 \pm 0.5^{a}_{AB}$	$115.1 \pm 8.5^{b}_{A}$	$97.6 \pm 14.0^{a}_{A}$	$112.6 \pm 20.8^{a}_{AB}$	$120.1 \pm 4.2^{a}_{A}$	$653.3 \pm 60.8^{a}_{BC}$	$702.3 \pm 14.0^{a}_{B}$	$658.0 \pm 4.5^{a}_{A}$
Capucines	$107.6 \pm 12.0^{a}_{AB}$	$100.1 \pm 2.0^{a}_{BC}$	$132.7 \pm 13.0^{a}_{A}$	$75.1 \pm 29.8^{a}_{A}$	$112.6 \pm 5.5^{a}_{AB}$	$130.2 \pm 2.5^{a}_{A}$	$798.4 \pm 8.7^{b}_{CD}$	$696.8 \pm 14.5^{a}_{B}$	$771.1 \pm 6.0^{b}_{A}$
Capotes	$102.6 \pm 25.5^{a}_{AB}$	$105.1 \pm 8.8^{a}_{C}$	$132.7 \pm 17.0^{a}_{A}$	$130.2 \pm 41.0^{a}_{A}$	$160.2 \pm 17.0^{a}_{AB}$	$132.7 \pm 18.8^{a}_{A}$	$545.6 \pm 6.0^{a}_{AB}$	$675.5 \pm 40.8^{a}_{B}$	$681.5 \pm 99.1^{a}_{A}$
Fines	$92.6 \pm 15.5^{a}_{AB}$	$87.6 \pm 1.3^{a}_{ABC}$	$105.1 \pm 0.5^{a}_{A}$	$100.1 \pm 23.3^{a}_{A}$	$242.8 \pm 17.0^{a}_{B}$	$92.6 \pm 6.8^{a}_{A}$	$433.0 \pm 73.1^{a}_{A}$	$562.4 \pm 35.8^{a}_{AB}$	$668.8 \pm 43.0^{a}_{A}$
Gruesas	$70.1 \pm 3.5^{a}_{A}$	$67.6 \pm 11.0^{a}_{A}$	$95.1 \pm 22.8^{a}_{A}$	$65.1 \pm 7.5^{a}_{A}$	$82.6 \pm 8.3^{a}_{AB}$	$87.6 \pm 37.0^{a}_{A}$	$485.6 \pm 43.8^{a}_{A}$	$507.1 \pm 79.3^{a}_{A}$	$624.7 \pm 49.1^{a}_{A}$

[z] Values (means ± SE) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) test at a 95% confidence level ($P < 0.05$) (n = 12). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.

[y] Abbreviations: $ABTS^{\bullet+}$, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical; FRAP, ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

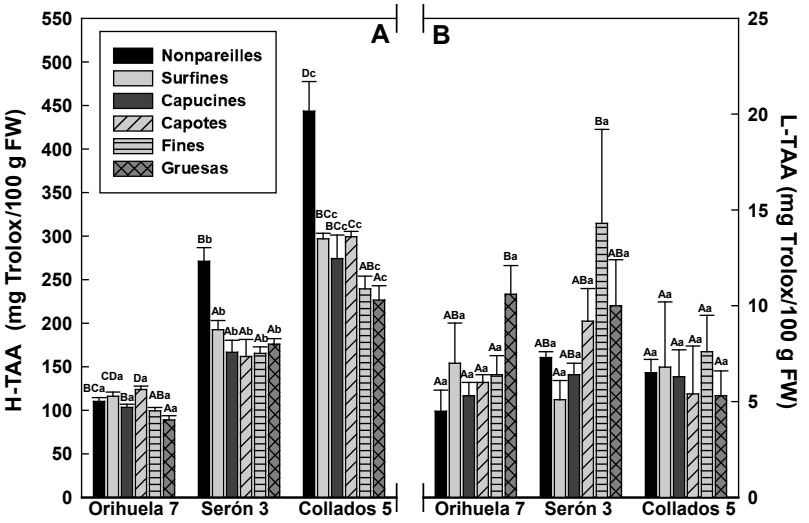

Fig. 2. H-TAA (A) and L-TAA (B) in the different development stages of 'Orihuela 7', 'Serón 3', and 'Collados 5' caper cultivars. Different capital letters indicate significant differences between stages and different lowercase letters between cultivars ($P < 0.05$) (n = 12). Error bars represent the SE of the mean. Abbreviations: H-TAA, hydrophilic total antioxidant activity; L-TAA, lipophilic total antioxidant activity.

velopment stage. H-TAA decreased slightly as the size of capers increased, while the L-TAA increased slightly, in the 'Orihuela 7' and 'Serón 3' cultivars.

Total polyphenols, flavonoids and flavonols content

The total polyphenols content ranged from 675.5–849.4 mg GAE/100 g FW (Nonpareilles) to 458.1–525.8 mg GAE/100 g FW (Gruesas), indicating the content of polyphenols decreased as the capers developed (Table 3). The content of flavonols was 68–95% of the content of flavonoids in the Nonpareilles stage and increased to values of 83–96% in capers in the Gruesas stage. On the other hand, the flavonoid and flavonol contents were significantly higher in the Nonpareilles stage of 'Collados 5' and the Capotes stage of 'Serón 3' cultivars.

Chlorophylls and carotenoids content

The carotenoids content (Table 4) ranged between 1.3–3.3 mg/100 g FW and was very similar in the three cultivars, except for the Nonpareilles and Surfines stages in 'Orihuela 7' that had significantly higher values. In addition, the level of carotenoids did not change during the development of the capers, except in the Gruesas stage of 'Orihuela 7' in which it decreased. The total chlorophylls content (Table 4) ranged between 7.3–21.2 mg/100 g FW, but it was higher in most of the stages of 'Collados 5', except for the Nonpareilles and Gruesas stages of 'Orihuela 7' and 'Serón 3', respectively. The chlorophyll a content was higher than chlorophyll b in the three cultivars (data not shown), and the chlorophyll a/chlorophyll b ratio ranged from 1–2.2 in 'Orihuela 7', 1–1.7 in 'Serón 3', and 1–2.7 in 'Collados 5' cultivars. There was no clear trend between the development stages regarding the chlorophyll content, but there were significant differences between them.

Pearson's correlation coefficients

Correlations between antioxidant activity, total polyphenols, flavonoids, flavonols and carotenoids in the three cultivars are shown in Table 5. In the 'Collados 5' cultivar, total polyphenols correlated positively with $ABTS^{\bullet+}$, FRAP, and DPPH antioxidant activities. However, flavonoids and flavonols were correlated with $ABTS^{\bullet+}$ and DPPH activities. In the 'Orihuela 7' cultivar, total polyphenols correlated with $ABTS^{\bullet+}$, FRAP, and DPPH activities and flavonoids with $ABTS^{\bullet+}$ and DPPH activities. On the other hand, in 'Serón 3', total polyphenols correlated with $ABTS^{\bullet+}$ and DPPH activities, while flavonoids only were correlated with $ABTS^{\bullet+}$

Table 3. Total polyphenols, flavonoids and flavonols contents in the different development stages of 'Orihuela 7', 'Serón 3', and 'Collados 5' caper cultivars.

Stage	TOTAL POLYPHENOLS (mg GAE/100 g FW)			FLAVONOIDS (mg RE/100 g FW)			FLAVONOLS (mg RE/100 g FW)		
	ORIHUELA 7	SERÓN 3	COLLADOS 5	ORIHUELA 7	SERÓN 3	COLLADOS 5	ORIHUELA 7	SERÓN 3	COLLADOS 5
Nonpareilles	$675.5 \pm 92.8^{a}_{B}$	$719.2 \pm 10.3^{a}_{B}$	$849.4 \pm 78.6^{a}_{A}$	$466.0 \pm 15.8^{a}_{C}$	$440.3 \pm 8.8^{a}_{A}$	$729.5 \pm 0.9^{b}_{B}$	$316.2 \pm 11.6^{a}_{AB}$	$342.4 \pm 22.3^{a}_{A}$	$691.7 \pm 6.7^{b}_{B}$
Surfines	$564.4 \pm 29.2^{a}_{AB}$	$566.5 \pm 103^{a}_{AB}$	$652.3 \pm 46.3^{a}_{A}$	$447.5 \pm 37.6^{a}_{BC}$	$557.8 \pm 18.9^{a}_{B}$	$490.6 \pm 2.2^{a}_{A}$	$400.7 \pm 17.7^{a}_{B}$	$534.8 \pm 1.6^{b}_{C}$	$439.7 \pm 3.1^{a}_{A}$
Capucines	$552.2 \pm 4.8^{a}_{AB}$	$699.1 \pm 87.0^{a}_{B}$	$811.4 \pm 81.8^{a}_{A}$	$415.8 \pm 10.1^{a}_{BC}$	$535.1 \pm 5.7^{b}_{B}$	$490.5 \pm 30.4^{ab}_{A}$	$368.8 \pm 13.0^{a}_{AB}$	$465.6 \pm 26.4^{b}_{BC}$	$430.6 \pm 5.9^{ab}_{A}$
Capotes	$555.7 \pm 16.6^{a}_{AB}$	$739.0 \pm 43.1^{a}_{B}$	$757.7 \pm 94.6^{a}_{A}$	$386.9 \pm 12.0^{a}_{ABC}$	$566.5 \pm 11.1^{c}_{B}$	$482.1 \pm 8.7^{b}_{A}$	$347.2 \pm 11.0^{a}_{AB}$	$511.3 \pm 13.8^{c}_{C}$	$431.4 \pm 14.0^{b}_{A}$
Fines	$508.5 \pm 72.8^{a}_{AB}$	$532.1 \pm 18.9^{a}_{A}$	$644.6 \pm 64.2^{a}_{A}$	$374.5 \pm 3.6^{a}_{AB}$	$526.6 \pm 14.2^{b}_{B}$	$571.3 \pm 3.2^{c}_{A}$	$334.3 \pm 6.4^{a}_{AB}$	$477.6 \pm 23.8^{b}_{BC}$	$462.1 \pm 1.6^{b}_{A}$
Gruesas	$458.1 \pm 6.1^{a}_{A}$	$476.6 \pm 33.4^{a}_{A}$	$525.8 \pm 71.2^{a}_{A}$	$334.8 \pm 40.7^{a}_{A}$	$442.0 \pm 25.5^{a}_{A}$	$478.6 \pm 59.1^{a}_{A}$	$283.4 \pm 65.6^{a}_{A}$	$424.6 \pm 12.4^{a}_{B}$	$397.5 \pm 43.3^{a}_{A}$

[z] Values (means ± SE) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) test at a 95% confidence level ($P < 0.05$) (n = 12). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.

[y] Abbreviations: GAE, gallic acid equivalents; RE, rutin equivalents.

Table 4. Carotenoids and total chlorophylls contents in the different development stages of 'Orihuela 7', 'Serón 3', and 'Collados 5' caper cultivars.

Stage	CAROTENOIDS (mg/100 g FW)			TOTAL CHLOROPHYLLS (mg/100 g FW)		
	ORIHUELA 7	SERÓN 3	COLLADOS 5	ORIHUELA 7	SERÓN 3	COLLADOS 5
Nonpareilles	$3.0 \pm 0.16^{b}_{B}$	$1.8 \pm 0.34^{a}_{A}$	$2.1 \pm 0.05^{a}_{A}$	$15.9 \pm 0.07^{c}_{F}$	$12.1 \pm 0.05^{a}_{D}$	$14.9 \pm 0.04^{b}_{B}$
Surfines	$3.3 \pm 0.44^{b}_{B}$	$1.8 \pm 0.07^{a}_{A}$	$1.3 \pm 0.02^{a}_{A}$	$13.2 \pm 0.11^{b}_{E}$	$7.3 \pm 0.19^{a}_{A}$	$14.0 \pm 0.003^{c}_{A}$
Capucines	$3.1 \pm 0.45^{a}_{B}$	$2.0 \pm 0.04^{a}_{A}$	$1.7 \pm 0.94^{a}_{A}$	$7.3 \pm 0.19^{a}_{A}$	$11.3 \pm 0.18^{b}_{C}$	$16.2 \pm 0.12^{c}_{C}$
Capotes	$2.8 \pm 0.20^{a}_{B}$	$2.3 \pm 0.25^{a}_{A}$	$2.3 \pm 0.46^{a}_{A}$	$10.9 \pm 0.09^{a}_{D}$	$10.8 \pm 0.11^{a}_{C}$	$16.2 \pm 0.12^{b}_{C}$
Fines	$2.5 \pm 0.15^{a}_{AB}$	$1.9 \pm 0.44^{a}_{A}$	$2.8 \pm 0.83^{a}_{A}$	$9.8 \pm 0.10^{b}_{C}$	$8.9 \pm 0.03^{a}_{B}$	$21.2 \pm 0.11^{c}_{D}$
Gruesas	$1.7 \pm 0.28^{a}_{A}$	$2.0 \pm 0.08^{a}_{A}$	$1.3 \pm 0.29^{a}_{A}$	$8.9 \pm 0.09^{a}_{B}$	$17.8 \pm 0.1^{c}_{E}$	$14.9 \pm 0.06^{b}_{B}$

[z] Values (means ± SE) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) test at a 95% confidence level ($P < 0.05$) (n = 12). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.

activity. Total polyphenols, flavonoids, and flavonols are important bioactive compounds that in general contribute to hydrophilic antioxidant activity (H-TAA). These compounds were positively correlated with H-TAA in the 'Collados 5' cultivar. However, flavonoids and flavonols were negatively correlated in the 'Serón 3' cultivar. Finally, only a negative correlation was found between L-TAA and carotenoids content in 'Orihuela 7'.

Protein and sugar content

The protein content was very similar in most development stages of 'Serón 3' and 'Collados 5' cultivars (Fig. 3), and in both cases was much higher than the content of the 'Orihuela 7' cultivar that had contents between 3.5 and 5 times lower. In the three cultivars, the protein content showed a tendency to decrease as the capers developed.

The total sugar content (Table 6) was much higher in

Table 5. Pearson´s correlations coefficients for assays of 'Orihuela 7' (a), 'Serón 3' (b), and 'Collados 5' (c) caper cultivars' constituents.

Assay or Constituent	Total Polyphenols (mg GAE/100 g FW)	Flavonoids (mg RE/100 g FW)	Flavonols (mg RE/100 g FW)	Carotenoids (mg/100 g FW)
ABTS (mg Trolox/100 g FW)	[a] 0.980***	[a] 0.910**	[a] 0.319	
	[b] 0.717	[b] 0.755*	[b] 0.459	
	[c] 0.865**	[c] 0.787*	[c] 0.896**	
FRAP (mg Trolox/100 g FW)	[a] 0.734*	[a] 0.520	[a] 0.109	
	[b] −0.222	[b] 0.591	[b] 0.582	
	[c] 0.816**	[c] 0.023	[c] 0.214	
DPPH (mg Trolox/100 g FW)	[a] 0.767*	[a] 0.809*	[a] 0.337	
	[b] 0.728	[b] −0.172	[b] −0.489	
	[c] 0.671	[c] 0.926***	[c] 0.979***	
H-TAA (mg Trolox/100 g FW)	[a] 0.578	[a] 0.574	[a] 0.612	
	[b] 0.299	[b] −0.607	[b] −0.756*	
	[c] 0.742*	[c] 0.813**	[c] 0.926***	
L-TAA (mg Trolox/100 g FW)				[a] −0.795*
				[b] 0.306
				[c] 0.430

[z] Asterisks indicate significant differences: * $P<0.1$, ** $P<0.05$, *** $P<0.01$, without * $P\geq0.1$.

[y] Abbreviations: ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); FRAP, ferric reducing antioxidant power; DPPH, radical 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalents; RE, rutin equivalents; H-TAA, total antioxidant activity of the hydrophilic fraction; L-TAA: total antioxidant activity of the lipophilic fraction.

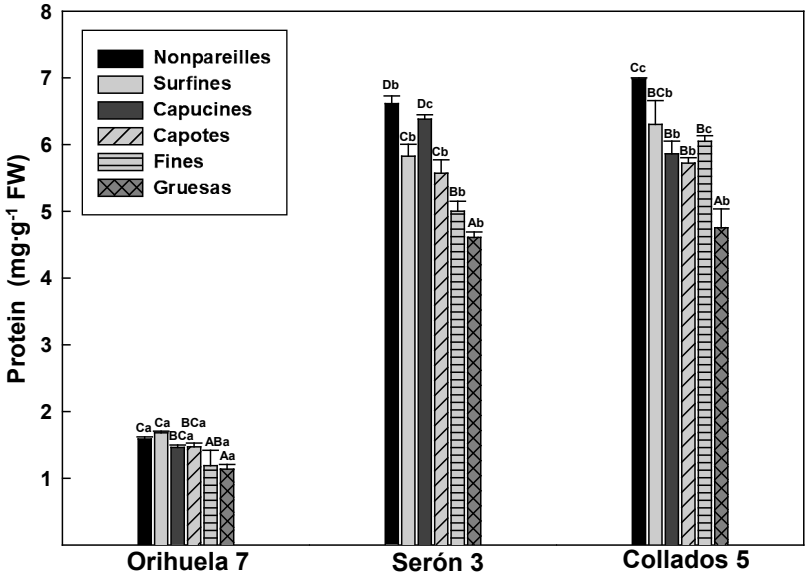

Fig. 3. Proteins in the different development stages of 'Orihuela 7', 'Serón 3', and 'Collados 5' caper cultivars. Different capital letters indicate significant differences between stages and different lowercase letters between cultivars ($P < 0.05$) (n = 12). Error bars represent the SE of the mean.

Table 6. Totals sugars, glucose, and fructose contents in the different development stages of 'Orihuela 7', 'Serón 3', and 'Collados 5' caper cultivars.

Stage	Totals sugars (g/100 g FW)			Glucose (g/100 g FW)			Fructose (g/100 g FW)		
	ORIHUELA 7	SERÓN 3	COLLADOS 5	ORIHUELA 7	SERÓN 3	COLLADOS 5	ORIHUELA 7	SERÓN 3	COLLADOS 5
Nonpareilles	$3.2\pm0.32^{a}_{AB}$	$16.1\pm0.06^{b}_{D}$	$19.0\pm0.01^{c}_{D}$	$3.0\pm0.27^{a}_{B}$	$8.3\pm0.25^{b}_{C}$	$11.8\pm0.22^{c}_{D}$	$0.3\pm0.11^{a}_{A}$	$7.8\pm0.19^{b}_{C}$	$7.2\pm0.21^{b}_{D}$
Surfines	$3.6\pm0.39^{a}_{AB}$	$8.6\pm1.08^{b}_{C}$	$8.9\pm0.10^{b}_{C}$	$2.9\pm0.51^{a}_{B}$	$4.1\pm0.37^{ab}_{B}$	$4.9\pm0.07^{b}_{C}$	$0.8\pm0.12^{a}_{AB}$	$4.5\pm1.45^{a}_{B}$	$4.0\pm0.02B^{a}_{C}$
Capucines	$3.6\pm0.92^{a}_{B}$	$6.3\pm0.48^{ab}_{AB}$	$7.1\pm0.31^{b}_{B}$	$2.8\pm0.66^{a}_{B}$	$4.6\pm0.94^{a}_{B}$	$3.0\pm0.003^{a}_{AB}$	$0.9\pm0.28^{a}_{B}$	$1.7\pm0.46^{a}_{A}$	$4.1\pm0.31^{b}_{C}$
Capotes	$4.0\pm0.32^{a}_{B}$	$6.9\pm0.94^{b}_{BC}$	$5.2\pm0.36^{ab}_{A}$	$2.7\pm0.05^{a}_{B}$	$4.8\pm0.64^{b}_{B}$	$2.4\pm0.37^{a}_{AB}$	$1.3\pm0.27^{a}_{B}$	$2.2\pm0.30^{ab}_{A}$	$2.8\pm0.002^{b}_{A}$
Fines	$4.6\pm0.48^{a}_{B}$	$4.3\pm0.04^{a}_{A}$	$7.2\pm0.46^{b}_{B}$	$3.2\pm0.49^{a}_{B}$	$1.2\pm0.18^{a}_{A}$	$4.1\pm1.08^{a}_{BC}$	$1.4\pm0.01^{a}_{B}$	$3.1\pm0.22^{b}_{B}$	$3.1\pm0.62^{ab}_{AB}$
Gruesas	$2.0\pm0.04^{a}_{A}$	$5.1\pm0.41^{b}_{AB}$	$4.6\pm0.55^{b}_{A}$	$1.1\pm0.08^{a}_{A}$	$2.2\pm0.32^{a}_{A}$	$2.0\pm0.49^{a}_{A}$	$0.8\pm0.10^{a}_{B}$	$2.9\pm0.41^{b}_{AB}$	$2.6\pm0.14^{a}_{A}$

[z] Values (means ± SE) followed by the same letter were not significantly different according to Fisher's least significant difference (LSD) test at a 95% confidence level ($P<0.05$) (n = 12). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.

'Collados 5' and 'Serón 3' compared to the 'Orihuela 7' cultivar, and was between 1.3 and 6 times higher, depending on the stage of development. The glucose content was higher than that of fructose in 'Orihuela 7' and did not change during caper development. However, the glucose and fructose contents were similar in 'Serón 3' and 'Collados 5' and both decreased during caper development.

Discussion

To date, no studies have been published on the changes in weight or the ratio length/diameter of capers between stages of development and different cultivars. 'Orihuela 7' capers was significantly heavier than those of 'Serón 3' and 'Collados 5' cultivars in most development stages. The initial shape of 'Orihuela 7' capers changed throughout development as they went from being the most elongated to rounded, and this shape matched in the Gruesas stage in the three cultivar.

These differences could be due to genetic or geographical variations. The quantification of the antioxidant activity in biological samples depends to a great extent on the method used. Three methods were used to evaluate the antioxidant abilities of capers of *Capparis spinosa* cultivars. Bouriche et al. (2011) found that a methanolic extract of *Capparis spinosa* buds from Algeria exhibited a strong scavenging activity against DPPH radicals (53 $\mu g \cdot mL^{-1}$), while a methanolic extract of *C. spinosa* fresh fruit averaged 9.06, 6.13, and 8.13 mmol Trolox·kg^{-1} FW by the FRAP, DPPH and $ABTS^{\bullet+}$ methods, respectively (Allaith, 2016). These latter values were higher than those we found by the FRAP (45.1–242.8 mg Trolox/100 g FW) and $ABTS^{\bullet+}$ (67.6–192.7 mg Trolox/100 g FW) methods, but much lower than those determined by the DPPH method (433–1542.5 mg Trolox/100 g FW). Germano et al. (2002) reported that a methanolic extract of capers showed strong activities in a DPPH assay (EC_{50}: 177.451 $\mu g \cdot mL^{-1}$). The differences observed between these values can be explained, at least in part, by the use of different solvents, extraction methods employed and different caper cultivars.

The hydrophilic fractions of the three caper cultivars exhibited a higher antioxidant activity, between 89–443 mg Trolox/100 g FW, in line with the results for caper fruits of Bahrain (Allaith, 2016). The higher antioxidant activity found in the hydrophilic fraction in caper fruits by FRAP (12.8 mmol·kg^{-1} FW), $ABTS^{\bullet+}$ (9.8 mmol·kg^{-1} FW) and DPPH (6.4 mmol·kg^{-1} FW) assays indicated the major contributors to the antioxidant activity of caper fruits were water soluble constituents or polyphenol compounds (Allaith, 2016), and could also be the case in capers. H-TAA decreased slightly as the size of the capers increased for all three cultivars.

Capers are rich in polyphenolic compounds and flavonoids that are usually associated with tolerance to high temperatures (Chedraoui et al., 2017). The concentrations of polyphenols and flavonoids vary depending on the extraction method, genetic factors, and climatic/growing conditions of different sites (Tagnaout et al., 2016). Arrar et al. (2013) determined polyphenols and flavonoids contents of 33.5 mg·g^{-1} DW and 15.8 mg·g^{-1} DW in *C. spinosa* flowers from Algeria; these values were very similar to those found in caper buds. Inocencio et al. (2000) observed a wide variation in the flavonoid contents of capers from different regions and they proposed that environmental and physiological factors could have important effects. The total flavonoids contents averaged 6.55 mg·g^{-1} FW, similar to the value obtained for the cultivars in the present study. Maldini et al. (2016) found that the levels of polyphenols and flavonoids in capers of wild and cultivated *C. spinosa* plants collected from different areas of Sardinia (Italy) ranged from 98–149 mg/100 g FW and 82–117 mg/100 g FW, respectively, levels that were five times lower than those reported in this study, indicating that those levels could be no different in cultivated and wild capers. However, Tlili et al. (2010) showed that leaves and flower buds of *C. spinosa* from different locations in Tunisia were very rich in total polyphenols, containing an average of 3643 and

2621 mg/100 g FW, respectively. These values were much higher than those found in this study and showed great variability depending on the location. However, in our Spanish cultivars, no variability was found between capers from three locations, although the average values were higher for 'Collados 5'.

Some flavonols are known to have various pharmacological effects (anti-inflammatory, antimicrobial, anticancer, cardioprotective, anti-allergic, neuroprotective and analgesics) and they are used as food supplements on account of their high antioxidant activity (Tagnaout et al., 2016). Germano et al. (2002) attributed the antioxidant power of the methanolic extract of *Capparis spinosa* buds to the presence of the flavonol rutin. According to Tagnaout et al. (2016) this flavonol is synthesized by plants as an adaptation to arid and semi-arid climates and to give protection against various environmental stresses. In our cultivars, the flavonols content was 68–95% of the flavonoids content in the Nonpareilles stage, and it increased to values of 83–96% in capers in the Gruesas stage, suggesting that capers could be a very rich source of flavonols. Comparing the flavonols contents in the Capotes stage (347.2, 511.3, and 431.4 mg RE/100 g FW) with those reported for the edible part of onion (39–42 mg/100 g FW), a food rich in flavonols (Hendler and Rorvik, 2008), we found that capers are a superior source of flavonols.

In the literature, data on *C. spinosa* carotenoids are limited. In capers in different Tunisian regions, the total carotenoids content ranged between 411.3–3452.5 $\mu g \cdot g^{-1}$ FW (Tlili et al., 2009), much higher values than those found in capers from Spain. Later, Tlili et al. (2010) reported that the content of these compounds in flower buds ranged between 1.14–9.09 mg/100 g FW, values very similar to those in this study (1.3–3.3 mg/100 g FW). However, Özcan and Akgül (1998) reported that the total carotenoids in buds of *C. spinosa* were between 5.61–17.07 $\mu g \cdot g^{-1}$ DW—about 10 times less than in the current study. Ulukapi et al. (2016) found carotenoids content of 21.24 $mg \cdot kg^{-1}$ in capers from Turkey, values very similar to those found in capers from Spain. These differences may be due to cultivar variations, geographic locations, and different harvesting and extraction techniques (Tlili et al., 2009, 2010). Carotenoids are involved in photosynthesis (they transfer light energy to chlorophylls) and in photoprotection of plants over-exposed to sunlight. In capers, lutein (64.9%), β-carotene (24.4%), neoxanthin (6.7%) and violaxanthin (3.9%) were identified by Tlili et al. (2009); they could therefore be used to increase the intake of these compounds in food as they play an important role in human nutrition. The consumption of *C. spinosa* is not associated with any adverse effects according to the published literature, indicating that *C. spinosa* is safe to consume (Sher and Alyemeni, 2010).

Considering the correlation results (Table 5), a high correlation between total polyphenols and/or flavonoids with $ABTS^{\bullet+}$, FRAP and/or DPPH activities in the three cultivars was found. However, Yadav and Malpathak (2016) observed no correlation between the antioxidant activities and total polyphenols, and flavonoids contents of stem and leaves extracts of *Capparis moonii* from India. Other antioxidants different to polyphenols and flavonoids, such as volatile oils, carotenoids, alkaloids, steroids, tannins, glycosides, and vitamins may be responsible of the antioxidant activity (Yadav and Malpathak, 2016). It is well known that total polyphenols, flavonoids, and flavonols are important bioactive compounds that in general contribute to hydrophilic antioxidant activity (H-TAA). These compounds were positively correlated with H-TAA in the 'Collados 5' cultivar, but flavonoids and flavonols were negatively correlated in 'Serón 3'. Allaith (2016) observed that in the hydrophilic fractions of caper fruits the polyphenol content was positively correlated with FRAP (0.59), DPPH (0.610), and $ABTS^{\bullet+}$ (0.486) activities and flavonoids content (0.714, 0.587, and 0.652, respectively). In this study, L-TAA and carotenoids contents were negatively correlated in the 'Orihuela 7' cultivar. Ulukapi et al. (2016) observed that total carotenoids and total polyphenols content were positively correlated (0.983) in *Capparis spinosa* buds.

The protein content in the capers in this study was much lower than that found by Özcan and Akgül (1998) and Ulukapi et al. (2016). Their values were 33% and 9.45% respectively, but they determined crude proteins by the Kjeldahl method which does not measure the protein content directly, so that a conversion factor is needed to convert the measured nitrogen concentration to a protein concentration. UV-visible spectroscopic techniques are often preferred because they give rapid and reliable measurements, and are sensitive to low protein concentrations, and can detect protein concentrations as low as 0.001% total weight. The 'Orihuela 7' cultivar had contents between 3.5–5 times lower than the 'Serón 3' and 'Collados 5' cultivars, which had protein levels between 4.6–6.6 and 4.8–7 $mg \cdot g^{-1}$ FW, respectively.

In the literature, data on the sugars content of *C. spinosa* are very limited. Özcan and Akgül (1998) found the reducing sugar content ranged from 3.84–4.69 g/100 g FW in capers, while Ulukapi et al. (2016) found that the glucose content was 8.1 $g \cdot kg^{-1}$ FW, fructose was 14.5 $g \cdot kg^{-1}$ FW and saccharose was 3.6 $g \cdot kg^{-1}$ FW in capers from Turkey. All these values were much lower than those found in Spanish cultivars that ranged between 2–19 g/100 g FW; however, no saccharose was detected in any sample and the glucose content was higher than that of fructose in 'Orihuela 7'. However, the glucose and fructose contents were similar in 'Serón 3' and 'Collados 5'. The highest sugar contents observed in 'Serón 3' and 'Collados 5' could be due to the fact that they were acting like osmolytes to allow water intake by caper plants in soil with lower water availa-

bility or more saline than that of 'Orihuela 7', which was located in a more humid orchard soil. The higher protein contents in these cultivars could act as osmoprotectors because salt stress and water deficit lead to the formation of reactive oxygen species and increased antioxidative enzyme activities in plants (Parida and Das, 2005). In addition, the higher content of proteins and total sugars in 'Serón 3' and 'Collados 5' suggests that the capers of these two cultivars could be of greater benefit from a nutritional point of view than those of 'Orihuela 7'.

The differences found between the three cultivars studied here in the different parameters investigated could be due to genetic or environmental differences because they were located in very different places.

In conclusion, no significant differences were found in the total antioxidant activity of capers quantified by the $ABTS^{\bullet+}$, DPPH, and FRAP methods between cultivars, but the 'Collados 5' cultivar had higher TAA values, especially by the DPPH method. The H-TAA content was much greater than the L-TAA content in the three cultivars, with H-TAA being between 32 to 68 times higher in 'Collados 5', while the L-TAA content was similar in the three cultivars. Total polyphenols, flavonoids, and flavonols contents were very high in all stages of caper development, and were very similar in the three cultivars. The flavonoid and flavonol content were significantly higher in the Nompareilles stage of development in the 'Collados 5' cultivar. The carotenoids content was very similar in the three cultivars and it did not change during caper development; however, for the TAA values obtained by the DPPH method, H-TAA, total polyphenols as well as protein and sugar contents, decreased as the capers developed and increased in size in all three cultivars. The results obtained in the present study draw attention to the antioxidant potential of capers, especially that of the 'Collados 5' cultivar, and showed that the smaller the capers were, the better the antioxidant and nutritional properties. An increased intake of capers in the diet, and their cultivation, should be encouraged.

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4.3. Publicación III

PUBLICACIÓN III
Antioxidant activity and the physicochemical composition of young caper shoots (*Capparis spinosa* L.) of different Spanish cultivars
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PUBLICACIÓN III: TRANSCRIPCIÓN LITERAL

Antioxidant activity and the physicochemical composition of young caper shoots (*Capparis spinosa* L.) of different Spanish cultivars

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Abstract

The cultivation of Capparis spinosa is widespread in the Mediterranean basin. The caper is a perennial and deciduous creeping shrub, of which different aerial parts are edible, such as the flower buds, fruit and caper shoots, however the level of consumption of caper shoots is less widespread. The caper shoots are quite typical of the south-eastern part of Spain and their consumption is usually as a side in salads. The present investigation tries to raise awareness of the nutritional value of this aerial part of the caper plant and promote higher levels of consumption of this specific part, by means of the study of different cultivars located in different geographic zones. According to the present investigation, it has been observed that the tender stems have good biochemical characteristics, providing higher nutritional value than the flower buds and caper fruits. The cultivar 'ORI 1' located in Orihuela (Alicante) has presented the best biochemical characteristics, due to its significantly higher values in polyphenols, flavonoids, flavonols and antioxidant activity. Therefore, it would be worthwhile to encourage the consumption of caper shoots, introducing them as one more ingredient in the Mediterranean diet. The caper is a high value crop, due to its ability to adapt to climate change and drought, in addition to the beneficial properties that the different aerial parts contribute to human health.

Keywords: Caper shoots, Total polyphenols, Antioxidant activity, Chlorophyll, Carotenoids

1. Introduction

Caper (*Capparis spinosa* L.) belongs to the Capparaceae family. It is a woody perennial winter-deciduous shrub with a summer cycle. It is a creeper plant which can reach 50-80 cm in height. The caper plant extends widely in the arid soil of the Mediterranean basin and in Spain it contributes to soil and water preservation, desertification and soil erosion control in their fragile ecosystems (Legua et al., 2013). It withstands high temperatures well and is resistant to long periods of drought thanks to its roots, which penetrate great depths into the earth searching for water, with some older plants reaching as far as 10 metres deep. It is able to grow in the most complicated places, where there is practically no soil such as in rock crevices and stone walls (Faran, 2014). The caper presents different edible aerial parts, the flower buds, caper fruits and the short non-lignified stems with young leaves or shoots. The flower buds present six developmental stages (Nompareilles, Surfines, Capucines, Capotes, Fines and Gruesas), while the caper fruits present three stages of development (Finos, Medianos and Gruesos), depending on its size (Legua et al., 2013). In addition, others edible aerial parts, and perhaps, less well-known are shoot (stem with young leaves). The last 10 cm of the stem is collected and consumed when it is green, since it is the most tender part. Young shoots with small leaves have also been pickled for use as a condiment and they are eaten as a vegetable (Sher and Alyemeni, 2010). The shoots have alternate leaves, round to oval, thick and bright and leaf stipules may transform into spines (Sher and Alyemeni, 2010). The leaves and flower buds have a high antioxidant activity, as well as a high presence of flavonoids and other phenolic compounds (Polat, 2007, Grimalt et al., 2019). Based on the literature, the caper plant is of highly diverse economic and medicinal importance (Faran, 2014). It is used to treat various human ailments including inflammation, gastro-intestinal problems, anemia, liver dysfunction, rheumatism, as well as being used as an antispasmodic analgesic; antihaemorrhoidal; aperient; diuretic; expectorant, etc. (Sher and Alyemeni, 2010). Furthermore, other properties have been given to the plant's young shoots throughout history. In Greece, young shoot infusions were used for the treatment of rheumatism, while in Libya caper shoots were investigated for anti-tumour activity (Rivera et al., 2003). Dried leaves of Capparis spinosa steeped in vinegar have been used in Syria for application to ulcers and scabs on the head (Rivera et al., 2003). Floral leaf and button infusions were used as a treatment for cold and respiratory infections, they have also been used internally for the curing of gastrointestinal infections, diarrhea, and dysentery and also to eliminate kidney stones (Sher and Alyemeni, 2010).

Consequently, the main objective of this paper is to increase knowledge about one of the least known aerial parts of Capparis spinosa, - its young shoots. This is the first paper that studies the antioxidant activity and physicochemical composition of caper shoots from plants similar in vigour and size of fifteen cultivars located in different geographical areas of Spain. A better understanding of Spanish cultivars is essential for the selection of high-quality caper genotypes that may be of interest for further nutraceutical studies.

2. Materials and Methods

2.1. Plant materials

Young shoots of caper plant from fifteen cultivars were hand-collected: 'ORI 1', 'ORI 2', 'ORI 3', 'ORI 4', 'ORI 5', 'ORI 6', 'ORI 7', 'ORI 8', 'ORI 9', 'ORI 10' and 'ORI 11' from Orihuela (Alicante) (38º5' N, 0º56' W, 23.6 masl), 'ALC 1' and 'ALC 2' from Alcayna (Murcia) (38º03' N, 1º12' O, 125 masl), and 'ALB 1', 'ALB 2' from Alberca (Murcia) (37º59' N, 1º07' O, 42 masl), respectively. The cultivars were grown in rainfed areas in Spain and harvested weekly from April to June in 2018 from adult plants, similar in vigour and size. The average annual rainfall and temperatures were about 307.8 mm and 17.97ºC (Orihuela), 401.2 mm and 17.9ºC (La Alcayna) and 356.6 mm and 18.8ºC (La Alberca) (SIAM, 2018). Young caper shoots samples were collected and lyophilized in a freeze dryer (Model LyoQuest Plus -85, TELSTAR, Japan) and then stored at -80°C in an ultra-low temperature freezer, premium range (New Brunswick Scientific, Edison, New Jersey US).

2.2. Determination of chlorophylls and carotenoids

Chlorophylls a and b were extracted from each sample using 85% acetone according to Official Methods (AOAC, 1990). Absorbance was read at 664 and 647 nm using a UNICAM Helios Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). The results were expressed as mg/100 g of dry weight (dw). Total carotenoids were extracted according to Valero et al. (2011), with acetone and diethyl ether to promote phase separation. The lipophilic phase was used to estimate the total carotenoids content by reading the absorbance at 450 nm using a and the results were expressed as mg of carotenoids/100 g dw, taking into account the Ɛ_(1%)^cm=2560.

2.3. Determination of proteins

The protein content was analysed by Bradford (1976) method using the Bio-Rad reactive. A standard curve of pure bovine serum albumin (BSA) was used for quantification according to Grimalt et al. (2019). The absorbance was measured at 595 nm and the results were expressed as mg protein/g dw.

2.4. Determination of total antioxidant activity

In this work, ABTS•+, FRAP, and DPPH• methods were used to describe the antioxidant activity of the samples. For the antioxidant activity determination, a methanolic extract was prepared with each sample to be analysed according to Grimalt et al. (2019). The ABTS•+ [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical scavenging assay and ferric reducing antioxidant power (FRAP) were carried out as described by Re et al. (1999) and Benzie and Strain (1996), respectively. Briefly, 10 µL of the extract was mixed with 990 µL of ABTS•+ or FRAP. After 10 min of reaction, the absorbance was measured at 734 nm for ABTS•+ and 593 nm for FRAP. While, the radical scavenging activity was evaluated using the DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams et al. (1995) with a modification in the reaction time. Briefly, 10 µL of the extract was 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 minutes. The decrease in absorbance was measured at 515 nm.

In addition, extract of caper shoots for hydrophilic (H-TAA) and lipophilic (L-TAA) total antioxidant activity determinations were prepared by mixing 0.15 g of sample with 20 mL Tris-acetate buffer pH 6.0, 30 mM CaCl2, and ethyl acetate (1:1, v/v). The mixture was sonicated for 15 minutes at 20 °C in a Ultrasonic cleaner (TierraTech, Model LT-150 PRO, Spain), homogenized and centrifuged to 15,000 x g for 20 minutes in a refrigerated centrifuge (SIGMA 3-18K, Germany) to separate the hydrophilic and lipophilic phases. The total antioxidant activity was quantified by the ABTS•+ method (Re et al.,1999) in the hydrophilic (H-TAA) and lipophilic (L-TAA) fractions of young caper shoots extracts. All the results were expressed as mg Trolox/100 g dw.

2.5. Determination of total polyphenols, flavonoids and flavonols

Total polyphenols were quantified in the hydrophilic phase according to Singleton et al. (1999) method slightly modified, using the Folin-Ciocalteu reagent. Briefly, 50 µL of sample were mixed with 2.5 mL of Folin- Ciocalteu reagent (1:10, v/v), 450 µL of phosphate buffer (pH 7.8) and 2 mL of sodium carbonate (75 g/L). A calibration curve was performed with gallic acid and the absorbance was measured at 760 nm. The results were expressed as mg of gallic acid equivalents (GAE)/100 g dw.

Methanolic caper shoots extracts were prepared to estimate the total flavonoids and flavonols using a slightly modified method of Argentieri et al. (2012) with 80% methanol, a weight/volume ratio of 1/50 and shaken for 24 hours. Total flavonoids were quantified by a slightly modified method of Chang et al. (2002). Briefly, the reaction mixture contained 0.5 mL extract, 1.5 mL 95% ethanol, 0.1 mL AlCl3, 0.1 mL sodium acetate and 2.8 mL distilled water. The reaction was maintained at room temperature for 30 minutes and the absorbance was subsequently measured at 415 nm. Total flavonols were quantified by the method of Kumaran et al. (2007) slightly modified. About 0.25 mL of each methanolic extract was mixed with 0.25 mL aluminium trichloride (20 mg/mL) and 1.5 mL sodium acetate (50 mg/mL) and the absorbance was measured at 440 nm. The results were expressed as mg of rutin equivalents (RE)/100 g dw.

2.6. Statistical analysis

Statistical analysis of the data was performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance test for mean comparisons. The method used to discriminate among the means (Multiple Range Test) was Fisher's LSD (Least Significant Difference) procedure at 95% confidence level ($P<0.05$). For all assays, three extracts were prepared per cultivar and two determinations were made for each of them (n=6). Principal component analysis (PCA) using Pearson correlation coefficient was carried out to study the statistical relationship between cultivars and it was performed using the software XLSTAT Premium 2016 (Addinsoft, New York, USA).

3. Results and Discussion

3.1. Chlorophylls and carotenoids content

The chlorophyll *a*, *b* and total chlorophyll content, as well as the carotenoid content in young shoots of different caper cultivars, presented significant differences between the different cultivars (Table 1). The 'ORI 5' cultivar had significantly higher values in chlorophylls a, b and totals with a content of 305.5, 389.6 and 694.9 mg/100 g dw, respectively. The 'ORI 3' cultivar did not show significant differences with respect to 'ORI 5', being the 'ORI 3' cultivar the second with a higher content of chlorophylls a, b and total (296.3, 364.0 and 660.1 mg/100 g dw, respectively), followed by 'ORI 6' (268.8, 383.5 and 652.1 mg/100 g dw, respectively) which did not show significant differences with both cultivars ('ORI 3' and 'ORI 5'). In general, cultivars located in the area of Orihuela had higher values of chlorophylls than cultivars located in the area of Alcayna and Alberca, this could be due to differences in soil and climate conditions. In fact, the average irradiance over 2018 in Orihuela (202,92 W/m2) was lower than that registered in Alcayna (206,46 W/m2) and Alberca (205,79 W/m2) (SIAM, 2018). In the case of 'ALC 2' cultivar, it had the lowest values in chlorophylls a, b and total (58.6, 21.2 and 79.8 mg/100 g dw, respectively). There are no previous studies that quantify the chlorophyll content in the young caper shoots. However, there were different studies carried out in other aerial parts of the caper plant. In flower buds of the caper plant the highest total chlorophyll content obtained was of 21.2 mg/100 g fw (Grimalt et al., 2019), which is much lower than that obtained in the present study. On the other hand, in caper fruits, the highest total chlorophyll content was of 25.0 mg/100 g fw (Grimalt et al., 2018). In view of the results obtained in this research, the aerial part with the highest chlorophyll contents were the caper shoots. Chlorophylls have positive effects on inflammation and oxidation. Several reports have demonstrated that a diet rich in plant pigments, such as chlorophylls, plays an important role in human health (Inanç, 2011).

As for the carotenoid content (Table 1), the highest value was presented by 'ORI 5' cultivar (112.2 mg/100 g dw), followed by 'ORI 4' (91.6 mg/100 g dw) and 'ORI 6' (84.1 mg/100 g dw) cultivars. The 'ALC 2' cultivar presented the lowest values in carotenoid content, with a total of 27.9 mg/100 g dw, followed by 'ALB 1' (38.9 mg/100 g dw), 'ALC 1' (39.5 mg/100 g dw) and 'ALB 2' (44.3 mg/100 g dw). In general, cultivars located in the area of Orihuela had higher carotenoid content than cultivars located in the area of Alcayna and Alberca, similar to that which was the case with chlorophylls, which could be due to the different climate conditions. The carotenoid content found in flower buds by Grimalt et al. (2019), was much lower compared to the present

research, being the highest value of 3.3 mg/100 g fw. In the case of the caper fruits, the values were lower, with a total of 1.7 mg/100 g fw (Grimalt et al., 2018). According to the study conducted by Tlili et al. (2010) in caper leaves, the results obtained were an average of 18.52 mg/100 g fw, being the highest value of 28.26 mg/100 g fw, these values were higher than those obtained in this current research (112.2 mg/100 g dw). In the young caper shoots there was a greater presence of carotenoids, compared to the flower buds and caper fruits. Carotenoids are important compounds for human nutrition. The beneficial effects derived from these compounds have been attributed to their antioxidant activity by scavenging free radicals (Tlili et al., 2010).

3.2. Protein content

The protein content obtained from the young caper shoots (Table 1) showed that 'ALB 2' cultivar presented the highest value, with a total of 1667 mg/100 g dw, followed by 'ORI 10' with a total of 1511 mg/100 g dw. While the 'ORI 1' and 'ORI 2' cultivars presented the lowest values, with a total of 1009 and 1073 mg/100 g dw, respectively. There are no previous studies that analyse the protein content in the young caper shoots, but there is a study about different aerial parts of capers that determined the crude protein content by the Kjedahl method (Ulukapi et al., 2016), which does not directly measure the protein content, so a conversion factor is needed to convert the measured nitrogen concentration into a protein concentration. Visible UV spectroscopic techniques are often preferred because they provide fast and reliable measurements. In the study conducted by Grimalt et al. (2018) in caper fruits, the results obtained were much lower (1.1 mg/g fw) than those obtained in the present work (1667 mg/100 g dw). It has been observed that the protein content was higher in the shoots than in other aerial parts of the caper plant.

3.3. Total polyphenols, flavonoids and flavonols

Polyphenolic compounds are known to have antioxidant activity. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Bhoyar et al., 2018). The content of total polyphenols determined by the Folin-Ciocalteu method for young caper shoots of cultivars analysed presented significant differences between the different cultivars (Table 2). The 'ORI 1' cultivar showed the highest value with a total content of 3483.01

mg GAE/100 g dw, followed by 'ORI 4' (2861.77 mg GAE/100 g dw) and 'ORI 2' (2840.94 mg GAE/100 g dw) cultivars. The 'ORI 2' and 'ORI 4' cultivars did not show significant differences. The 'ALB 1', 'ORI 11' and 'ALB 2' cultivars presented the lowest values of total polyphenols content (2068.34, 2097.63 and 2200.77 mg GAE/100 g dw, respectively), without significant differences between them.

There are not many studies prior to the present investigation on the polyphenol content in caper shoots, Gull et al. (2018) reported values about ten times lower than those of the present work (287 mg GAE/100 g dw). The content of total polyphenols of young caper shoots was lower than the contents reported by Tlili et al. (2010) (3643.29 mg/100 g fw), Arrar et al. (2013) (57.0 mg GAE/g fw) and Mansour et al. (2016) (427.27 mg GAE/g dw), but it was higher than that reported by Bhoyar et al. (2018) (24.78 mg GAE/g dw) for leaves of C. spinosa. Compared with other commonly used crops such as mango (208 mg GAE/100 g fw) (Ribeiro et al., 2007) or blueberry (525 mg GAE/100 g fw) (Prior et al., 1998), the caper shoots presented a higher content in polyphenols and they are, therefore, an excellent natural source of these compounds, known as effective antioxidants. The effectiveness of different parts of the caper plant could also be attributed to the concentration of these compounds (Gull et al., 2018).

The cultivar 'ORI 1' presented the highest values in flavonoids and flavonols contents with 3276.80 and 1685.50 mg RE/100 g dw, respectively (Table 2). 'ORI 10' was the second cultivar with a higher content of flavonoids, followed by 'ORI 4' (2729.37 and 2686.99 mg RE/100 g dw, respectively). On the contrary, 'ORI 11' and 'ALB 1' cultivars were those with a lower content (1614.08 and 1771.17 mg RE/100 g dw, respectively). Gull et al. (2018) obtained lower results than the present study, with a total of 215.3 mg/100 g dw in caper shoots. However, the content of flavonoids in caper leaves obtained by Arrar et al. (2013) (11.2 mg RE/g fw) was far higher than those of the present study. On the contrary, the flavonoid content in caper shoots was much higher than that reported by Bhoyar et al. (2018) (5.69 RE/g dw) for caper leaves. With respect to the flavonols content, the second cultivar with a greater presence of these compounds was 'ORI 4' followed by 'ORI 11' with a total of 1575.48 and 1572.41 mg RE/100 g dw, respectively, both cultivars did not show significant differences. The 'ALC 1' (719.87 mg RE/100 g dw) and 'ORI 3' (915.75 mg RE/100 g dw) cultivars showed the lowest flavonols content. There are no previous studies that analyse the flavonols content in the young caper shoots. The content of flavonols obtained by Mzoughi et al. (2019) in leaves of wild Swiss chard were much higher than those obtained in the present work, with a total of 22.69 mg RE/g fw.

The content of total polyphenols, flavonoids and flavonols has been determined in different edible aerial parts of the caper plant. In flower buds the contents of total polyphenols (849.4 mg GAE/100 g fw), flavonoids (729.5 mg RE/100 g fw) and flavonols (691.6 of mg RE/100 g fw) (Grimalt et al., 2019) were higher than those obtained in young caper shoots. However, the contents of total polyphenols (119.2 mg GAE/100 g fw), flavonoids (81.6 mg RE/100 g fw) and flavonols (39.9 mg RE/100 g fw) in caper fruits (Grimalt et al., 2018) were lower than those obtained for flower buds and young shoots of caper.

Environmental factors, such as light, temperature and humidity, play an important role in the accumulation of phenolics in plants (Zhang et al., 2016). Caper shoots are rich in polyphenol and flavonoid compounds that are normally associated with high temperature tolerance (Chedraoui et al., 2017), but the average annual temperatures were very similar in the three areas (Orihuela: 17.97ºC, La Alcayna: 17.9ºC and La Alberca: 18.8ºC) (SIAM, 2018). Meanwhile, most plants likely exhibit a similar response to temperature, whereby the concentration of phenolic compounds can be promoted by low temperatures but inhibited by high temperatures (Zhang et al., 2016). In addition, the difference in the concentration of polyphenolic compounds is related to the extraction method, genetic and climatic factors of the areas where the plant grows and the conditions of the different sites (Tagnaout et al., 2016). Our results suggested that high air humidity (AH), lower annual rainfall and lower irradiance could have triggered a higher level of these compounds, conditions that occurred in Orihuela (AH: 68.7%, 307.8 mm, 202,92 W/m2) (SIAM, 2018) whose cultivars presented the highest levels of total polyphenols and flavonoids. Some authors suggest that the polyphenolic compounds are relatively stable with respect to the different extraction methods (Makris and Rossiter, 2002).

3.4. Total antioxidant activity (TAA)

The total antioxidant activity (TAA) was quantified in young caper shoots by three methods: ABTS•+, DPPH•, and FRAP (Table 3). It is necessary to use several methods to better compare the results obtained in the analysis of the antioxidant activity, and their choice is based on the matrix of the sample and on the chemical nature of the compounds to be evaluated (Prior et al., 2005). The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. DPPH• and ABTS•+ tests provide information about the activity of the test compounds with a stable free radical and its effects are thought to be due to their hydrogen donating ability. The 'ORI 4' cultivar presented the highest value (1813.63 mg Trolox/100 g dw), followed by 'ORI 10' (1593.37 mg Trolox/100 g dw) and 'ORI 1' (1399.64 mg

Trolox/100 g dw) cultivars by the ABTS•+ method. The 'ALB 2' and 'ALB 1' cultivars showed the lowest value with a TAA of 608.46 and 768.40 mg Trolox/100 g dw. The total antioxidant activity determined by the DPPH• method showed values between 907.57 for 'ORI 11' and 2066.68 mg Trolox/100 g dw for 'ALB 2' cultivars. FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe3+-TPTZ) complex and produces a coloured complex of ferrous tripyridyltriazine (Fe2+-TPTZ) (Benzie and Strain, 1996), but it only detects compounds with redox potentials less than 0.7 V. The TAA determined by the FRAP method presented the higher value for 'ORI 10' cultivar with a value of 1924.76 mg Trolox/100 g dw, followed by 'ORI 1' and 'ORI 11' cultivars (1899.23 and 1880.21 mg Trolox/100 g dw, respectively), that did not show significant differences between them. The lowest value was presented by 'ORI 5' cultivar with a TAA of 709.33 mg Trolox/100 g dw.

The DPPH• activity of most cultivars studied was lower than their ABTS activity (Table 3), which agrees with Bhoyar et al. (2011) for caper leaves. However, 'ALB 1' and 'ALB 2' cultivars presented the highest values for DPPH and the lowest for ABTS activities. ABTS•+ method allows the evaluation of hydrophilic and lipophilic antioxidants since the ABTS•+ radical is soluble in both organic and aqueous media. However, the DPPH• method is less adapted to measure lipophilic antioxidants. This could be the reason why 'ALB 1' and 'ALB 2' present the lowest ABTS•+ activity and the highest DPPH• since these cultivars have a lower content of carotenoids, L-TAA and total polyphenols. While 'ORI 4', which is the one with the highest ABTS•+ activity, has a high content of carotenoids, L-TAA and total polyphenols (Tables 1, 2, 3). The total antioxidant activity obtained by these three methods, ABTS•+, DPPH• and FRAP, in Capparis decidua leaves, flowers and fruits by Zia-Ul-Haq et al. (2011) showed higher values than those obtained for caper shoots in the present research. Factors such as geographical area, soil type, genotype and the part of the plant analysed, could provide significant differences in the antioxidant activity of a sample. Besides, factors like stereo-selectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals (Yu et al., 2002).

In addition, the total antioxidant activity was quantified by the ABTS•+ method in the hydrophilic (H-TAA) and lipophilic (L-TAA) fractions of young caper shoots extracts (Table 3). It was observed that H-TAA and L-TAA showed very different values depending on the cultivar. The H-TAA values remained between 233.38 and 1291.36 mg Trolox/100 g dw, 'ORI 8' cultivar being the one with the highest H-TAA and 'ORI 3' the one with the lowest amount of H-TAA. The L-TAA values remained in a range between 462.52 mg Trolox/100 g dw in 'ORI 5' and 95.21 mg Trolox/100 g dw in 'ORI 2' cultivars, respectively.

There are no previous studies on the hydrophilic and lipophilic total antioxidant activity in young caper shoots, although there are previous studies for different edible aerial parts. Grimalt et al. (2018) determined H-TAA values of 68.3 and L-TAA of 24.0 mg Trolox/100 g fw in caper fruits, these being values lower than those obtained for young caper shoots. In view of the results obtained, it was observed that the young shoots of the caper had a higher antioxidant activity, both hydro and lipophilic.

Principal component analysis (PCA) biplot (axes F1 and F2: 59.38%), with all the biochemical compounds and the different cultivars of young caper shoots have been represented in Figure 1. The PCA is a very useful tool to show graphically, which compounds predominate in young shoots of different cultivars. Three well-differentiated groups could be observed. In the upper quadrant seven cultivars were grouped, which had a very similar composition. The 'ORI 1', 'ORI 4', 'ORI 7', 'ORI 8', 'ORI 9', 'ORI 10' and 'ALC 2' cultivars, were associated with a high number of biochemical compounds, mainly at a high antioxidant activity, ABTS•+, FRAP, H-TAA and L-TAA, and polyphenolic compounds (total polyphenols, flavonoids and flavonols). Next, in the lower left quadrant, the 'ORI 11', 'ALB 1', 'ALB 2' and 'ALC 1' cultivars were associated with proteins content and DPPH• activity. Finally, in the lower right quadrant the presence of chlorophylls and carotenoids in greater quantity was found in 'ORI 2', 'ORI 3', 'ORI 5' and 'ORI 6' cultivars.

4. Conclusions

According to the objective of the present investigation, it has been determined that the caper shoots of Capparis spinosa L. have good biochemical characteristics, the aerial part being that which contains a higher content of chlorophylls, carotenoids and a greater presence of antioxidant activity compared with other studies on biochemical properties in different aerial parts of the Capparis spinosa. Caper shoots could play a very important role in human nutrition.

Of the different cultivars studied in the present investigation, those with the best biochemical properties have been 'ORI 1', 'ORI 5' and 'ALB 2'. The 'ORI 1' cultivar was very interesting due to its high content of polyphenols, flavonoids and flavonols, as well as its high total antioxidant activity by ABTS•+ and FRAP methods and H-TAA. The 'ORI 5' cultivar stands out due to its high content of total chlorophylls, carotenoids and L-TAA. In the case of the 'ALB 2' cultivar, it is of interest due to its high protein content and total antioxidant activity by the DPPH• method.

Credit author statement

Mar Grimalt prepared the plant material, participated in the physicochemical measurements, statistical analyses and drafted the manuscript. Asunción Amorós and María Soledad Almansa designed the experiment, proposed and supeervised the research. María Soledad Almansa rewrote the manuscript. Francisca Hernández and Pilar Legua revised the manuscript. María Soledad Almansa and Francisca Hernández carried out the revision proposed by the reviewers.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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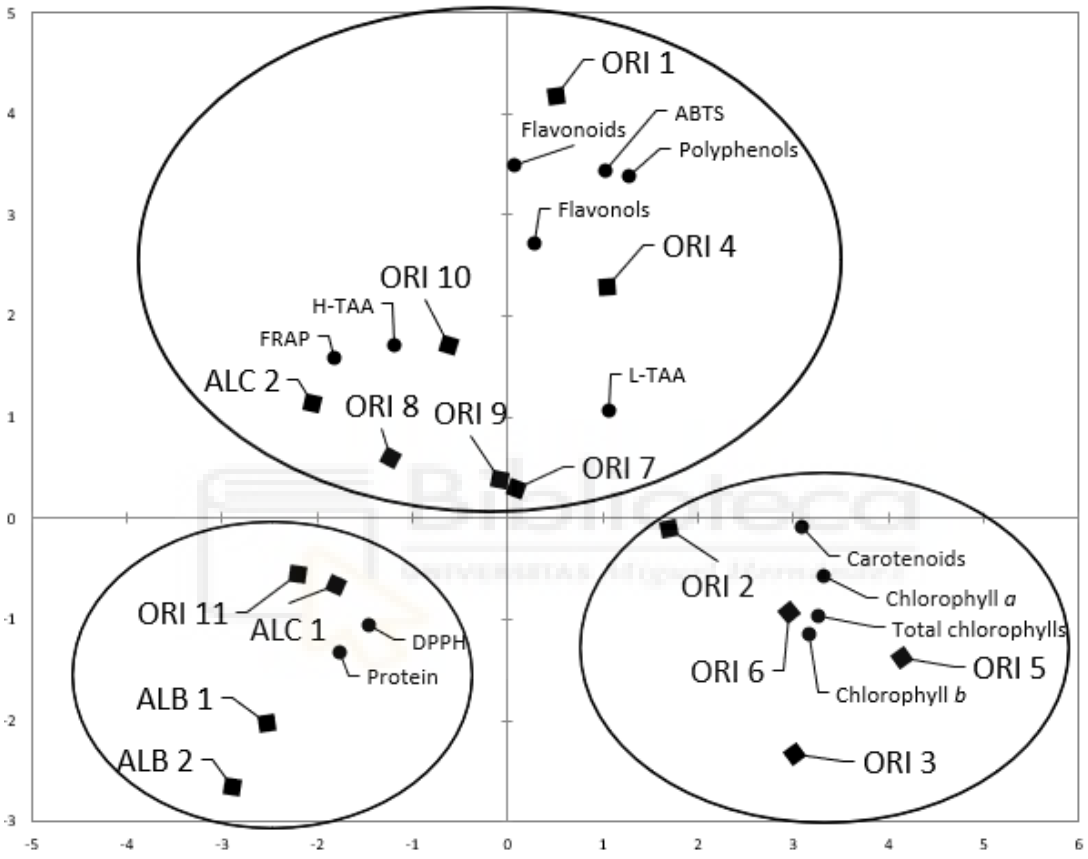
FIGURES

Figure 1. Principal component analysis (PCA) of young caper shoots of different Spanish cultivars (◆) and biochemical compounds (●).



TABLES

Table 1. Chlorophylls (*a*, *b* and total), carotenoids and protein contents in young caper shoots of different Spanish cultivars.

Cultivar	**Chlorophyll *a*** (mg/100 g dw)	**Chlorophyll *b*** (mg/100 g dw)	**Total chlorophylls** (mg/100 g dw)	**Carotenoids** (mg/100 g dw)	**Protein** (mg/100 g dw)
'ORI 1'	168.3 ± 26.6 de	128.7 ± 67.5 b	296.9 ± 89.2 de	55.6 ± 9.7 cd	1009 ± 35 a
'ORI 2'	244.6 ± 28.9 f	242.6 ± 85.7 c	486.9 ± 112.7 f	75.0 ± 3.4 fg	1073 ± 43 ab
'ORI 3'	296.3 ± 20.3 g	364.0 ± 10.2 d	660.1 ± 30.4 g	70.9 ± 1.2 ef	1110 ± 23 abc
'ORI 4'	191.4 ± 11.5 e	63.6 ± 7.1 ab	255.0 ± 18.6 bcde	91.6 ± 6.2 h	1206 ± 16 cde
'ORI 5'	305.5 ± 17.9 g	389.6 ± 23.6 d	694.9 ± 40.5 g	112.2 ± 7.8 i	1127 ± 32 bcd
'ORI 6'	268.8 ± 6.8 fg	383.5 ± 38.9 d	652.1 ± 42.8 g	84.1 ± 0.3 gh	1232 ± 20 efg
'ORI 7'	160.7 ± 9.5 cde	48.0 ± 5.2 ab	208.6 ± 14.5 abcde	74.0 ± 1.3 fg	1194 ± 25 cd
'ORI 8'	129.6 ± 8.9 bcd	37.1 ± 4.1 ab	166.7 ± 12.8 abcd	53.4 ± 3.9 cd	1363 ± 30 h
'ORI 9'	159.6 ± 21.1 cde	116.2 ± 54.3 ab	275.6 ± 64.3 cde	60.3 ± 3.1 de	1308 ± 25 fgh
'ORI 10'	196.6 ± 15.6 e	128.2 ± 50.1 b	324.5 ± 48.5 e	51.7 ± 5.2 bcd	1511 ± 17 i
'ORI 11'	90.9 ± 5.4 ab	31.2 ± 3.4 ab	122.0 ± 8.7 ab	45.2 ± 2.7 bc	1333 ± 16 gh
'ALC 1'	119.1 ± 9.0 bc	41.7 ± 2.9 ab	160.8 ± 11.9 abcd	39.5 ± 0.5 ab	1143 ± 26 bcd
'ALC 2'	58.6 ± 8.4 a	21.2 ± 3.2 a	79.8 ± 11.5 a	27.9 ± 1.9 a	1083 ± 44 ab
'ALB 1'	90.1 ± 9.1 ab	33.9 ± 3.1 ab	124.0 ± 12.1 ab	38.9 ± 6.0 ab	1310 ± 101 fgh
'ALB 2'	116.2 ± 22.5 bc	43.1 ± 8.5 ab	159.2 ± 31.0 abc	44.3 ± 1.4 bc	1667 ± 45 j

Values (means ± standard error) followed by different letters in the same column indicate significant differences between cultivars according to Fisher's least significant difference (LSD) procedure at 95% confidence level (P<0.05) (n=6).

Table 2. Total polyphenols, flavonoids and flavonols contents in young caper shoots of different Spanish cultivars.

Cultivar	Total polyphenols (mg GAE/100 g dw)	Flavonoids (mg RE/100 g dw)	Flavonols (mg RE/100 g dw)
'ORI 1'	3483.01 ± 115.86 f	3276.80 ± 114.00 e	1685.50 ± 91.94 d
'ORI 2'	2840.94 ± 182.41 e	2557.90 ± 375.13 bcde	1361.46 ± 193.80 **cd**
'ORI 3'	2537.16 ± 181.60 cde	1940.35 ± 298.00 abc	915.75 ± 42.63 ab
'ORI 4'	2861.77 ± 50.85 e	2686.99 ± 266.35 cde	1575.48 ± 245.07 d
'ORI 5'	2337.51 ± 80.86 abc	1908.69 ± 185.09 ab	1309.12 ± 162.49 bcd
'ORI 6'	2594.70 ± 84.94 cde	2225.86 ± 260.01 **abcd**	1560.02 ± 144.50 d
'ORI 7'	2536.02 ± 28.27 cde	2256.45 ± 179.27 abcd	1368.63 ± 238.24 cd
'ORI 8'	2558.09 ± 98.71 cde	2277.55 ± 125.51 abcd	1497.49 ± 170.79 cd
'ORI 9'	2614.19 ± 117.09 cde	2289.56 ± 93.32 abcd	1454.54 ± 60.00 cd
'ORI 10'	2779.34 ± 142.01 de	2729.37 ± 377.51 de	1406.58 ± 69.64 cd
'ORI 11'	2097.63 ± 42.67 a	1614.08 ± 238.07 a	1572.41 ± 157.57 d
'ALC 1'	2450.42 ± 144.89 bcd	2097.47 ± 385.56 abcd	719.87 ± 34.99 a
'ALC 2'	2455.11 ± 99.84 bcd	2290.29 ± 215.29 abcd	1566.92 ± 78.33 d
'ALB 1'	2068.34 ± 128.47 a	1771.17 ± 233.62 a	1142.26 ± 146.33 bc
'ALB 2'	2200.77 ± 91.96 ab	2071.12 ± 303.40 abcd	1143.69 ± 36.28 bc

Values (means ± standard error) followed by different letters in the same column indicate significant differences between cultivars according to Fisher's least significant difference (LSD) procedure at 95% confidence level (P<0.05) (n=6).

Table 3. Total antioxidant activity, hydrophilic (H-TAA) and lipophilic (L-TAA) antioxidant activity contents in young caper shoots of different Spanish cultivars.

Cultivar	ABTS$^{\bullet+}$ (mg Trolox/100 g dw)	FRAP (mg Trolox/100 g dw)	DPPH$^{\bullet}$ (mg Trolox/100 g dw)	H-TAA (mg Trolox/100 g dw)	L-TAA (mg Trolox/100 g dw)
'ORI 1'	1399.64 ± 204.74 gh	1899.23 ± 343.65 de	986.66 ± 77.59 ab	1059.87 ± 294.56 cde	386.79 ± 15.00 efgh
'ORI 2'	1238.45 ± 95.86 efg	1431.68 ± 144.67 bcd	1183.39 ± 21.53 abcd	267.03 ± 17.07 ab	95.21 ± 7.65 a
'ORI 3'	886.29 ± 158.19 abcd	1187.14 ± 135.41 ab	962.38 ± 42.55 ab	233.38 ± 59.69 a	338.32 ± 35.28 de
'ORI 4'	1813.63 ± 92.86 i	1030.21 ± 55.82 ab	1404.4 ± 235.53 cd	974.75 ± 180.58 cde	419.96 ± 18.07 hi
'ORI 5'	1080.52 ± 93.86 cdef	709.33 ± 7.01 a	997.92 ± 91.86 ab	980.46 ± 151.08 cde	462.52 ± 23.41 i
'ORI 6'	1047.98 ± 23.78 bcdef	1019.45 ± 113.63 ab	929.84 ± 39.80 a	1028.43 ± 95.54 cde	395.04 ± 10.96 fgh
'ORI 7'	1171.88 ± 22.53 defg	1068.76 ± 127.65 ab	1085.27 ± 50.06 abc	902.76 ± 116.97 cde	398.36 ± 25.51 gh
'ORI 8'	1075.26 ± 107.13 cdef	1200.41 ± 64.58 b	1038.22 ± 77.34 ab	1291.36 ± 196.90 e	341.07 ± 12.60 def
'ORI 9'	1267.99 ± 85.1 fg	1264.49 ± 233.52 bc	1113.06 ± 153.93 abc	785.10 ± 127.65 cd	337.15 ± 16.20 de
'ORI 10'	1593.37 ± 130.40 hi	1924.76 ± 26.28 e	1293.77 ± 195.98 bcd	992.88 ± 329.96 cde	343.87 ± 8.32 defg
'ORI 11'	944.86 ± 87.60 bcde	1880.21 ± 232.77 de	907.57 ± 56.07 a	923.91 ± 47.82 cde	332.65 ± 15.91 cde
'ALC 1'	865.27 ± 79.59 abc	1683.98 ± 255.01 cde	918.33 ± 48.31 a	1253.75 ± 213.88 de	328.42 ± 30.42 bcd
'ALC 2'	1177.13 ± 91.61 defg	1363.35 ± 75.59 bc	1088.03 ± 67.08 abc	1150.98 ± 117.46 cde	341.95 ± 11.50 def
'ALB 1'	768.40 ± 49.81 ab	1495.51 ± 229.02 bcde	1479.49 ± 260.81 d	739.97 ± 152.65 bc	279.36 ± 9.50 bc
'ALB 2'	608.46 ± 27.03 a	1180.39 ± 57.07 ab	2066.68 ± 71.33 e	795.52 ± 154.62 cd	265.88 ± 19.96 b

Values (means ± standard error) followed by different letters in the same column indicate significant differences between cultivars according to Fisher's least significant difference (LSD) procedure at 95% confidence level (P<0.05) (n=6).

4.4. Publicación IV

PUBLICACIÓN IV
Polyphenol Compounds and Biological Activity of Caper (*Capparis spinosa* L.) Flowers Buds
Aneta Wojdyło*, Paulina Nowicka, **Mar Grimalt**, Pilar Legua, Maria Soledad Almansa, Asunción Amorós, Ángel Antonio Carbonell-Barrachina and Francisca Hernández Plants 2019, 8, 539 Doi:10.3390/plants8120539

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Article

Polyphenol Compounds and Biological Activity of Caper (*Capparis spinosa* L.) Flowers Buds

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Abstract: The aim of the study was to analyze potential health-promoting components of caper flower buds (*Capparis spinosa* L.) at six stages of development in two cultivars. Polyphenol compounds (flavonols, hydroxycinnamic acids, flavan-3-ols) were identified by Liquid Chromatography–quadrupole Time–of–Flight –Mass Spectrofotometer/Mass Spectrofotometer (LC-qTOF-MS/MS) and quantified by Ultra Performance Liquid Chromatography–Photodiode Array-Fluorescence Detector (UPLC-PDA-FL). Moreover, antioxidant properties (ABTS+•, FRAP, and ORAC), anti-diabetic potential (α-amylase and α-glucosidase), and anti-aging activity (acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)) of the buds were examined. Total phenolic compounds in the investigated caper varied from 10,720 to 3256 mg/100 g dry weight (DW), and depended on a genotype and growing stage of caper flowers. Among six different growing stages, the one named 'nonpareilles' was characterized by significantly higher content of polyphenols than the remaining five stages. The flavonols in caper flowers represented a mixture of different glycosylated quercetin, kaempferol, myricetin, and isorhamnetin derivatives, accounting for 38%–67%, 15%–36%, 4%–7%, and 0.8%–3%, respectively, of total flavonols,. Their contents strongly depended on the growth stage. 'Nonpareilles' and 'surfines' were richer in flavonols than 'fines' and 'gruesas'. Of the six investigated growth stages, 'nonpareilles' accumulated the greatest amounts of bioactive compounds that correlated with antioxidant and anti-diabetic properties, and were more potent BuChE than AChE inhibitors.

Keywords: caper; nonpareilles; surfines; capucines; capotes; fines; gruesas; LC-qTOF-MS/MS; antioxidant activity; anti-diabetic activity; cholinesterase's inhibition

1. Introduction

Polyphenol compounds are secondary metabolites commonly found in different plant organs, such as in flowers, berries, fruits, roots, leaves, and stems. Currently, polyphenol compounds are receiving considerable attention for their health benefits and capability of preventing the diseases of modern civilization such as cardiovascular or diabetic (hypertriglyceridemia and hyperglycemia) disorders. They also show anti-obesity, anti-inflammatory, anti-bacterial, anti-tumor,

and anti-hepatotoxic activities [1,2]. Plants have become increasingly important, not only in the pharmaceutical but also in the food industry, due to the presence of physiologically active phytochemicals capable of imparting diverse health benefits with diet [3].

Capparis species are grown for their medicinal properties and as food sources. Medicinal plants *Capparis* sp. belong to the *Capparidaceae* family. The most popular species include *C. spinosa, C. decidua,* and *C. ovata*, and less known are *C. sepiaria, C. tomentosa,* and *C. shumilis* [2–5].

Caper grows abundantly in wild arid regions of Asia, Africa, Saudi Arabia, and Europe, especially in the Mediterranean basin. Caper consumables are mainly flower buds and berries. Additionally, different parts of caper (roots) have been used in traditional Chinese, Iranian, Moroccan, Pakistani, Egyptian, and Arabian medicine [5]. Nowadays, it is widely cultivated in Mediterranean countries such as Turkey, Morocco, Algeria, as well as France, Spain, Greece, and Italy. Average annual global production of caper is estimated at 10,000 tons, and Spain is one of the main European producers, with a cultivation area of about 2,600 ha and annual production 500–1000 tons [2,4]. Capers are consumed for their flavor and digestive properties in fresh salads, pizza, and after processing as pickle or products of lactic acid fermentation [6,7].

Caper berries contain a wide range of bioactive compounds such as alkaloids, flavonoids, steroids, terpenoids, and tocopherols [2–5,7]. A few papers [2–5] have reviewed some of the chemical compounds and health benefits of *C. spinosa* in different aspects, including its potential for sustainability. However, the literature contains limited data on flavonoid identification, indicating that capers contain only quercetin, isorhamnetin, and kaempferol of -3-*O*-rutinoside [4,8,9]. NMR techniques yielded one quercetin 3-*O*-6′-α-L-rhamnosyl-6′-*O*-ß-D-glucosyl-ß-D-glucoside in *C. spinosa* berries. Other works reported the presence of some derivatives of (+)-catechin and (epi)-catechin [7] or some derivatives of benzoic acids [10].

Despite this, scientific literature on caper biological activity and polyphenols is still insufficient, especially regarding caper flowers. Health benefits, especially antioxidant potential of food, depend on the type and amount of flavonoids. Research reports published to date have compared bioactive compounds in different caper organs (flowers, berries, leaves, seeds) [11–13], different cultivars, and genotypes (both cultivated and wild) [14]. Some papers also investigated the influence of selected processes on the retention of bioactive compounds in final commercial or non-commercial products [6,7,15,16]. To the best of our knowledge, no paper has been published on the content of phenolic compounds in caper flowers at different stages of development, that is, 'nonpareilles', 'surfines', 'capucines', 'capotes', 'fines', and 'gruesas'.

Considering the wide interest in caper, we designed this study with the aim (i) to identify and quantify polyphenolic compounds (flavonoids and hydroxycinnamic acids) from two of the most often cultivated cultivars in Spain, and (ii) to determine biological activity (antioxidant, anti-diabetic, and cholinesterase inhibition properties) at different growth stages of caper flowers: 'nonpareilles', 'surfines', 'capucines', 'capotes', 'fines', and 'gruesas'.

2. Results and Discussion

2.1. Identification of Phenolic Profile

Table 1 and Figure 1 present details of Liquid Chromatography– quadrupole Time–of–Flight –Mass Spectrophotometer/Mass Spectrophotometer (LC-qTOF-MS/MS) analysis—retention times (recorded on total ion current (TIC) chromatograms), λ_{max}, main ions and formulas for deprotonated molecules $[M - H]^-$ and main fragment ions in MS/MS of 34 flavonols, 10 hydroxycinnamic acids, and five flavan-3-ols.

Table 1. Phenolic compounds identified by qTOF-MS/MS in caper flowers.

No.	Name of Compounds	R_t	λ_{max}	MS [M − H]− (*m*/*z*)	MS/MS [M − H]−(*m*/*z*)			
	Hydroxycinnamic Acid							
1	*p*-Coumaric acid	3.41	310	163.04	119.05			
2	5-Caffeoylquinic acid	3.51	285, 326	353.04	191.06/179.03/173.05			
3	4-Caffeoylquinic acid	4.43	285, 326	353.01				
4	*trans*-5-*p*-Coumaroylquinic acid	5.29	311	337.04	163.05			
5	*cis*-5-*p*-Coumaroylquinic acid	5.34	311	337.04	163.05			
6	3-Feruloylquinic acid	5.59	325	367.05	193.03/191.02/173.09			
7	5-Feruloylquinic acid	9.39	325	367.05	191.02/135.06			
8	4-Feruloylquinic acid	9.54	325	367.05	193.03/173.09/134.09			
9	Coumaric acid-*O*-hexoside	10.26	275, 314	325.03	163.11/119.12			
10	Sinapic acid	10.95	337	223.06	205.13/179.03/164.06			
	Flavonols				−308	−162	−146	aglycone
11	Quercetin-3-*O*-rutinoside-7-hexoside	3.85	285, 352	771.06	463.06	609.06		301.02
12	Quercetin-3-*O*-rutinoside-hexoside-7-*O*-rhamnoside	3.93		917.11	609.11	755.11	463.04	30.,02
13	Kaempferol-3-*O*-rutinoside-hexoside-7-*O*-rhamnoside	4.25		901.12	593.12	739.12		285.95
14	Isorhamnetin-3-*O*-rutinoside-hexoside-7-*O*-rhamnoside	4.45		931.08		607.08	623.08	315.05
15	Quercetin-3-*O*-rutinoside-7-*O*-hexoside	4.50	202, 256, 351	771.06	463.06	609.06		301.02
16	Quercetin-3-*O*-rutinoside-7-*O*-hexoside	4.90	266, 352	771.06	463.06	609.06	625.06	301.02
17	Kaempferol-3-*O*-rutinoside-7-*O*-hexoside	5.04	342	755.07		593.07		285.95
18	Isorhamnetin-3-O-rutinoside	5.09	271, 337	623.03	315.03			315.00
19	Isorhamnetin-3-*O*-rutinoside-7-*O*-hexoside	5.21	264, 326	785.07	477.07	623.07		315.05
20	Myricetin-3-*O*-rutinoside	5.29	274, 355	625.02	317.02			317.09
21	Myricetin-3-*O*-rutinoside (isomer)	5.46	274, 355	625.03	317.03			317.09
22	Quercetin-3-*O*-rutinoside-7-*O*-rhamnoside	5.56		755.07	447.07		609.07	301.02
23	Isorhamnetin-3-O-rutinoside-7-*O*-hexoside	5.69		785.08	477.08	623.08		315.08
24	Quercetin-3-*O*-rutinoside-7-*O*-rhamnoside (isomer)	5.90	254, 353	755.07	447.07		609.07	301.02
25	Quercetin-3-*O*-rutinoside	6.33	252, 348	609.03	301.03			301.02
26	Kaempferol-3-*O*-(2-rhamnoside)-rutinoside	6.41	264, 347	739.08	431.08		593.08	285.95
27	Isorhamnetin-3-*O*-(2-rhamnoside)-rutinoside	6.50	253, 347	769.08	461.08		623.08	315.95
28	Quercetin-3-*O*-rutinoside (rutin)	6.66	256, 354	609.03	301.03			301.02
29	Quercetin-3-*O*-hexoside-7-*O*-hexoside	6.80	253, 330	625.02		463.02		301.02
30	Kaempferol-3-*O*-rutinoside	6.90	254, 346	593.03	285.03			285.95
31	Isorhamnetin-3-*O*-rutinoside	7.02	255, 266sh, 351	623.04	315.04	461.04		315.03
32	Kaempferol-3-*O*-rutionoside-7-*O*-hexoside	7.28	264, 348	755.07	447.07	593.07		285.95

Table 1. *Cont.*

No.	Name of Compounds	R_t	λ_{max}	MS [M − H]− (*m*/*z*)	MS/MS [M − H]−(*m*/*z*)			
	Hydroxycinnamic Acid							
33	Kaempferol-3-*O*-rutinoside (isomer)	7.38	254, 348	593.03	285.03			285.95
34	Isorhamnetin-3-*O*-rutinoside (isomer)	7.55	253, 352	623.04	315.04			315.03
35	Isorhamnetin-3-*O*-hexoside)	7.83	254, 336, 365	477.04		315.05		315.03
36	Kaempferol-3-*O*-rutinoside (isomer)	7.85	264, 346	593.03	285.03			285.95
37	Kaempferol-3-*O*-rutinoside-7-*O*-rhamnoside	8.97		753.09	445.09	591.09	607.09	285.05
38	Myricetin-3-*O*-hexoside	9.62	355	479.11		317.11		317.11
39	Quercetin-3-*O*-(2-rhamnoside)-hexoside	9.87	366	609.05		447.05	463.05	301.05
40	Myricetin-3-*O*-rhamnoside	10.30	355	463.10			317,1	317.08
41	Quercetin-3-*O*-(2-rhamnoside)-hexoside	10.47	316	609.64		447.64	463.64	301.02
42	Myricetin-3-*O*-rhamnoside (isomer)	10.98	355	463.10			317.10	317.08
43	Kaempferol	11.43		285.95				285.95
44	Quercetin	11.71	256, 268sh, 352	301.04				301.02
	Flavan-3-ols							
45	(+)-Catechin	3.41	278	289.06	245.14			
46	Procyanidin B2	3.73	280	577.03	289.04/245.14			
47	Procanidin C1	4.14	280	865.07	577.08/289.04/245.14			
48	(−)-Epicatechin	4.61	278	289.06	245.14			
49	Procyanidin dimer	5.56	280	577.04	289.04/245.14			

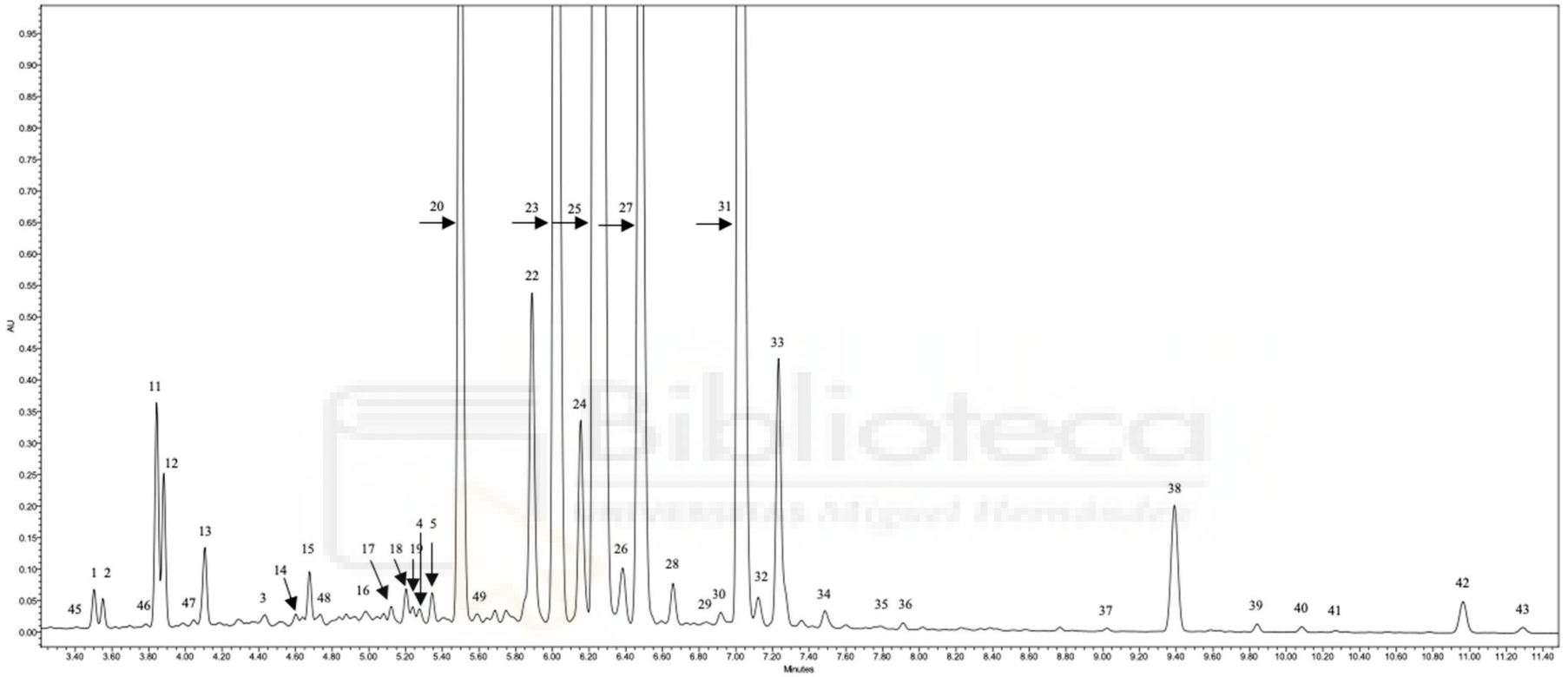

Figure 1. Typically chromatogram of caper flowers. The names of the compounds are presented in Table 1.

2.1.1. Flavonol Glycosides

Caper flowers contained a total of 34 different flavonol compounds, including derivatives of aglycones of quercetin (*m/z* 301), kaempferol (*m/z* 285), isorhamnetin (*m/z* 315), and myricetin (*m/z* 317). An analysis of chemical structure of individual aglycones ([Aglc-H]$^-$) showed a cut off for sugars at *m/z* 162 (glucose or galactose), *m/z* 146 (rhamnoside), and *m/z* 308 (rhamnohexosyl as rutinoside). We did not observe any sugars substituted by *p*-coumarate, malonate acetate, or other compounds. Some UV spectra of compounds **12**, **13**, **14**, **22**, **23**, **34**, **37**, and **42** were undetectable due to their low abundance, especially of flavonoid-*O*-tri- or –tetra-glycosides. Therefore, their identification was based on an exhibited deprotonated molecular ion of a flavonoid and/or literature data.

We identified two caper aglycones as kaempferol (**43**; *m/z* 285.95) and quercetin (**35**; Rt:11.71 min; UV 256, 268sh, 296sh, 374 nm: *m/z* 301.04). They were also detected in capers from Sardinia by Maldini et al. [14].

Compounds **35**, **38**, **40**, and **42,** belonging to flavonoid-*O*-monoglycosides, showed in their MS fragmentation a loss of 162 amu (hexosyl radical) or 146 amu (rhamnosyl radical) that yielded an ion of a deprotonated aglycone as a base peak.

In their MS/MS fragmentation pattern, a loss of 162 amu was observed for only two compounds (**35** and **38**), and the deprotonated aglycone lost either 317 amu (isorhamnetin) or 315 amu (myricetin). Therefore, the compounds were identified as isorhamnetin-3-*O*-hexoside (**35**) and myricetin-3-*O*-hexoside (**38**). Compounds **40** and **42** were characterized as mono-rhamnoside isomers of myricetin. Monoglycosides such as quercetin and isorhamnetin of -3-*O*-glucoside were previously documented by Siracusa et al. [17].

Compounds **18, 20, 21, 25, 28, 30, 31, 33**, **34**, **36**, **39**, and **41,** belonging to flavonoid-*O*-diglycosides, showed in their MS fragmentation a loss of 308 amu ((rhamno)hexosyl radical) that yielded the ion of an aglycone. Compounds **20** and **21** were characterized as myricetin-3-*O*-derivatives; compounds **18**, **31,** and **34** as isorhamnetin-3-*O*-derivatives; compounds **30**, **33**, and **36** as kaempferol-3-*O*-derivatives; and compounds **25**, **28**, **29**, **39,** and **41** as quercetin-3-*O*-derivatives. Compounds **18**, **20**, **21**, **25**, **28**, **30**, **31**, **33**, **34,** and **36** could correspond to flavonol-3-*O*-rutinoside ((rhamno)hexosyl radical—308 amu). In the MS fragmentation of compounds **39** and **41,** the losses of 146 amu (rhamnosyl radical) and 162 amu (hexosyl radical) indicated that these two sugars were situated on different phenolic hydroxyl groups of aglycones. However, Abu-Reidah, Gil-Izquierdo, Medina, and Ferreres [18] and Ferreres, Grosso, Gil-Izquierdo, Fernandes, Valentão, and Andrade [19] suggested a presence of an interglycosidic 1→2 bond. Additionally, the loss of 146 + 18 amu demonstrated that these compounds usually have the 1→2 bond.

Moreover, a loss of two sugars as 324 amu (di-hexosyl radical) from compound 29 indicated the presence of diglycosides with an interglycosidic 1→6 bond, which is very difficult to break down, as suggested by numerous authors [18,20]. Therefore, this compound can be characterized as quercetin-3-*O*-hexoside-hexoside (**29**). As already indicated, similar results were reported by other authors [19]. The flavonoid-*O*-diglycoside group contained some pairs of compounds, for example, quercetin-3-*O*-rutinoside (**25/28**), kaempferol-3-*O*-rutinoside (**33/36**), isorhamnetin-3-*O*-rutinoside (**31/34**), and myricetin-3-*O*-rutinoside (**20/21**). All the pairs are a combination of structure or confirmation type of sugar (i.e., glucose, galactose).

Some diglycosideflavonols, for example, quercetin-, kaempferol-, and isorhamnetin-3-*O*-rutinoside were previously identified in fermented caper berries [7] as documented by Siracusa et al. [17]. However, Inocencio et al. [8] found only quercetin- and kaempferol-3-*O*-rutinoside in different commercial pickled capers produced in Mediterranean countries.

The second largest group of chemicals with a characteristic structure of flavonoid-*O*-triglycosides identified in capers included compounds **11**, **15**, **16, 17, 19**, **22–24**, **26, 27**, **32**, and **37**. The compounds classified as flavonoid-triglycosides that after deprotonation yielded the ion of the aglycone as base peak turned out to be quercetin (**11**, **15**, **16**, **22**, **24**), kaempferol (**17**, **26**, **32**, **37**), and isorhamnetin (**19**, **23**, **27**).

MS/MS fragmentation of the resulting ions [((M − H)-162)]- (**11**, **15**, **16**, **17**, **19**, **23**, **32**), derived from some triglycoside compounds, was caused by a separation of a hexoside from the rest of the molecule (aglycon + 308 amu). The glycosidic fraction in position 3, indicating diglycosides with an interglycosidic 1→6 bond, was very difficult to break down. Additionally, in these types of compounds, we identified some pairs (**15**/**16**, **17**/**32,** and **19**/**23**) labeled as quercetin-, kaempferol-, and isorhamnetin of -3-*O*-rutinoside-7-hexoside, respectively.

Losses of [(M − H) -146 (rhamnosyl radical)] in compounds **22, 24,** and **37** yielded the deprotonated aglycone + 308 amu (rhamnoside). These compounds were identified as derivatives of -3-*O*-rutinoside-7-*O*-rhamnoside of quercetin (**22**/**24**) and kaempferol (**37**). Similar compounds were identified in edible parts and by-products of date palm (*Phoenix dactylifera* L.) [18].

Compounds **26** and **27** presented deprotonated molecular ions other than the remaining flavonoid-*O*-triglycosides (Table 1). After a loss of one hexose and two rhamnoses, their MS spectra showed a deprotonated aglycone of kaempferol (*m/z* 285). Additionally, the loss of 266 amu (146 + 120) fragment was observed in the MS of a compound produced by the internal cleavage of a rhamnose (−146 amu) at position 6 and involved a hexose from positions 0 and 2 (−120 amu). Similarly to other researchers [20], we found that the loss of 164 (146 + 18, radical rhamnosyl + water) and 146 (radical rhamnosyl) indicated a new glycosylation with another rhamnose moiety. These compounds were then identified as -3-*O*-(2-rhamnoside)-rutinoside of kaempferol and isorhamnetin, respectively. Compound **26** was previously identified in fermented caper berries [7].

Compounds **12**, **13,** and **14** with the highest molar mass at *m/z* 917.11, 901.12, and 931.08 were quercetin-, kaempferol-, and isorhamnetin of -3-*O*-rutinoside-hexoside-7-*O*-rhamnoside, respectively. MS/MS fragmentation showed ions obtained after losing the rhamnosyl at position 7 ([M − H-146). The hexoside must be localized at position 6, as inferred by the presence of the ion [(M − H-162) produced by an internal cleavage of the hexose at positions 0 and 2 and involving the rutinoside at position 6, which is of difficult fragmentation. As far as we know, flavonoids with these masses have not been identified before in capers, but were detected in other plants such as *Bauhinia forficate* L. by Ferreres et al. [16].

Some compounds identified in this work, for example, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucoside-7-*O*-rhamnoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-rhamnorutinoside, and isorhamnetin-3-*O*-rutinoside were also identified in wild caper berries [17,21–23]. Kaempferol-3-rutinoside, quercetin-3-rutinoside, quercetin-7-rutinoside, and quercetin-3-glucoside-7-rhamnoside were isolated from stems and leaves of *C. spinosa* [22]. *C. spinosa* also produced quercetin-3-*O*-[6′-rhamnosyl-6′-glucosyl]-glucoside [22]. Apart from these compounds, compounds **12–14, 17, 19, 23, 24, 26,** and **27** have never before been identified and quantified in caper berries.

2.1.2. Flavan-3-ols

Our caper flower samples yielded five compounds in monomer, dimer, and trimer form, belonging to flavan-3-ols. Identification of compounds **45**, **46**, **47**, and **48** was attained by their comparison with authentic standards. Their *m/z* 289 was exactly the same as for commercial compounds, allowing us to identify compounds **45** and **46** as (+)-catechin and (−)-epicatechin, respectively. Compounds **47** and **49** are a dimer and a trimer of procyanidins with characteristic *m/z* 577 and 865, respectively. MS/MS spectra of these ions showed the retro-Diels–Alder fragmentation as a loss of phloroglucinol A-ring (loss of 126 amu), of heterocycles (loss of 152 amu), and rupture of the interflavan linkage (loss of 288 amu). The last polymeric procyanidin was characterized as procyanidin dimer (**49**) with *m/z* 577, and after MS/MS with *m/z* 289 and 245. Previously, only Jiménez-López et al. [7] identified two dimers and two trimers of procyanidins and (−)-epicatechin.

2.1.3. Hydroxycinnamic Acid Derivatives

Hydroxycinnamic acid derivatives were the second group after flavonol glycosides that contributed to the final concentration of polyphenols in caper flowers. Compounds **1**–**10** were identified on the

basis of their mass and UV spectra characteristic of hydroxycinnamic acid derivatives. Only a few hydroxybenzoic acids (cinnamic acid, *p*-hydroxybenzoic acid, protocatechuric acid, and vanillic acid) and only a few hydroxycinnamic acids (chlorogenic acid, ferulic acid, coumaric-glucoside, 4-feruloylquinic acid, and sinapic acid) were previously identified [24], but in different works and in different parts of *C. spinosa*, such as stem, leaves, flowers, roots, or berries [7,14,17]. Two compounds (**1** and **9**) had fragmentation typical of *p*-coumaric acid at *m/z* 163 and 119 after decarboxylation of coumaric acid ($[M - H]^-$ -CO_2). Compound **9** showed an ion at *m/z* 325 with a daughter ion *m/z* 163 ($[M - H)^-$ -hexose), most probably glucose. Therefore, compounds **1** and **9** were tentatively annotated as *p*-coumaric and coumaric acid-*O*-hexoside, respectively. The presence of coumaric acid-*O*-hexoside was previously reported by other authors [7].

Compounds **2** and **3** exhibited a deprotonated molecular ion at *m/z* 353, and also yielded a fragment ion at *m/z* 191. The MS/MS spectrum *m/z* 191 was likely due to quinic acid ([quinic acid-H]$^-$) ion resulting from a cleavage of the C–O bond of the ester linkage typical of caffeoylquinic acid. Compound **3** yielded near equal *m/z* 173 and 179 at MS/MS, whereas for compound **2,** *m/z* 179 predominated. Therefore, compounds **2** and **3** were identified as 5-caffeoylquinic acid and 4-caffeoylquinic acid, respectively. They were also compared with authentic standards. The present identification corroborated the data published for caper berries by Maldini et al. [14] and Siracusa et al. [17].

Mass spectra of compounds **4** and **5** revealed dominant ions at *m/z* 337, and gave anion signals at *m/z* 191 (quinate) and low intensity ion *m/z* 163 (coumarate), characteristic of 5-*p*-coumaroylquinic acid. Targeted MS/MS experiments showed the same fragmentation patterns without any other intense ions [24]. This confirmed that compounds **4** and **5** were *trans*-5-*p*- and *cis*-5-*p*-coumaroylquinic acid, respectively. 5-*p*-Coumaroylquinic acid was the most abundant *p*-coumaroylquinic acid and was previously identified in caper berries by Siracusa et al. [17].

Fragment ions after MS/MS at *m/z* 191 ($[M - H]^-$—quinic acid) and 173 ($[M - H]^-$—ferulic acid) were observed in three compounds (**6**–**8**), indicating them as derivatives of quinic and ferulic acids with a characteristic pseudomolecular ion at *m/z* 367.

These three compounds are feruloylquinic acid (FQA) with region-isomers eluted as 3-, 5-, and 4-feruloylquinic acid. The three positional isomers were identified by their distinct fragmentation—3-FQA gave an intense MS/MS ion at *m/z* 193 [ferulate], whereas 4-FQA yielded an abundant *m/z* 173 ion and weak ions *m/z* 193 and 191, and for 5-FQA we measured a strong ion at *m/z* 191 (quinate) and a weak ion at *m/z* 173, as suggested by Parveen et al. [24] and Jaiswal et al. [25]. An earlier paper [14] confirmed only the presence of ferulic acid in caper berries, and other authors identified one of these isomers (4-FQA) [17].

We found *m/z* 223.06 with fragments *m/z* 205 ($[M - H]^-$—H_2O), 179 ($[M - H]^-$—CO_2), and 163 ($[M - H]^-$—OCH_3-OCH_3). This compound was evaluated as sinapic acid (**10**) after comparison with an authentic reference substance. Sinapic acid is widely distributed in the Brassicaceae family, and Capparis belongs to Capparidaceae, a family closely related to Brassicaceae. The presence of sinapic acid has already been reported in caper leaves [26].

2.2. Phenolic Compounds' Quantitative Profile

Phenolic composition of capers at different growing stages has not been recognized so far. Total phenolic compounds in the investigated caper varied from 10720 to 3256 mg/100 g dry weight (DW) (Table 2), and depended on a genotype and growing stage.

Table 2. Phenolic composition of caper flowers at different stage of development (mg/kg DW) ‡.

Cultivars	Stages of Development	Hydroxycinnamic Acid				Flavonols					F-3-ols	∑ Total polyphenols
		dpCA	dCA	dpQCA	dFQA	SA	dQ	dK	dISO	dM		
ORI.7	nonpareilles	46.4 ± 2.3	42.4 ± 2.4	18.5 ± 1.4	67.2 ± 2.1	8.3 ± 1.5	6254.9 ± 56	1511.0 ± 14	619.2 ± 12	1643.8 ± 23	513.2 ± 23	10724.9
	surfines	33.3 ± 2.1	30.1 ± 1.3	19.4 ± 1.6	90.1 ± 3.8	11.2 ± 1.1	5218.3 ± 57	2331.3 ± 15	618.3 ± 13	1607.4 ± 32	304.3 ± 19	10263.6
	capucines	19.3 ± 1.4	19.2 ± 2.1	20.8 ± 2.5	34.6 ± 2.6	9.6 ± 1.5	2245.6 ± 34	2133.5 ± 23	437.0 ± 21	1084.0 ± 11	670.7 ± 32	6674.3
	capotes	31.4 ± 2.1	16.6 ± 1.7	16.5 ± 1.7	24.3 ± 3.1	11.6 ± 1.6	2711.6 ± 21	1694.1 ± 14	355.2 ± 15	1046.1 ± 18	507.7 ± 26	6415.1
	fines	18.9 ± 1.6	20.2 ± 1.6	11.5 ± 1.4	25.4 ± 1.6	1.4 ± 0.4	1657.6 ± 32	815.0 ± 24	125.5 ± 17	361.6 ± 25	305.0 ± 29	3342.2
	gruesas	10.8 ± 0.9	22.9 ± 2.1	3.5 ± 2.6	13.7 ± 1.3	2.0 ± 0.2	1453.2 ± 19	979.1 ± 21	119.3 ± 10	317.7 ± 21	315.0 ± 32	3237.3
ORI.10	nonpareilles	32.9 ± 3.1	15.6 ± 2.7	5.4 ± 0.8	47.0 ± 1.9	5.0 ± 0.7	2375.6 ± 26	1885.1 ± 27	229.2 ± 9	760.6±25	208.0 ± 37	5564.5
	surfines	29.5 ± 2.7	14.8 ± 2.5	7.5 ± 1.1	51.1 ± 2.7	8.9 ± 1.1	2528.9 ± 21	1753.0 ± 31	230.8 ± 11	804.9 ± 31	344.0 ± 35	5773.4
	capucines	34.0 ± 4.1	17.2 ± 1.9	6.9 ± 0.5	14.4 ± 3.1	13.0 ± 0.9	2660.7 ± 22	1302.6 ± 16	141.0 ± 11	802.9 ± 27	366.8 ± 28	5359.4
	capotes	29.4 ± 2.5	15.3 ± 2.7	5.4 ± 0.9	21.5 ± 2.6	13.1 ± 1.1	2603.6 ± 32	1052.3 ± 14	225.7 ± 16	1018.7 ± 28	511.9 ± 26	5497.0
	fines	10.9 ± 1.8	15.6 ± 2.3	2.5 ± 0.3	14.7 ± 2.9	0.5 ± 0.2	2257.8 ± 15	503.4 ± 19	200.2 ± 14	430.1 ± 34	547.2 ± 21	3982.9
	gruesas	6.4 ± 0.4	11.5 ± 1.3	1.8 ± 0.4	14.4 ± 1.1	0.7 ± 0.1	2124.8 ± 19	532.5 ± 17	202.2 ± 18	322.6 ± 18	476.9 ± 22	3693.9
Stage of development	nonpareilles	a	a	a	a	b	a	c	a	a	a	a
	surfines	b	b	a	a	a	b	a	a	a	b	a
	capucines	c	bc	ab	b	ab	c	b	b	b	a	b
	capotes	d	c	b	c	ab	c	d	c	b	b	b
	fines	de	c	c	d	c	c	e	c	c	c	c
Cultivars	ORI.7	a	a	a	a	a	a	a	a	ab	a	a
	ORI.10	b	b	b	b	b	b	b	b	b	b	b

‡ Results are expressed as mean ± standard deviation of three determinations. F-3-ols—flavan-3-ols; dQ—derivatives of quercetin; dK—derivatives of kaempferol; dISO—derivatives of isorhamnetin; dM—derivaties of myricetin; dpCA—derivatives of *p*-coumaric acids; dCA—derivatives of caffeoylquinic acids; dpQCA—derivatives of *p*-coumaroylquinic acids; dFQA—derivatives of feruloylquinic acids; SA—sinapic acid; F-3-ols—sum of polymeric procyanidins; a, b, c letter were significantly different ($p < 0.05$) according to Duncan's test;

Phenolic content in Orihuela nº 7 (ORI.7) sample was significantly higher than in Orihuela nº 10 (ORI.10). Among six different growing stages, the one called 'nonpareilles' was characterized by significantly higher content of polyphenols than the other stages. Nonpareilles caper flowers of ORI.7 showed three times higher content of phenolic compounds than 'fines' or 'gruesas' flowers, but for ORI.10 the differences were smaller. The content of phenolic compounds in caper is higher than in some exotic and common fruits and vegetables. Consumers value food with a high level of bioactive substances, such as polyphenols, as they are known to be the most abundant antioxidants in our diet.

Our analyses of caper phenolic compounds identified flavonols as the major polyphenolic group, representing on average 80% to 95% of all phenolics, irrespective of the growing stage. Flavan-3-ols (with abundance of 3% to 14%) took the second place, and phenolic acids (1% to 5%) were the third. These results corroborated those from previous reports [6,9,15], but our work focused mainly on flavonol content. High content of flavonols may reflect plant response to biotic and abiotic stress or acclimation to environmental stressors such as heat, cold, UV radiation, drought, salinity, or an attack of herbivores or pathogens [27]. Several studies showed the effect of temperature and sunlight on flavonoid accumulation in the flower and berry or fruit skin. Wang and Zheng [27] found that strawberries grown at 18/12 °C generally had the lowest anthocyanin, flavonol (quercetin-3-*O*-glucoside and quercetin-3-*O*-glucoronide), and phenolic acid contents, whereas at 30/22 °C their content was the highest. Solovchenko and Schmitz-Eiberger [28] demonstrated quercetin glycosides to be the principal group of flavonoids accumulated in apple skin in response to high sunlight. Lee et al. [29] reported a positive correlation between the duration of exposure to sunshine and flavonoid content in the leaves of *Angelica keiskei*.

Ultraviolet C (UV-C) induced accumulation of flavonols and activity of the enzymes of phenylpropanoid pathway at the beginning of growth during the first 3 days, but later on the effects ceased [30]. Additionally, strong sun exposure improves synthesis and glycosylation of flavonols with various sugar molecules and synthesis of larger number of compounds from the flavonoid biosynthesis pathway, with dihydrokaempferol serving as a parent compound for kaempferol, quercetin, isorhamnetin, and myricetin [30].

These results confirm that capers are a very rich source of phenolic compounds, especially flavonols, flavan-3-ols, and hydroxycinnamic acid. Total flavonols in caper flowers comprise a mixture of different glycosylated quercetin, isorhamnetin, kaempferol, and myricetin derivatives.

Quercetin, kaempferol, myricetin, and isorhamnetin derivatives represented, respectively, 38%–67%, 15%–36%, 4%–7%, and 0.85–3% of total flavonols in caper flowers, but their contents strongly depended on the growth stage. 'Nonpareilles' and 'surfines' were richer in flavonols than fines and gruesas. The content of flavonols in 'gruesas', as compared with 'nonpareilles' dropped by 4.3, 1.5, 5.2, and 5.2 times for quercetin, kaempferol, isorhamnetin, and myricetin derivatives in ORI.7 capers. A similar tendency was observed for ORI.10 capers. The smallest differences between individual stages were spotted for 'nonpareilles' and 'surfines' or for 'capucines' and 'capotes'. Small caper flowers of the size below 7–8 mm were richer in flavonols than larger flowers. A similar effect was observed for honeysuckle berries and the content of anthocyanins [31].

Diglycoside structure (77%–85%) of flavonols predominated over monoglycosides (11%–21%), triglycosides (2%–6%), and tetraglycosides or aglycone (<2%). Quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside were the major flavonols. The most abundant among the remaining identified flavonoids were myricetin-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside-7-*O*-hexoside, and isorhamnetin-3-*O*-hexoside (Table 2). Siracusa et al. [17] and Inocencio et al. [8] reported -3-*O*-rutinoside of quercetin, kaempferol, and isorhamnetin as major flavonoids in *C. spinosa*, which was concurrent with this work.

The minor flavonols in caper flowers identified for the first time included isorhamnetin-3-*O*-rutinoside-hexoside-7-*O*-rhamnoside, kaempferol-3-*O*-rutinoside-7-*O*-hexoside, myricetin-3-*O*-hexoside, isorhamnetin-3-*O*-rutinoside-7-*O*-hexoside, and isorhamnetin-3-*O*-(2-rhamnoside)-rutinoside.

Quercetin-3-*O*-rutinoside content in caper flowers was higher than in onion (~120 mg/100 g), thyme (~ 2490 mg/100 g), or buckwheat (~5350 mg/100 g), and it was one of the most common quercetin glycosides [8]. Caper flowers therefore seem to be an abundant source of quercetin-3-*O*-rutinoside and the other flavonols. Inocencio et al. [8] suggested that 10 g of *C. spinosa* would provide approximately 65 mg of flavonoid glycosides in our diet. Flavonol derivatives, especially quercetin, are very important for human health [18]. Their consumption reduces the risk of cardiovascular disease due to their anti-hypertensive and anti-platelet aggregating properties, and decreases low-density lipoprotein (LDL) cholesterol levels [17]. Flavonol accumulation in fruit skin as a result of sunlight exposition is well documented and is the most important environmental factor inducing flavonol biosynthesis. Fruits with sun-exposed peel have higher levels of anthocyanins and flavonols than those grown in the shade [30].

Polymeric procyanidins classified as flavan-3-ols were determined using phloroglucinol methods. They showed that polymeric procyanidins in caper flowers consisted mainly of polymer unit of (−)-epicatechin rather than (+)-catechin. In our study, polymeric procyanidins in the total phenolic pool accounted for no more than 14%, and their concentration ranged from 200 mg to 700 mg/100 g. Jiménez-López et al. [7] postulated that fermented caper still contained (–)-epicatechin and (epi)catechin dimer and trimer in the amount of 160 mg/100 g. Regarding flavonols, 'nonpareilles' had similar content of polymeric procyanidins to 'gruesas'. 'Capucines', 'capotes', and 'fines; exhibited higher levels of polymeric procyanidin than capers at other growth stages. Zhang et al. [1] reported that accumulation of flavan-3-ols in Cabernet Sauvignon grape depended on a developmental stage and corresponded to supplemental UV light. In fact, UV radiation increased the levels of flavan-3-ol during the berry development but not in the mature berries [1]. Francesca et al. [6] and Maldini et al. [14] did not identify any compounds belonging to flavan-3-ols in caper berries.

Quantitative analysis of polymeric procyanidins revealed their higher content in ORI.7 capers versus ORI.10 sample. Some researchers [8] reported that flavan-3-ols present in caper extracts might be responsible for antiparasitic activity and act as synthetic phenolic anthelmintics.

Hydroxycinnamic acids belonged to a minor group of caper flower polyphenols. Their concentration ranged from 178.6 to 71.8 mg/100 g for ORI.7 and from 283.4 to 44.8 mg/100 g for ORI.10. Similarly to flavonols, the content of hydroxycinnamic acids in 'nonpareilles' was higher (1.2 and 6.3 times for ORI.7 and ORI.10, respectively) than in 'gruesas'. The predominant hydroxycinnamic acids in caper flowers were feruloylquinic acid (19%–51% and 45%–61% for ORI.7 and ORI.10, respectively) and caffeoylquinic acid (17%–32% and 5%–26% for ORI.7 and ORI.10, respectively). The remaining acids were present at low concentrations, that is, sinapic acid constituted less than 2%–14%. Literature data on hydroxycinnamic acids are sparse.

Siracusa et al. [17] identified 5- and 4-caffeoylquinic, 5-*p*-coumaroylquinic, and 4-feruloylquinic acids, and postulated 5-caffeoylquinic and 4-feruloylquinic acids as the major compounds. Jiménez-López et al. [7] identified some hydroxycinnamic acids in fermented caper (coumaric acid-*O*-hexoside), but did not quantify them.

Hydroxycinnamic acids such as 5-caffeoylquinic and caffeoylquinic acid are good sources of antioxidants *in vitro* that protect low-density lipoprotein (LDL) from oxidation and, therefore, supposedly prevent various age-related diseases. Sinapic acid present in caper flowers conveys their bitter taste and astringency, similarly to rape products.

2.3. Biological Potential of Caper Flowers

We evaluated biological potential of caper flowers at different growth stages on the basis of their antioxidant activity (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation ($ABTS^{\bullet+}$), ferric-reducing antioxidant power (FRAP), and Oxygen Radical Absorbance Capacity (ORAC), antidiabetic activity (α-amylase and α-glucosidase), and cholinesterase inhibition (acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)). The biological potential of caper flowers clearly depended on their content of bioactive compounds and growth stage.

The ORAC for nonpareilles was significantly higher than for 'gruesas' (1.7 times for ORI.7 and 1.9 times for ORI.10). 'Nonpareilles' capers of ORI.7 exhibited higher antioxidant activity (27.7 mM Trolox/100 g) than 'nonpareilles' ORI.10 (19.3 mM Trolox/100 g) (Table 3). Capers at 'gruesas' stage had the lowest antioxidant activity of 16.8 and 10.7 mM Trolox/100 g for ORI.7 and ORI.10, respectively. The ABTS and FRAP assays showed the same trend as the ORAC assays.

α-Amylase and α-glucosidase catalyze digestion of oligo- and disaccharides into absorbable monosaccharides. Caper flower extracts inhibited these enzymes, with IC_{50} values ranging from 3.74 to 0.93 mg/mL for α-glucosidase and from 3.68 to 1.52 mg/mL for α-amylase. ORI.7 'nonpareilles' extract turned out to be an effective potential inhibitor of α-glucosidase and α-amylase. This was not true for the ORI.10 sample, where 'gruesas' extract much more effectively inhibited α-amylase and 'capotes' extract showed high inhibitory potential towards α-glucosidase. Our results are comparable with those reported by Adriano Mollica et al. [26], where aqueous extracts of *C. spinosa* exhibited a potent anti-hyperglycemic activity in diabetic rats. Other authors [32] suggested alkaloids, saponins, terpenes, and phenolics as major compounds responsible for anti-diabetic effect.

As far as we know, this is the first report on cholinesterase inhibition by caper flowers. The ability of caper flowers to inhibit AChE and BuChE was evaluated *in vitro*. In all cases, the inhibition closely depended on the growth stage and cultivar. In the ORI.7 sample, maximum inhibition of AChE and BuChE reached 18.3% and 31.0%, respectively, and in ORI.10 sample it was 28.1% and 33.8%, respectively. 'Nonpareilles' flowers turned out to be the most potent inhibitors. Moreover, the suppression of BuChE was more effective than that of AChE. This is desirable, as peptidase activity of butyrylcholinesterase controls the development and progression of Alzheimer's disease. The extracts capable of BuChE inhibition may also prevent the disease progression caused by β-amyloid protein accumulation, as they help to diffuse the β-amyloid plaques [33]. Alzheimer's disease is the most common cause of dementia in the elderly, being characterized by degeneration of cholinergic neurons in specific areas of the brain associated with higher intellectual functions, memory, and consciousness [16].

Polyphenolics are known for their high biological activity that is determined by their individual composition. Ferreres et al. [16] postulated that antioxidant activity of flavonoids results from their structure, especially a free hydroxyl group at 4' present in all derivatives, a double bond between C2-C3 present in all identified compounds, a free hydroxyl group at 3' found only in quercetin and kaempferol (**34**, **35**; Table 1), and a di-hydroxyl group at *orto* position in B-ring found only in quercetin and myricetin derivatives. Therefore, a possible explanation of high antioxidant activity of caper flowers, especially at 'nonpareilles' stage, is high concentration of quercetin and its derivatives.

Studies focused on anti-diabetic properties and cholinesterase inhibition often investigated the structure of analyzed compounds. Mandatory structural features for anti-AChE activity include the presence of *O*-glycosylation at C7 rather than at C3 that diminishes cholinesterase inhibition, a double C2–C3 bond present only in some compounds identified in caper flowers, and methoxylation at C4, not detected in caper flowers but determined for C3 in isorhamnetin derivatives that had no effect on this activity [19,34,35]. For BuChE inhibition, methoxylation at C4 is not so important [36]. Quercetin-3-*O*-galactoside (absent in caper flowers) at 90 μg/mL was capable of reducing AChE by 55% [36]. Caper flowers contained mainly compounds with -3-*O*-glycosylation (mono, di, tri, tetra), so we did not expect them to be effective cholinesterase inhibitors.

As postulated by Ferreres et al. [16], anti-diabetic activity requires a hydroxyl group at position 4' typical for quercetin, myricetin, and kaempferol structure, and a hydroxyl group at position 3' typical for compounds of quercetin, isorhamnetin, and myricetin structure.

Table 3. Antioxidant (ABTS, FRAP, ORAC), anti-diabetic (α-amylase and α-glucosidase), and cholinesterase's (AChE and BuChE) inhibition of caper flowers at different stages of development ‡.

Cultivars	Stages of Development	Antioxidant Activity [mmol Trolox/100 g]			Anti-Diabetic Activity [IC_{50}; mg/mL]		Cholinesterase's Inhibition [% of Inhibition]	
		ABTS	FRAP	ORAC	α-amylase	α-glucosidase	AChE	BuChE
ORI.7	nonpareilles	6.92 ± 0.54	7.51 ± 0.11	27.66 ± 1.43	3.15 ± 0.11	2.98 ± 0.11	18.3 ± 0.1	31.0 ± 2.4
	surfines	6.89 ± 0.12	7.23 ± 0.47	25.52 ± 1.11	2.10 ± 0.05	2.47 ± 0.13	15.9 ± 0.3	20.1 ± 1.8
	capucines	6.53 ± 0.32	7.03 ± 0.72	22.97 ± 0.99	1.94 ± 0.21	2.32 ± 0.14	14.5 ± 0.7	28.4 ± 3.8
	capotes	6.05 ± 0.14	6.35 ± 0.32	22.25 ± 0.57	1.72 ± 0.13	2.20 ± 0.11	13.8 ± 1.7	20.5 ± 1.1
	fines	5.16 ± 0.21	6.76 ± 0.54	20.79 ± 1.17	1.30 ± 0.11	1.89 ± 0.11	12.1 ± 1.0	18.0 ± 1.2
	gruesas	3.56 ± 0.11	4.48 ± 0.38	16.77 ± 1.21	0.93 ± 0.07	1.52 ± 0.09	10.5 ± 0.9	11.4 ± 0.9
ORI.10	nonpareilles	6.82 ± 0.21	7.64 ± 0.51	19.27 ± 0.99	3.74 ± 0.99	4.46 ± 0.15	28.1 ± 2.0	33.8 ± 2.3
	surfines	5.83 ± 0.11	6.45 ± 0.58	18.55 ± 0.60	3.23 ± 0.21	3.68 ± 0.19	17.8 ± 1.3	25.2 ± 2.1
	capucines	5.45 ± 0.43	6.54 ± 0.32	16.45 ± 2.32	2.67 ± 0.15	2.38 ± 0.10	15.4 ± 0.9	23.4 ± 1.6
	capotes	2.43 ± 0.11	3.68 ± 0.24	15.29 ± 1.12	2.14 ± 0.21	2.34 ± 0.13	13.5 ± 1.3	20.4 ± 1.4
	fines	2.01 ± 0.09	2.83 ± 0.44	13.59 ± 1.43	1.71 ± 0.32	2.02 ± 0.06	13.4 ± 1.4	19.2 ± 1.8
	gruesas	0.54 ± 0.04	1.69 ± 0.37	10.09 ± 2.01	1.45 ± 0.15	1.97 ± 0.89	10.4 ± 1.2	8.6 ± 1.2
Stage of development	nonpareilles	a	a	a	a	a	a	a
	surfines	a	a	ab	a	b	b	ab
	capucines	a	ab	ab	ab	c	bc	ab
	capotes	b	b	b	b	c	c	b
	fines	b	b	b	bc	d	c	b
Cultivars	ORI.7	a	a	a	a	a	a	a
	ORI.10	b	b	b	b	b	b	b

‡ The results are shown as mean ± standard deviation of three assays performed in triplicate. AChE—acetylcholinesterase; BuChE—butyrylcholinesterase. (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (ABTS•+), ferric-reducing antioxidant power (FRAP), and Oxygen Radical Absorbance Capacity (ORAC), a, b, c letter were significantly different ($p < 0.05$) according to Duncan's test;

Table 4 presents Pearson's correlation coefficient for polyphenol content and biological activity: antioxidant activity (ORAC, ABTS, FRAP), cholinesterase inhibition (AChE, BuChE), and antidiabetic activity (α-amylase and α-glucosidase). Significant and strong correlation can be noticed between ORAC and flavonols (sum of quercetin, kaempferol, isorhamnetin, and myricetin derivatives; above $r = 0.684$) and phenolic acid (sum of caffeic acid, *p*-coumaroylquinic acid, and feruloylquinic acid). We also found a positive correlation between phenolic compounds and cholinesterase inhibition (AChE, BuChE) and antidiabetic activity (α-amylase and α-glucosidase).

Table 4. Pearson's correlation coefficients between in vitro biological activity methods and polyphenols content in caper flowers.

Accession	ABTS	FRAP	ORAC	AChE	BuChE	α-amylase	α-glucosidase
∑ Flavonols	0.666	0.598	0.805	0.393	0.547	0.497	0.357
∑ Quercetin derivatives	0.474	0.419	0.684	0.285	0.408	−0.301	−0.220
∑ Kaempferol derivatives	0.855	0.791	0.737	0.550	0.641	0.025	−0.438
∑ Isorhamnetin derivatives	0.556	0.466	0.786	0.226	0.423	−0.422	−0.181
∑ Myricetin derivatives	0.652	0.585	0.790	0.348	0.571	−0.217	−0.403
∑ Aglycone of flavonols	0.460	0.425	0.635	−0.170	0.278	−0.140	−0.257
∑ Mono-*O*-glycosides of flavonols	0.546	0.513	0.677	0.490	0.601	0.638	0.464
∑ Di-*O*-glycosides of flavonols	0.480	0.425	0.689	0.291	0.412	0.434	0.258
∑ Tri-*O*-glycosides of flavonols	0.546	0.468	0.806	0.183	0.379	0.224	0.105
∑ Tetra-*O*-glycosides of flavonols	0.479	0.420	0.784	0.027	0.251	0.092	−0.023
∑ Phenolic acid	0.571	0.582	0.287	0.866	0.765	0.925	0.871
∑ *p*-CA derivatives	0.737	0.730	0.694	0.605	0.732	0.085	−0.607
∑ CA derivatives	0.506	0.487	0.772	0.120	0.309	−0.458	−0.398
∑ *p*-QCA derivatives	0.747	0.699	0.906	0.102	0.445	−0.286	−0.371
∑ FQA derivatives	0.676	0.628	0.753	0.498	0.496	−0.268	−0.233
SA	0.496	0.460	0.428	0.204	0.434	0.270	−0.516
∑ flavan-3-ols	−0.239	−0.307	−0.009	−0.403	0.005	−0.268	0.153

pCA—derivatives of *p*-coumaric acids; CA—derivatives of caffeoylquinic acids; *p*-QCA—derivatives of *p*-coumaroylquinic acids; FQA—derivatives of feruloylquinic acids; SA—sinapic acid; correlation is significant at the 0.05 level.

However, we only noticed a negative correlation between polymeric procyanidins and the biological activity test, except for BuChE, where it was not significant.

Mono- and di-flavonols played a greater role in biological activity (especially antioxidant activity (ORAC), α-amylase, and BuChE inhibition) than aglycone or tetra-flavonols. Additionally, phenolic acids showed higher anti-diabetic potential and anticholinesterase activity than antioxidant activity (FRAP > ABTS > ORAC). These outcomes demonstrated that we should not ignore the role of non-phenolic components of caper flowers, as they may exhibit still unknown synergic or antagonist effects. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3. Materials and Methods

3.1. Chemicals

Flavonols (quercetin-, kaempferol-, myricetin, and isorhamnetin: -3-*O*-glucoside, -3-*O*-galactoside, -3-*O*-rutinoside) and hydroxycinnamic acid (sinapic, *p*-coumaric, 5-caffeoylquinic acids) with a purity of HPLC standards were obtained from Extrasynthese (Lyon, France). The LC–MS and chromatography grade solvents as methanol and acetonitrile were procured from POCH S.A. (Gliwice, Poland). Formic acid and the remainder of solvents were purchased from Sigma-Aldrich (Darmstadt, Germany). Deionized water was made using a HLP SMART 1000s purification system (Hydrolab, Gdansk, Poland). The supernatant before all LC–MS and UPLC analysis was filtered through a Hydrophilic PTFE 0.20 µm membrane (Millex Samplicity Filter; Merck, Darmstadt, Germany).

3.2. Plant Material

The caper flowers (*Capparis spinosa* L.) at six stages of development of cultivars Orihuela nº7 (ORI.7) and Orihuela nº10 (ORI.10) were collected at the experimental field station of Miguel Hernández University in the province of Alicante, Spain (38°5'N, 0°56'W, 23.6 masl) and classified by size (Figure 2) as 'nonpareilles' (∅ 0–7 mm), 'surfines' (∅ 7–8 mm), 'capucines' (∅ 8–9 mm), 'capotes' (∅ 9–11 mm), 'fines' (∅ 11–13 mm), and 'gruesas' (∅ > 13 mm) (Boletin Oficial del Estado, 1984). The samples were hand-collected provided from University Miguel Hernández, Orihuela, Spain, in September 2017, and were taxonomically identified by an expert botanist from the Department of Plant Sciences and Microbiology using the protocol by García-Rollán [37]. One voucher of each cultivar is kept in the Miguel Hernández University herbarium (#072010 and #102010). All samples were freeze-dried, ground in a laboratory mill, and stored at −80 °C to prevent degradation until analysis.

Figure 2. Flower bud classification by size.

3.3. Separation, Identification, and Quantification of Polyphenolic Compounds

For polyphenolic compounds, three individual sample replicates from each freeze-dried caper (max. 0.5 g each) were extracted by 5 mL of 1% formic acid in a 30% methanol solution, as described previously by Wojdyło et al. [38]. Identification and quantification of phenolic compounds by LC-qTOF-MS/MS and UPLC-PDA-FL, respectively, was analyzed as described previously by [38]. The analysis of polymeric procyanidins by phloroglucinol method was performed according to the protocol described previously by [39]. The results were expressed as miligrams per 100 grams dry weight (DW).

3.4. Determination of Biologically Activity: Anti-Oxidant, Anti-Diabetic, and Cholinesterase Inhibition

Freeze-dried caper samples weighing (~0.5 g) were extracted with 10 mL of methanol:H_2O:acetic acid (80%:20%:1%, v/v/v) following the procedure described previously by Wojdyło et al. [38].

The $ABTS^{\bullet+}$ (2,2′-azine-bis-(3-ethylene-benzothiazoline-6-sulfonic acid) scavenging test was based on measuring the decrease in the color intensity inversely proportional to the antioxidant content measured by Re et al. [39]. An $ABTS^{\bullet+}$ solution was prepared with an absorbance of 0.700 ± 0.02 at

a wavelength of 734 nm. Caper extracts and the $ABTS^{\bullet+}$ solution were mixed, and after 6 min the absorption at the wavelength was measured as above. Distilled water was the blank.

The FRAP method involves determining the ability to reduce Fe3+ ions by antioxidant substances contained in sea buckthorn extracts to the blue Fe^{2+} ions complex, according Benzie et al. [40]. The absorbance of the caper extract was measured 10 min after the addition of FRAP reagent (acetate buffer, 2,4,6-Tris(2-pyridyl)-s-triazine, in HCl, and $FeCl_3 \times 6H_2O$ in a volume ratio of 10:1:1, v:v:v) at a wavelength of 593 nm.

The analysis of oxygen radical absorbance capacity (ORAC) consists of spectrofluorometric measurement of the decrease in fluorescence caused by oxidation of a fluorescent substance under the influence of free radicals; according Ou et al. [41]. Samples containing sea buckthorn extract, phosphate buffer, and fluorescein were incubated at 37 °C throughout the analysis period. 2,2′-Azobis(2-amidinopropane)dihydrochloride was added and spectrofluorometric measurement and was performed every 5 min at excitation wavelength 493 nm and emission wavelength 515 nm. The blank was a phosphate buffer. The antioxidant activity of the tested samples was obtained by comparing the surface under the fluorescence decrease curves over time with the surface for Trolox solution.

The antioxidant activity ($ABTS^{\bullet+}$, FRAP, ORAC) was expressed as mmol of Trolox per 100 grams.

The α-amylase and α-glucosidase inhibitory effect of the caper extracts was assayed according to the procedure described previously by Wojdyło et al. [38,42].

Briefly, analysis of anti-α-amylase inhibitory activity was based on spectrophotometric measurement of color change as a result of reaction of iodine in potassium iodide with remaining starch after enzymatic hydrolysis. After incubation at 37 °C, for caper extract samples with starch solution and α-amylase, the reaction was stopped using 0.4 M HCl, and solution of potassium iodide was added to obtain color. Reference samples contained phosphate buffer instead of enzyme. Absorbance was measured at 600 nm.

The analysis of α-glucosidase inhibitory activity consisted of the reaction of the enzyme with a β-D-glucosidase substrate, producing a yellow solution upon cleavage. Basic samples containing caper extract and enzyme were incubated as above. After addition of substrate, the mixture was incubated again at 37 °C and measurement was made at 405 nm. As in the above analysis, the reference samples contained buffer instead of enzyme.

The results of α-amylase and α-glucosidase were expressed as IC_{50} (mg/mL).

Cholinesterase inhibition was measured as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) methods described by [38,43]. The reaction mixture was composed of caper extract sample, Tris–HCl buffer (pH 8), acetylthiocholine iodide or S-butyrylthiocholine iodide, and 5,5′-dithiobis(2-nitrobenzoic acid), and after incubation at 37 °C for 10 min, AChE or BuChE solution was added. Absorbance was measured after 15 min at a wavelength of 412 nm. The results were expressed as percent of inhibition.

All determinations of biological activity were assayed in triplicate and performed using the UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan).

3.5. Statistical Analysis

Mean values ± standard deviation were conducted for polyphenolic compounds in caper samples. The mean values were subjected to analysis of variance and Duncan's multiple range test for mean comparison ($p = 0.05$) and Pearson's correlation by using Statistica version 13.0 (Stat-Soft, Kraków, Poland).

4. Conclusions

This is the first study that characterized the phenolic profile of caper flowers by LC–qTOF-MS/MS and provided a fingerprint for future quality control of this species. The research confirmed that caper flowers are a valuable source of polyphenolics with biological activity. The main constituents of

the flowers included flavonols (quercetin, kaempferol, myricetin, and isorhamnetin), phenolic acids, and flavan-3-ols. Nine compounds were reported for the first time in caper flowers, which have never before been identified and quantified in caper berries. Total phenolic compounds in the investigated caper varied from 10,720 to 3256 mg/100 g DW, and depended on a genotype and growing stage. Of the six investigated growth stages, nonpareilles accumulated the greatest amounts of bioactive compounds that correlated with anti-oxidant and anti-diabetic properties, and were more potent BuChE than AChE inhibitors. Both analyzed caper cultivars (ORI.7 and ORI.10) are promising, but should be constantly improved through breeding programs to refine their functional properties. Total polyphenol compounds in nonpareilles was 3982.9 and 3693.9 mg/100 g DW for ORI.7 and ORI.10, respectively, and the dominant fraction were flavanols (~80%–85% of total polyphenol fraction). Further studies on caper flower composition should investigate nutritional values, and it is necessary to have more data from clinical trials and toxicity tests.

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4.5. Publicación V

PUBLICACIÓN V

Volatile Profile in Different Aerial Parts of Two Caper Cultivars

(*Capparis spinosa* L.)

Mar Grimalt, Lucía Sánchez-Rodríguez, Francisca Hernández, Pilar Legua, Ángel A. Carbonell-Barrachina, María S. Almansa, and Asunción Amorós

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Research Article

Volatile Profile in Different Aerial Parts of Two Caper Cultivars (*Capparis spinosa* L.)

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This research presents, for the first time, full volatile profiles of four aerial parts of caper plants (*Capparis spinosa* L.) from southeastern Spain. Volatile compounds in caper leaves and stems (together), flowers, flower buds, and fruits from two cultivars were identified and quantified using headspace-solid phase microextraction (HS-SPME) and gas chromatography with a mass spectrometry detector (GC-MS). Forty-three volatile compounds were identified in the caper shoots, 32 in caper flowers, with only 18, 10, and 6 compounds being found in flower buds, leaves, and fruits, respectively. The predominant compound in all studied materials was methyl isothiocyanate, with nerolidol, trans-2-hexenal, and nonanal playing key roles in flowers, leaves, and flowers buds, respectively. The two studied cultivars had the same volatile compounds but at very different concentrations, although the two studied cultivars are cultivated under the same climatic and agronomic conditions. Additionally, the predominant compounds, especially methyl isothiocyanate (6882 $mg \cdot kg^{-1}$ fw in flower buds of ORI 3 cultivar), can be separated and concentrated for future applications in food technology.

1. Introduction

Capparis spinosa L. (Capparaceae) has its origin in the regions of Central or Western Asia, but now, it is distributed in Southern Europe, North and East Africa, Madagascar, Southwest and Central Asia, Philippines, Indonesia, Papua New Guinea, Australia, and Oceania [1]. The species of the genus *Capparis* have been widely studied and used for edible or medicinal purposes, for its content in bioactive compounds and its numerous beneficial effects on antisclerosis, antihypertensive, anti-inflammatory, analgesic, anti-asthmatic, antihyperlipidemic, hepatoprotective, antibacterial, and antifungal agents and diuretics, astringents, and tonics properties [2]. The infusion of stems and root crust were used as antidiarrheal and febrifuge remedies; fresh fruits were used for sciatica and dropsy; the combination of dried fruit and powder with honey was used to treat colds, gout, sciatica, and back pain, and it was also applied in the body for the treatment of epilepsy. Besides, the seeds were used to treat female sterility and to alleviate toothache [3]. Currently, the flower buds are marketed together with the caper fruit and the tenderest stems (the last 10 cm); before their commercialization, they are conserved in brine and are used as condiments and ingredients in salads and snacks.

There are works describing the main physicochemical characteristics of the caper fruits at 3 phenological states [4] and of the flower buds at 6 stages of development [5] and the phenological profile in Spanish cultivars [6]. Besides, there are few previous studies describing the volatile profiles *Capparis spinosa* L. cultivars. Cultivars from Morocco, Iran,

and the Eolian Archipelago have been studied by Brevard et al. [7], Afsharypuor et al. [8], Romeo et al. [9], and Condurso et al. [10], respectively. The first two works used distillation-based and volatile extraction techniques. The volatile compounds generally show a great variability, which depends on climatological conditions, cultivar, maturity, and storage conditions among other factors [11].

According to our knowledge, this is the first work studying the chemical volatile profile of capers grown in Spain (Orihuela, Alicante), and it is intended to evaluate the different aerial parts: stems and leaves, flowers, flower buds, and caper fruit. The main innovative aspect of this study is that capper flowers are evaluated for the first time. They are edible, have a large size with white petals, delicate violet stamens, and specific aroma. These flowers can be introduced in modern dishes to make them more visual attractive, but their aroma and flavor must be also attractive to consumers and specially not having off-flavors. The rest of the aerial capper parts are also edible and have specific aroma and flavor. Thus, it is interesting to know the volatile composition of all cappers parts to be able to fully understand their potential role in modern cuisine, among other applications. For that purpose, headspace solid phase microextraction (HS-SPME) was used to isolate the volatile compounds, while the gas chromatography with a mass spectrometry detector (GC-MS) and FID detector (GC-FID) were used to identify and quantify these compounds, respectively. These analyses were conducted in the aerial parts of caper plants of two cultivars grown on the same plot. The information generated will help us understand the cultivar differences that may exist in the composition of volatile compounds in two cultivars grown under the same agronomic conditions.

2. Materials and Methods

2.1. Plant Material and Sample Processing. This research has been carried out in the different aerial parts of two caper cultivars, "Orihuela 3" ("ORI3") and "Orihuela 7" ("ORI7"). This caper cultivars were collected at the experimental field station of Miguel Hernandez University located in Orihuela (Alicante, Spain, 02°03′50″E, 38°03′50″N, and 25 masl). According to the agroclimatic classification of Papadakis [12], the experimental plot has a subtropical Mediterranean climate. The annual mean temperature is 19°C, with mild winters (11°C in January) and hot summers (28°C in August). A mean annual precipitation of 300 mm was recorded for the year of study, mostly falling in spring and autumn. The soil was a clay loam Xerofluvent [13], which showed high active calcium carbonate and low organic matter content, electrical conductivity, available phosphorus, and potassium exchange levels (Table 1). The irrigation water had a Cl^- concentration of 71–84 mg L^{-1} and an electrical conductivity of between 1.4 and 1.6 dS m^{-1}. The irrigation was with a dropper, watering 3 h per day, 5 days a week in the months of June–September, but in the months of October–December, watering was carried out 2 h per day, 3 days a week. A background fertilizer was made in autumn with 400 kg·ha^{-1} of 18% calcium superphosphate, 150 kg·ha^{-1} of 50% KCl, and 100 kg·ha^{-1} of 21% $(NH_4)_2SO_4$. A pruning was carried out in late autumn, leaving only the live vine as recommended by Melgarejo [14].

The plant material selection was manually carried out in July 2017. The different aerial parts collected during the process were classified into stems and leaves (together), flowers, flower buds, and caper fruits. Three different batches of samples were prepared using 10 uniform sampling units (stems and leaves, flower, flower buds, or fruits) of each cultivar; sampling units were manually picked at the same ripening stage and immediately were transported to the laboratory for preparation and further analyses. In this way, 30 sampling units per cultivar were used for the analyses. Once in the laboratory, the samples were washed with tap water for 2 min, frozen using liquid nitrogen, grinded for 10 s in a grinder (Taurus Aromatic Ver II; Taurus Group, Barcelona, Spain), and stored at −80°C until analyses were conducted. Once the collection period was over, the relevant analyses were carried out according to the aerial part to be determined.

2.2. Extraction Procedure of Volatile Aroma Compounds. The extraction of the volatile fraction was conducted using HS-SPME, and the analysis was carried out following the protocol developed by Cano-Lamadrid et al. [15]. Briefly, 5 g of the grinded sample was weighed, introduced in a 50 mL vial, and mixed with 15 mL of ultrapure water, 1.5 g of NaCl, and 15 μg of internal standard (β-ionone 15 mg kg^{-1}). The vial was placed in a bath at 36°C (to simulate the mouth temperature and establishing proper conditions to study the orthonasal olfaction), and after equilibration (15 min), a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 2 cm fiber was exposed to the headspace during 30 min. Later, desorption of the volatile compounds from the fiber coating was performed in the injection port of the GC-MS during 3 min at 230°C. Extraction experiments were run in triplicate.

2.3. Chromatographic Analyses. First, the separation and identification of the volatile compounds was performed using a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP-5050A mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan). The GC-MS system was equipped with a TRACSIL Meta.X5, column (30 m × 0.25 mm × 0.25 μm film thickness; Teknokroma S. Coop. C. Ltd., Barcelona, Spain). Analyses were carried using He as carrier gas at a column flow rate of 0.6 mL·min^{-1} and a total flow of 13 mL·min^{-1} in a split ratio of 2 : 1. The oven program was the next: (i) 50°C for 0 min; (ii) increase of 3°C min^{-1} from 50°C to 170°C and hold for 1 min; and (iii) increase of 25°C·min^{-1} from 170°C to 300°C and hold for 1.8 min. The temperatures of the injector and detector were 230°C and 300°C, respectively. Samples were run using the scan mode in the range 40–350 m/z.

Identification was performed using 3 methods: (i) retention indices of each compound, (ii) GC-MS retention times (authentic standard), and (iii) mass spectra (Wiley 09

Table 1: Soil characteristics of the experimental plot.

Parameters*	
pH	8.37
Electrical conductivity ($dS \cdot m^{-1}$)	0.46
Sand (%)	26.2
Loam (%)	37.2
Clay (%)	36.6
Active $CaCO_3$ (%)	13.6
Oxidisable organic C ($g \cdot kg^{-1}$)	9.67
Total Kjeldahl N ($g \cdot kg^{-1}$)	1.44
Available P ($mg \cdot kg^{-1}$)	64
Exchangeable K ($g \cdot kg^{-1}$)	0.44
Exchangeable Ca ($g \cdot kg^{-1}$)	3.67
Exchangeable Mg ($g \cdot kg^{-1}$)	0.65

*Values on a dry matter basis.

MS library (Wiley, New York, NY, USA) and NIST14 (Gaithersburg, MD, USA)).

Finally, the quantification ($mg \cdot kg^{-1}$ fresh weight, fw) of the volatile compounds was performed on a gas chromatograph, Shimadzu 2010, with a flame ionization detector, FID. The column and chromatographic conditions were those previously reported for the GC-MS analysis. The injector temperature was 200°C, and N_2 was used as carrier gas ($1\ mL \cdot min^{-1}$). β-Ionone was used as internal standard, and the areas from all compounds were normalized using its area; this compound was chosen after checking that it was not present in the volatile profiles of the samples under study. This analysis was run in triplicate.

2.4. Statistical Analysis. One-way analysis of variance (ANOVA) and Tukey's multiple range test were performed to compare experimental data and to determine significant differences among cultivars ($p < 0.05$). Principal component analysis (PCA) using Pearson correlation was also carried out. The software XLSTAT Premium 2016 (Addinsoft, New York, USA) and Statgraphics Plus (Version 3.1, Statistical Graphics Corp., Rockville, MA, USA) were used.

3. Results and Discussion

In the present research, 43 different volatile compounds have been identified in the four parts (flowers, leaves, flower buds, and caper fruits) of the caper plant under analysis (Tables 2–5). Results indicated that the predominant volatile component in all aerial parts of the caper was methyl isothiocyanate (chemical family: isocyanate). This compound is responsible for the pungent scent of the caper plant. In the flowers, leaves, stems, and flower buds, there were significant differences in the methyl isothiocyanate content between the cultivars "ORI3" and "ORI7" (710 and 2184 $mg \cdot kg^{-1}$ fresh weight (fw), 2654 and 1277 $mg \cdot kg^{-1}$ fw, and 6882 and 667 $mg \cdot kg^{-1}$ fw, respectively). In the study on the volatile composition of *Capparis spinosa* L. by Afsharypuor et al. [8] using leaves, fruits, and roots, the predominant compound was also methyl isothiocyanate, which agreed with current results. Sulfur compounds showed the highest amount in these cultivars, with methyl isothiocyanate being the main component. Isothiocyanates are derived from glucosinolates by enzymatic hydrolysis and are mainly found in Brassicaceae, although they are also identified in other families, such as Capparaceae and Caricaceae [10]. The glucosinolates are part of plant antiherbivore defenses, and methyl isothiocyanate can be considered as the most representative compound of the aromatic fraction of capers and derives from the degradation of glucocapparin [17].

Another compound having a high content was nonanal; in general, aldehydes provide oily and green flavor notes to plant matrices. Nonanal is one of the volatile compounds responsible for the characteristic "fresh green aroma" of fruits and vegetables. They are normally synthesized in the green organs of plants in response to wounds, and it is also the characteristic aroma of fruits and vegetables. The nonanal compound obtained from aldehydes C6 and C9 that have been derived from hydroperoxide lyase convert both types of hydroperoxide fatty acid derivatives into aldehydes that are often reduced in alcohols by alcohol dehydrogenases [17]. These C6/C9 aldehydes and alcohols have their origin in the biosynthesis of fatty acids, which is based on a pastidic set of acetyl-CoA generated from pyruvate, the final product of glycolysis [18]. The trends for nonanal were different according to the cultivar; "ORI7" has more contents than "ORI3" in flowers and caper fruits (3.5 and 1.7 $mg \cdot kg^{-1}$ fw and 29.2 and 1.32 $mg \cdot kg^{-1}$ fw, respectively), and in the case of leaves and flower buds, "ORI3" presented higher contents than "ORI7" (4.63 and 2.05 $mg \cdot kg^{-1}$ fw and 772.5 and 2.57, respectively). However, it is important to highlight that factors such as soil type, climatic conditions, stage of growth, and other factors can influence the volatile composition of plants, and specific analysis must be run to confirm results showed in this study.

Alcohols and aldehydes, derived from the oxidative degradation of fatty acids, form the volatile complex of most of the green leaf plants as cited by Romeo et al. [9]. According to the bibliography consulted, there are only few studies evaluating the volatile composition of the aerial parts of the caper plant (flowers, leaves, flower buds, and caper fruits). Regarding the flowers, there are not many previous studies of complete flowers. There are studies such as those carried out by Ascrizzi et al. [19], which determine the content of volatile compounds of the different parts of the flower, such as sepals, petals, and stamens, as well as complete flowers, leaves, and floral buttons. The results have been expressed in percentage; therefore, they are not fully comparable to the results showed in the present research. In any case, these authors reported that the predominant compound in flowers was methyl benzoate, as compared to methyl isothiocyanate (26882 $mg \cdot kg^{-1}$ fw in "ORI3" of flower buds). This may be due to the factors such as cultivar, farming practices, soil type, and method of volatiles isolation [20, 21].

Table 2: Volatile compounds of *Capparis spinosa* L. in flowers detected using headspace solid phase microextraction (HS-SPME) with GC-MS.

		RT	RI		Chemical family	ANOVA	ORI 3 ($mg \cdot kg^{-1}$)	ORI 7 ($mg \cdot kg^{-1}$)	Descriptors *
			Exp.	Lit.					
Flowers									
1	Methyl isothiocyanate	2.917	747	742	Isocyanates	**	709.80 b	2183.99 a	Pungent
2	3-Hexanol	3.752	827	811	Alcohol	**	34.70 b	96.64 a	Alcohol; medicinal
3	1-Hexanol	3.85	836	854	Alcohol	NS	19.76	20.76	Green; woody
4	Butyl isothiocyanate	4.989	924	943	Isocyanates	NS	4.54	6.38	—
5	Isobutyl isothiocyanate	5.42	947	na	Isocyanates	NS	3.53	5.19	—
6	Butyl 2-propenoate	5.502	951	na	Ester	**	0.17 b	1.06 a	—
7	1-Octen-3-ol	5.876	973	978	Alcohol	NS	0.64	0.95	Herbaceous; earthy; mushroom; green
8	6-Methyl-5-hepten-2-one	6.002	979	985	Ketone	**	1.66 b	4.30 a	Green; oily
9	Decane	6.266	998	1000	Hydrocarbon	**	2.16 a	0.03 b	—
10	2-Ethylhexanol	7.061	1024	1025	Alcohol	**	0.53 b	1.17 a	—
11	Limonene	7.273	1032	1033	Terpene	**	3.66 a	2.45 b	Lemon; orange; sweet
12	Benzeneacetaldehyde	7.659	1046	1045	Aldehyde	**	8.57 b	44.26 a	—
13	3,5-Octadien-2-one	9.059	1098	1098	Ketone	**	0.68 a	0.14 b	—
14	Undecane	9.133	1102	1100	Hydrocarbon	**	25.701 a	0.01 b	Faint
15	Methyl benzoate	9.213	1104	1102	Ester	**	0.04 b	103.27 a	Fruity
16	Nonanal	9.381	1106	1104	Aldehyde	**	1.74 b	3.50 a	Green; lemon; lime; meaty; oily; rose
17	Methyl octanoate	9.943	1122	1120	Ester	**	0.56 b	1.24 a	Green; citrus; fruity
18	Benzyl cyanide	10.642	1142	1148	Aldehyde	**	0.87 b	12.58 a	—
19	Octanoic acid	11.375	1162	1169	Acid	**	1.74 a	0.77 b	Cheese; oily
20	Decanal	13.102	1209	1203	Aldehyde	**	1.63 a	0.22 b	Floral; citrus; sweet
21	Linalyl acetate	15.058	1254	1257	Ester	NS	1.22	0.05	fruity; floral; sweet; pear
22	Nonanoic acid	15.417	1267	1273	Acid	**	1.36 a	0.05 b	Cheesy; waxy; tallow
23	Benzyl isocyanide	15.733	1275	na	Isocyanate	**	1.02 b	4.91 a	—
24	Benzyl isothiocyanate	19.767	1370	1361	Isocyanate	**	16.89 b	98.10 a	Cabbage
25	3-Methylindole	20.76	1393	1396	Indol	**	26.99 a	6.31 b	Fatty
26	Isoamyl benzoate	22.913	1445	1462	Ester	NS	17.94	8.97	Sweet
27	Neryl acetone	23.23	1452	1457	Ketone	NS	0.36	0.78	Sweet, fruity and floral
28	β-Farnesene	23.453	1457	1457	Terpene	**	5.46 b	26.60 a	Apple; orange; grapefruit juice; lime; pear
29	α-Farnesene (isomer 1)	25.033	1495	1504	Terpene	**	20.29 a	0.43 b	Apple; orange; grapefruit juice; lime; pear
30	α-Farnesene (isomer 2)	25.609	1509		Terpene	**	1.75 a	0.64 b	Apple; orange; grapefruit juice; lime; pear
31	Methyl laureate	25.74	1528	1524	Ester	NS	0.18	0.89	—
32	Nerolidol	27.974		1713	Terpene	NS	143.75	193.73	Apple; green; citrus; woody; rose

RT, retention time; RI, retention indexes; Lit., literature (NIST 2011); Exp., experimental; NS, not significant at $p < 0.05$; ** significant at $p < 0.05$. Values (mean of 3 replications) in each row followed by the same letter were not significantly different ($p < 0.05$). *Descriptors reference [16].

Thirty-two volatile compounds were identified and quantified in flowers (Table 2); nerolidol was the predominant compound after methyl isothiocyanate. Significant differences between "ORI3" and "ORI7" cultivars were found with nerolidol content being 144 and 194 $mg \cdot kg^{-1}$ fw, respectively. The sensory descriptors of this compound are wood, green, apple, citrus, or rose and can be attractive for consumers. In the current study, an important terpene content was also found in flowers as limonene, β-farnesene, α-farnesene (isomers 1 and 2), and nerolidol, which have fruity aroma notes such as lemon, orange, sweet, lime, pear, or grapefruit. This can be due, among other facts, to terpenes acting as chemical messages for insects and other animals, which is essential to carry out pollination [9]. One of the important uses of the caper throughout the history has been its medicinal use, which is linked to its high terpene content and their high antimicrobial activity [22]. Regarding total terpene content in flower in this research, "ORI3" had 174.9 $mg \cdot kg^{-1}$ as compared to 223.8 $mg \cdot kg^{-1}$ of the cultivar "ORI7." It also found a high content in benzyl isothiocyanate (chemical family: isocyanate); although there were significant differences between both cultivars, the cultivar "ORI7"

Table 3: Volatile compounds of *Capparis spinosa* L. in leaves and stems detected using headspace solid phase microextraction (HS-SPME) with GC-MS.

		RT	RI		Chemical family	ANOVA	ORI 3 ($mg \cdot kg^{-1}$)	ORI 7 ($mg \cdot kg^{-1}$)	Descriptors *
			Exp.	Lit.					
Leaves									
1	Methyl isothiocyanate	2.90	739	742	Isocyanate	**	2653.52 a	1276.89 b	Pungent
2	trans-2-Hexenal	3.84	841	848	Aldehyde	**	307.46 a	160.80 b	Almond; apple; green; vegetable; plum; sweet
3	Butyl isothiocyanate	4.98	926	943	Isocyanate	NS	2.98	1.56	—
4	Isobutyl isothiocyanate	5.40	949	na	Isocyanate	**	22.83 a	6.52 b	—
5	1-Heptanol	5.64	951	970	Alcohol	NS	0.83	1.28	—
6	2,4-Heptadienal (isomer 1)	6.33	999	999	Aldehyde	NS	3.73	4.29	—
7	Octanal	6.40	1002	1001	Aldehyde	**	2.03 a	0.50 b	Fatty; honey; citrus; fruity
8	2,4-Heptadienal (isomer 2)	6.70	1013	1012	Aldehyde	NS	9.22	7.79	—
9	Nonanal	9.35	1106	1071	Aldehydes	NS	4.63	2.05	Apple; fatty; green; lemon; lime; oily; rose; nutty; waxy; meaty; melon; grape
10	Benzyl isocyanide	19.73	1369	1353	Isocyanate	**	43.09 a	14.91 b	—

RT, retention time; RI, retention indexes; Lit., literature (NIST 2011); Exp., experimental; NS, not significant at $p < 0.05$; ** significant at $p < 0.05$. Values (mean of 3 replications) in each row followed by the same letter were not significantly different ($p < 0.05$). *Descriptors reference [16].

Table 4: Volatile compounds of *Capparis spinosa* L. in flower buds detected using headspace solid phase microextraction (HS-SPME) with GC-MS.

		RT	RI		Chemical family	ANOVA	ORI 3 ($mg\,kg^{-1}$)	ORI 7 ($mg\ kg^{-1}$)	Descriptors *
			Exp.	Lit.					
Flower buds									
1	Ethyl p-hydroxybenzoate	2.407	1528	na	Isocyanate	**	99.12 a	0.11 b	—
2	Methyl isothiocyanate	2.836	736	747	Isocyanate	**	6881.76 a	667.34 b	Pungent
3	trans-2-Hexenal	3.778	839	848	Aldehyde	**	854.39 a	6.03 b	Almond; apple; green; vegetable; plum; sweet
4	2,4-Heptadienal (isomer 1)	6.295	999	999	Aldehyde	**	67.33 a	1.10 b	—
5	Octanal	6.392	1002	1001	Aldehyde	**	43.37 a	0.16 b	Fatty; honey; citrus; fruity
6	2,4-Heptadienal (isomer 2)	6.663	1012	1013	Aldehyde	NS	10.09	3.42	—
7	Limonene	7.208	1032	1033	Terpene	**	26.01 a	0.22 b	Lemon; orange; sweet
8	Benzeneacetaldehyde	7.606	1045	1045	Aldehyde	**	507.75 a	0.03 b	—
9	1-Octanol	8.223	1068	1070	Alcohol	**	22.27 a	0.99 b	Fatty; woody; citrus; waxy
10	3,5-Octadien-2-one	9	1095	1098	Ketone	**	49.19 a	0.45 b	—
11	Methyl benzoate	9.156	1101	1106	Isocyanate	**	650.18 a	2.80 b	Fruity
12	Nonanal	9.318	1105	1104	Aldehyde	**	772.46 a	2.57 b	Green; lemon; lime; meaty; oily; rose
13	Ethyl benzoate	11.433	1168	1153	Isocyanate	NS	0.90	1.12	Anise; banana; berry; cherry; grape; floral; minty; plum; vanilla
14	Octanoic acid	11.588	1163	1169	Acid	**	77.59 a	0.05 b	Cheese; oily
15	Decanal	13.05	1208	1203	Aldehyde	**	88.42 a	0.37 b	Floral; citrus; sweet
16	Nonanoic acid	15.363	1266	1273	Acid	**	205.99 a	0.33 b	Cheesy; waxy; tallow
17	Methyl 2-hydroxy-5-methylbenzoate	17.614	1319	na	Isocyanate	**	148.47 a	1.93 b	—
18	Decanoic acid	19.54	1364	1371	Acid	**	36.08 a	0.02 b	Fatty; citrus

RT, retention time; RI, retention indexes; Lit., literature (NIST 2011); Exp., experimental; NS, not significant at $p < 0.05$; **significant at $p < 0.05$. Values (mean of 3 replications) in each row followed by the same letter were not significantly different ($p < 0.05$). *Descriptors reference [16].

Table 5: Volatile compounds of *Capparis spinosa* L. in fruits detected using headspace solid phase microextraction (HS-SPME) with GC-MS.

		RT	RI		Chemical family	ANOVA	ORI 3 ($mg \cdot kg^{-1}$)	ORI 7 ($mg \cdot kg^{-1}$)	Descriptors *
			Exp.	Lit.					
Fruits									
1	Methyl isothiocyanate	2.93	733	747	Isocyanate	NS	1991.75	2352.37	Pungent
2	trans-2-Hexenal	3.84	836	848	Aldehyde	**	0.93 b	20.05 a	Almond; apple; green; vegetable; plum; sweet
3	2,4-Heptadienal (isomer 1)	6.32	999	1009	Aldehyde	NS	0.08	10.30	—
4	Limonene	7.13	999	1009	Terpene	**	0.39 b	13.31 a	Lemon; orange; sweet
5	4-Heptenal	8.52			Aldehyde	NS	0.56	8.92	Green; vegetable; fatty
6	Nonanal	9.33	1077	985	Aldehyde	**	1.32 b	29.19 a	Apple; fatty; green; lemon; lime; oily; rose; nutty; waxy; meaty; melon; grape

RT, retention time; RI, retention indexes; Lit., literature (NIST 2011); Exp., experimental; NS, not significant at $p < 0.05$; **significant at $p < 0.05$. Values (mean of 3 replications) in each row followed by the same letter were not significantly different ($p < 0.05$). *Descriptors reference [16].

presented a higher concentration (98.1 $mg \cdot kg^{-1}$ fw) compared to cultivar "ORI3" (16.9 $mg \cdot kg^{-1}$ fw). This compound gives a slight cabbage smell to caper flowers. In this research could be observed that the predominant chemical family present in flowers was isocyanates. Moreover, 3-hexanol (chemical family: alcohol) also showed high contents, with significant differences between cultivars, "ORI7" 96.6 $mg \cdot kg^{-1}$ fw and "ORI3" 34.7 $mg \cdot kg^{-1}$ fw. On the other hand, β-farnesene (chemical family: terpene) also had a high content, with again differences between cultivars: "ORI7" 26.6 $mg \cdot kg^{-1}$ fw and "ORI3" 5.46 $mg \cdot kg^{-1}$ fw.

A total of 10 volatile compounds had been identified in the caper leaves (Table 3). The compounds trans-2-hexenal (aldehyde) presented a high content (after methyl isothiocyanate), with 308 and 161 $mg \cdot kg^{-1}$ fw in "ORI3" and "ORI7," respectively. This compound has an almond, apple, green, vegetable, plum, and sweet odor. It also showed an important content in benzyl isocyanide (chemical family: isocyanate), with "ORI3" having a higher content that "ORI7" (43.1 and 14.9 $mg \cdot kg^{-1}$ fw, respectively). There were no previous studies on the composition of volatiles in caper leaves. In the study made by El-Ghorab et al. [23] with *Capparis ovata* Desf. var. canescens cultivated in Turkey, it was reported that the most abundant compound in caper buds was benzyl alcohol (20.4%) and methyl isothiocyanate in leaves (20.0%). In the current experiment, new compounds have been reported probably due to different cultivars being studied, among other factors.

In flower buds, 18 volatile compounds (Table 4) had been identified, with trans-2-hexenal (aldehyde) predominating in the "ORI3" cultivar (854 $mg \cdot kg^{-1}$ fw), as compared to only 6.03 $mg \cdot kg^{-1}$ fw in "ORI7" samples, after methyl isothiocyanate in both cultivars. This aldehyde has an aroma resembling to vegetable, green, plum, apple, almond, green, and sweet. The second relevant compound in the caper flower buds was nonanal (aldehyde) with 772.46 and 2.57 $mg \cdot kg^{-1}$ fw in "ORI3" and "ORI7," respectively. This compound has an aroma resembling to green, lemon, lime, meaty, oily, and rose. Then is methyl benzoate (isocyanate) with 650 and 2.80 $mg \cdot kg^{-1}$ fw in "ORI3" and "ORI7," respectively. Also, benzeneacetaldehyde showed high contents, with high contents in the "ORI3" cultivar (508 $mg \cdot kg^{-1}$ fw) and only trace content in the cultivar "ORI7" (0.03 $mg \cdot kg^{-1}$ fw). The presence in food of aliphatic aldehydes, acids, and alcohols such as trans-2-hexenal, hexanal, nonanoic acid, and 1-octanol is related to fat oxidation reactions. These substances have been found in a variety of food products including meats and processed meats, fruits, as well as dairy and grain products. More specifically, they have been associated with green/grassy/vegetable aromatics in fruits and vegetables [10]. Caper flower buds have been previously studied; for instance, Romeo et al. [9] studied *Capparis spinosa* L. from the Eolian Archipelago. In general, contents reported in this previous study were lower than those found in the current experiment. For instance, in flower buds, methyl isothiocyanate had a content of 441 $mg \cdot kg^{-1}$ in the Eolian samples and 26882 $mg \cdot kg^{-1}$ in the Spanish ones; a similar trend was found for nonanal: 773 $mg \cdot kg^{-1}$ fw in "ORI3" and 11.32 ppm for the Eolian samples.

In fruits, only 6 volatile compounds were found (Table 5). Most of these compounds showed no significant differences between the two cultivars under study "ORI3" and "ORI7', R except for the cases of trans-2-hexenal (20.05 and 0.93 $mg \cdot kg^{-1}$ fw in "ORI7" and "ORI3," respectively), limonene (13.31 and 0.39 $mg \cdot kg^{-1}$ fw in "ORI7" and "ORI3, R respectively), and nonanal (29.2 and 1.3 $mg \cdot kg^{-1}$ fw in "Ori7" and "ORI3" respectively). Methyl isothiocyanate was identified as the predominant volatile compound, in the study by Afsharypuor et al. [8], found in fruits de caper plant.

Figure 1 shows the content of volatile compounds grouped by chemical family. In flowers, isocyanates followed

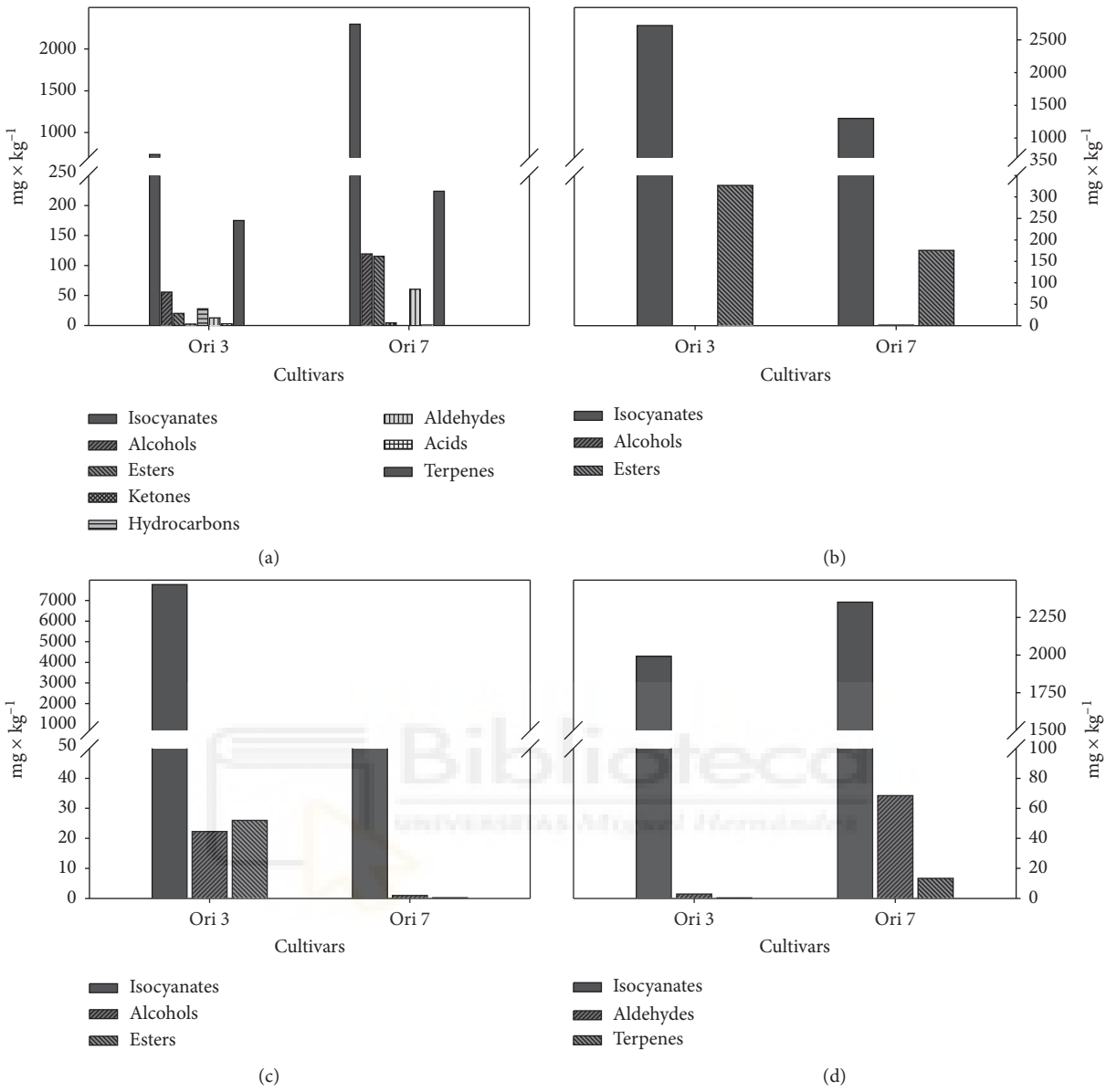

Figure 1: Identified families in both cultivars, "ORI3" and "ORI7," in the aerial parts of *Capparis spinosa* L. expressed in mg·kg^{-1} of fresh weight of flowers (a), leaf and stems (b), flower buds (c), and caper fruits (d).

by terpenes predominated in the volatile profiles of both cultivars (Figure 1(a)). Figure 1(b) represents the volatile profiles of caper leaves and shows more simple profiles, with isocyanates predominating over alcohols and esters. Figure 1(c) shows that the volatile contents in "ORI3" flower buds were much higher than those of the "ORI7" samples. Finally, capers fruits had significantly higher content of volatiles in "ORI7" as compared to "ORI3" samples (Figure 1(d)).

Figure 2 shows a principal component analysis (PCA) biplot (axes F1 and F2: 74.74%) where all the compounds and the different parts of caper and the two cultivars have been represented. The PCA is a very useful tool to show, in a graphic way, which compounds predominate in the different aerial parts of caper plants; 3 well-differentiated groups can be observed. In this way, in the upper left quadrant, the flowers of both cultivars were grouped, which had a very similar volatile composition. Flowers were associated with a high number of volatile compounds, including nerolidol and benzyl isothiocyanate; this high number of compounds is indicative of a complex aroma profile. Next, in the upper right quadrant, "ORI3" flower buds were positioned. The flower buds of "ORI7" showed a volatile profile similar to those of caper leaves and fruits of both cultivars "ORI3" and "ORI7." It is interesting to point out that methyl isothiocyanate was grouped close to "ORI3" flower buds, which are characterized by a more pungent flavor than that of the "ORI7" flower buds.

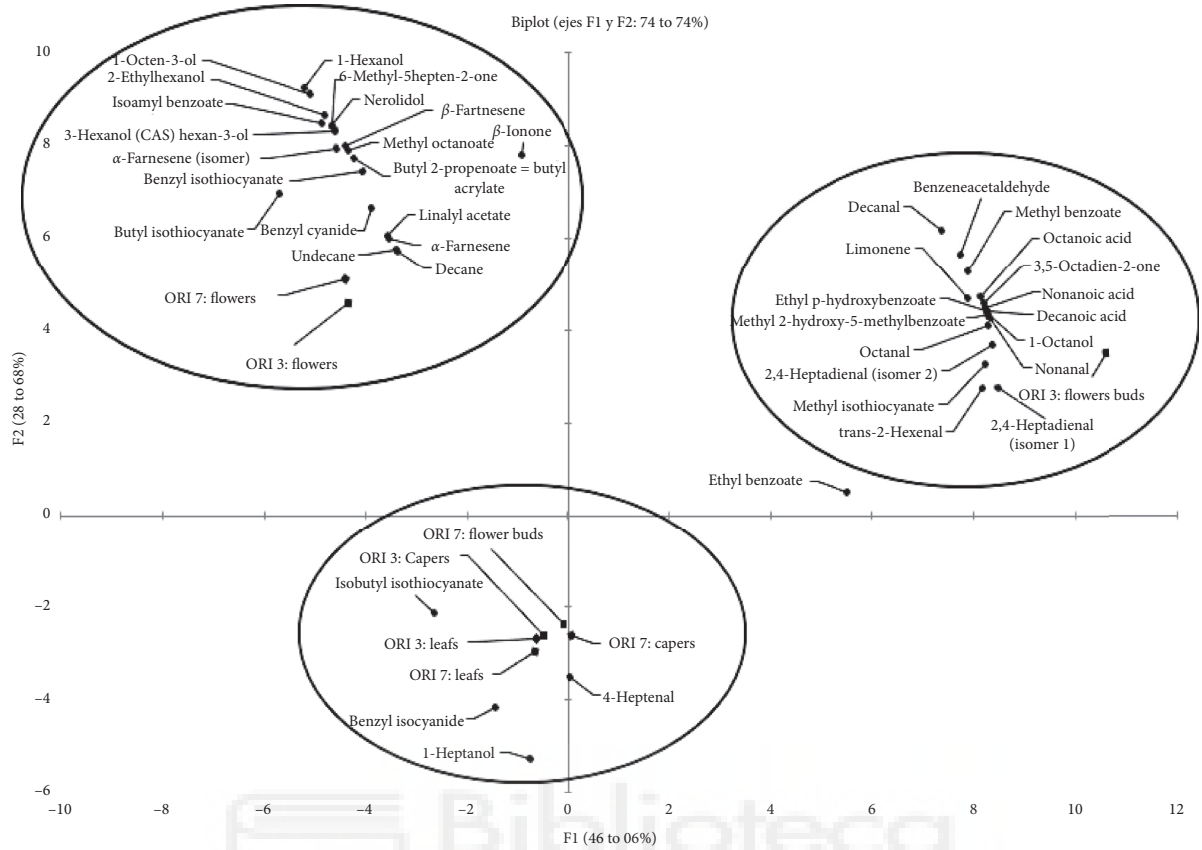

Figure 2: Principal component analysis (PCA) of different aerial parts of *Capparis spinosa* L. (●) and volatile compounds (◆).

4. Conclusions

Results showed that the two cultivars ("ORI3" and "ORI7") had the same volatile profile but compounds were present at very different concentrations, although plants were grown under the same climatic and agronomic conditions, indicating that they are cultivar-dependent. Caper flowers had the more complex profile, with 32 volatile compounds isolated, identified, and quantified: 5 isocyanates, 4 alcohols, 8 esters, 2 ketones, 2 hydrocarbons, 5 terpenes, 4 aldehydes, and 2 acids. Flower buds had a profile with 18 volatile compounds isolated, identified, and quantified: 5 isocyanates, 7 aldehydes, 1 terpene, 1 alcohol, 1 ketone, and 3 acids. Leaf capers had a profile with 10 volatile compounds: 4 isocyanates, 5 aldehydes, and 1 alcohol. Caper fruits had profiles with 6 volatile compounds, which do not have a strong aroma: 1 isocyanate, 4 aldehydes, and 1 terpene. The chemical family predominating the volatile profile of caper shoots was isocyanates, mainly due to the content of methyl isothiocyanate. In the caper flowers and fruits, the cultivar "ORI7" had higher contents of volatiles as compared to the "ORI3" samples. The opposite trend regarding cultivars was found in leaves and flower buds, with "ORI3" samples having the highest contents. The results demonstrated that volatiles profiles allowed distinguishing caper cultivars because they are cultivar-dependent and that capper flowers have a complex and rich volatile profile besides an attractive appearance that will make them very useful for modern cuisine purposes.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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4.6. Publicación VI

PUBLICACIÓN VI

Relationships between chemical composition, antioxidant activity and genetic analysis with ISSR markers in flower buds of caper plants (*Capparis spinosa* L.) of two subspecies *spinosa* and *rupestris* of Spanish cultivars

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RESEARCH ARTICLE

Relationships between chemical composition, antioxidant activity and genetic analysis with ISSR markers in flower buds of caper plants (*Capparis spinosa* L.) of two subspecies *spinosa* and *rupestris* of Spanish cultivars

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Abstract Genetic diversity and variability between populations is essential for the long-term survival of plant species as well as their adaptation to different habitats. The *Capparis spinosa* L. has two subspecies in Spain, *spinosa* with stipules thorny and *rupestris* without them. In Spain, the subspecies used for its cultivation is *spinosa*, which is difficult to manipulate due to its stipules thorny. The capers, unripe fruits and tender shoots are used as food. The caper plant is a rich source of phenolic compounds, due to that many flavonoids have been found in different parts of caper plant and in high quantities, which indicates that it is a good source of functional compounds both as food and for nutraceutical applications. There are no published works on the differences in biochemical and functional compounds of both subspecies, so in this work 32 varieties have been genetically analyzed to know their subspecies. Afterwards, various biochemical and functional parameters have been analyzed to find out if they present differences between both subspecies. From the results of the biochemical and functional parameters studied, there are no difference between the *spinosa* and *rupestis* subspecies, in all the parameters studied, except chlorophylls. There was more difference between the results of the subspecies *spinosa* among them, than with the subspecies *rupestris*. For all this, it can be concluded that the *rupestris* subspecies that does not present stipules thorniness can be cultivated, instead of the *spinosa* subspecies that does present them, without losing functional or nutritional characteristics of the caper buds.

Keywords Antioxidant activity · Flavonoids · Flavonols · Phenols · *Capparis spinosa* · ISSR

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Introduction

Genetic diversity and variability between populations is essential for the long-term survival of plant species (Wang et al. 2016), as well as their adaptation to different habitats. The family *Capparaceae* consists of 39 genera and 650 species, which are distributed in warm areas around the world. In the genus *Capparis*, about 250 species are known (Gull et al. 2015), they

could be native to the tropics and later spread to the Mediterranean basin and Central Asia (Zohary 1960). The *Capparis spinosa* L. is a common perennial winter-deciduous shrub with a summer cycle, and it has a creeping growth. It grows in North Africa, Europe, West Asia, Afghanistan and Australia (Inocencio et al. 2006; Fici 2014; Grimalt et al. 2019). The caper plant is largely cultivated in the Mediterranean basin. In Spain, the most valuable parts of *Capparis spinosa* used as food are the fresh aerial parts, especially the flower buds (capers), unripe fruits and young shoots. These are pickled or kept in brine and used as an appetizer or as a complement to meat, salads, pasta, and other foods (Argentieri et al. 2012; Grimalt et al. 2019). These plants have been used traditionally to prevent a high number of diseases such as diabetes, hepatitis, obesity and kidney problems (Anwar et al. 2016). In recent years, interest in consuming healthy foods has increased (Wu et al. 2004). The caper plant is a rich source of phenolic compounds, due to that many flavonoids have been found in different parts of capers and in high quantities, which indicates that it is a good source of functional compounds both as food and for nutraceutical applications (Grimalt et al. 2018, 2019; Wodyło et al. 2019).

On the other hand, the caper is a xerophytic shrub with a remarkable adaptability to harsh environments. Mediterranean countries are in a region of the world threatened by global warming and *C. spinosa* is a promising crop for arid or semi-arid regions within the climate change context, since the caper plant is highly tolerant to drought and heat stress (Grimalt et al. 2018).

C. spinosa present two different subspecies in wild populations and cultivated forms, *C. spinosa* L. subsp. *spinosa* and *C. spinosa* L. subsp. *rupestris* (Sibth. & Sm.) Nyman) in Spain (Mateo and Crespo 1995) and in Italy (Gristina et al. 2014). The subsp. *spinosa* is characterized, in the Mediterranean Region, for having branches spreading or erect, multiramified; stipules conspicuous and thorny, mostly recurved, decurrent at the base. While, the subsp. *rupestris* is characterized, in the Mediterranean Region, for having branches pendulous, unramified or few–ramified; stipules mostly setaceous or caducous, when persisting straight or slightly recurved, not decurrent at the base (Fici et al. 2014). Intermediate phenotypes also appear which increases the genetic diversity of this species (Gristina et al. 2014). From an agricultural point of view, the cultivation and collection of edible parts of this plant is greatly hampered by these stipules thorny. For this reason, it would be very successful to be able to cultivate the subsp. *rupestris*, which does not show stipules thorny, if this subsp. had a high content of compounds, antioxidant activity and nutritional power with respect to subsp. *spinosa*, which does present stipules thorny, and that is the subsp. more cultivated.

Undersanding the level of genetic diversity and the genetic structure of this species, we wanted to do genetic analysis of the collected samples to ensure that they belong to each of the subsp. mentioned or if they are intermediate phenotypes. For this analysis, we have used the inter-simple sequence repeats, also known as ISSRs. This DNA analysis technique is a quick and simple technique with low running costs and requiring only small quantities of template DNA. The production of large number of fragments and the reproducibility are other advantages of these markers (Reche et al. 2019), they do not require prior knowledge of the DNA sequence and can be universally applied as dominating markers (Liu et al. 2015). The ISSR markers have already been used successfully on *C. spinosa* (Saifi et al. 2011; Al-Safadi et al. 2014; Gristina et al. 2014; Liu et al. 2015; Tamboli et al. 2018; Rhimi et al. 2019; Ahmadi et al. 2020). Other DNA analysis techniques such as RAPD (Özbek and Kara 2013) and AFLP (Inocencio et al. 2005; Aichi-Yousfi et al. 2016) have also been used in this species. However, in all cases these techniques have been used to distinguish between several species of the genus *Capparis* or subspecies of *C. spinosa* with respect to morphological characters of the samples.

The objective of this work is to compare two subspecies of *Capparis spinosa*, the subspecies *spinosa*, which has stipules thorny, and the subspecies *rupestris*, which does not. This study is carried out in order to know if the two subspecies have similar chemical and functional characteristics, since the *spinosa* subspecies, which has stipules thorny, is the only one cultivated in Spain and its spines make agronomic work very difficult. If the subspecies *rupestris*, which does not have stipules thorny, had similar functional characteristics, it could be cultivated instead of the subspecies *spinosa*, which would greatly facilitate agronomic work. To our knowledge,

this is the first time this study has been done. For this, as specific objectives of the work, we would have to:

(i) to carry out their genetic analysis using ISSR markers of thirty-two Spanish cultivars of *Capparis*, to ensure the subspecies to which each sample belongs
(ii) to study the functional and nutritional properties of twenty-four Spanish cultivars of Capparis and finally
(iii) to study the relationship between the genetic profile and the functional and nutritional properties, with the purpose of knowing if *C. spinosa* subsp. *rupestris* (without stipules thorny) presents a compositional profile differentiate from *C. spinosa* subsp. *spinosa* (with stipules thorny), to know if subsp *rupestris* can be cultivated without losing functional and nutritional properties.

Materials and methods

Experimental conditions and plant material

During the vegetative growth stage of the caper plant (May) forty leaves from four plant (ten per plant) of thirty-two cultivars were hand-harvested in 2018. The cultivars were collected in five locations in the southeast of Spain of which 9 cultivars belong to the subsp. *rupestris* and 22 to the subsp. *spinosa* (Table 1). The leaves were immediately taken to the laboratory and frozen at – 80 °C until they were used for genetic analysis.

During the reproductive growth stage of the caper plant (June and July), forty flower buds (capers) from four caper plant (ten per plant) were hand-harvested. However, there were cultivars that presented pests and did not have the sufficient quality of the capers or were not present, so only 24 cultivars were taken (to study the functional and nutritional properties). Once in the laboratory, forty flower buds of each cultivar in the stage of development called surfines (diameter between 7–8 mm) were selected; since they are the stage most used for culinary purposes. Then, the flower buds were freeze-dried using a freeze dryer (Telstar Technologies LyoQuest-55) 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. After drying, the samples were stored vacuum-packed in a freezer at − 80 °C until analysis.

Genetic study

DNA extraction

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

Table 1 *Capparis* cultivars and site of collection

Population	Cultivars	Subspecies[a]	Site of collection (town, province, country)	Latitude, longitude, altitude (m)
EPSO	TE1, TE2, TE3, TE4, TE5, TE6, TE7, TE8, TE9,TE10	*rupestris*	Orihuela, Alicante, Spain	38°5′ N, 0°56′ W, 24
Serón	TS1, TS2, TS3, TS4, TS5, TS6, TS7,TS8	*spinosa*	Serón, Almería, Spain	37°21′ N, 2°32′ W, 822
Águilas	TA1, TA2, TA3, TA4, TA5,TA6, TA7	*spinosa*	Águilas, Murcia, Spain	37°24′ N, 1°34′ W, 21
La Alberca	TALB1, TALB2, TALB3, TALB4, TALB5	*spinosa*	La Alberca, Murcia, Spain	37°56′ N, 1°07′ W, 88
La Alcayna	TALCY1, TALCY2	*spinosa*	La Alcayna, Murcia, Spain	38°05′ N, 1 09′ W, 212

[a]Plant species were identified by an expert botanist from the Department of Applied Biology using the protocol by García-Rollán (1981)

PCR optimization and ISSR selection

We used 6 markers of the UBC primer set #9 of the University of British Columbia Biotechnology Laboratory (Vancouver, Canada), and 12 markers from the work of Al-Safadi et al. (2014) (Table 2). These markers were the more polymorphic in previous studies (Al-Safadi et al. 2014). Annealing temperature was optimised by running a gradient PCR between 45 and 60 °C. Annealing temperature of 53 °C obtained the best results. Amplification with each arbitrary primer was repeated twice and only those primers that produced reproducible and consistent bands were only selected for data generation.

PCR amplifications

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

Table 2 ISSR markers and primer sequence

Marker	Primer sequence
UBC807	AGAGAGAGAGAGAGAGT
UBC814	CTCTCTCTCTCTCTCTA
UBC817	CACACACACACACACAA
UBC820	GTGTGTGTGTGTGTGTC
UBC825	ACACACACACACACACT
UBC829	TGTGTGTGTGTGTGTGC
ISSR1	CACCACCACCACCACACCACT
ISSR2	GAGAGAGAGAGAGAGAGAC
ISSR7	CACACACACACACACACACAG
ISSR8	CTCTCTCTCTCTCTCTCTG
ISSR10	TCCTCCTCCTCCTCC
ISSR13	AGAGAGAGAGAGAGAGG
ISSR14	GAGAGAGAGAGAGAGAT
ISSR15	GAGAGAGAGAGAGAGAC
ISSR16	GAGAGAGAGAGAGAGAA
ISSR19	CTCTCTCTCTCTCTCTT
ISSR22	GTGTGTGTGGTGTGTA
ISSR43	ACACACACACACACACCTA

Electrophoresis conditions

Amplified products were loaded on 1.5% agarose gel and separated in 1 × TAE buffer at 100 V. The gels were visualized under UV after staining with ethidium bromide and documented using a gel documentation and image analysis system (Vilber Lourmat, Collégien, France).

Data analysis

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

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

Phylogenetic relationships among accessions were estimated from the molecular characterization data, using the package NTSYSpc 2.0 (Adams et al., 1998). Dendrogram was constructed using the Unweighted Pair Group Method with arithmetic averaging (UPGMA) clustering analysis based on the genetic similarity coefficient matrices (Nei and Li 1979). Statistical stability of the branches in the cluster was estimated by bootstrap analysis with 1000 replicates, using the Winboot software program (Yap and Nelson 1996). Population structure was estimated using a model based Bayesian procedure implemented in the software Structure v. 2.3.4 (Pritchard et al. 2000). An admixture model with correlated allele frequencies without prior population information was used. The most informative number of subpopulations was

identified using the K method (Evanno et al. 2005) with the aid of Structure Harvester (Earl and Vonholdt 2012). The estimated cluster membership coefficient matrices of the 20 runs were permuted so that all replicates have the closest match possible and then averaged across replicates, using the Greedy algorithm of the software CLUMMP (Jakobsson and Rosenberg 2007). To validate the predefined or the estimated population structure, we calculated pairwise Fst and Nei's standard genetic distance between populations (Nei and Li 1979). The reference distribution for *P* value calculation of the Fst analysis was calculated using 10,000 permutations. These analyses were performed with the Genalex 6.5 software (Peakall and Smouse 2012).

Biochemical, nutritional and functional parameters

In the capers the following parameters were measured in triplicate:

Chlorophylls *a* and *b* were extracted for each sample using 85% acetone according to Official Method AOAC (1990). The absorbance was read at 664 nm, using Helios Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). The results were expressed in mg 100 g^{-1} dry weight (dw).

Total carotenoids were extracted according to Valero et al. (2011), with acetone and diethyl ether to promote phase separation. The lipophilic phase was used to estimate the total carotenoid content and the absorbance was measured at 450 nm using the same Helios Gamma spectrophotometer named above. The results were expressed as mg of carotenoids 100 g^{-1} dw.

To determine the protein content, the Bradford method (1976) was used, using the Bio-Rad reagent. For the quantification, a standard curve of pure bovine serum albumin (BSA) was used, according to Grimalt et al. (2019). The absorbance was measured at 595 nm using the same Helios Gamma spectrophotometer named above. The results were expressed as mg g^{-1} dw.

To estimate the total flavonoids and flavonols, extracts of methanolic caper buds were used using 80% methanol and a weight-to-volume ratio of 1/50, stirring for 24 h (Argentieri et al. 2012). The total phenols were quantified according to Singleton et al. (1999), with slight modifications, using the Folin-Ciocalteu reagent and the calibration curve was performed with gallic acid. The absorbance was measured at 760 nm using the same Helios Gamma spectrophotometer named above. The results were expressed as mg GAE 100 g^{-1} dw.

Total flavonoids were quantified using the method of Chang et al. (2002) with some modifications. The reaction was kept at room temperature for 30 min and the absorbance was measured at 415 nm, using the same Helios Gamma spectrophotometer named above. Total flavonols were quantified using the Kumaran et al. (2007) method with some modifications, and absorbance was measured at 440 nm. The results of flavonoids and flavonols were expressed as mg rutin Eq. 100 g^{-1} dw.

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

The determination of the total antioxidant activity made by three methods FRAP, $ABTS^{+}$ and $DDPH^{\bullet}$. The ferric reducing ability of plasma (FRAP) and (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) ($ABTS^{+}$) antioxidant assays were determined following Benzie and Strain (1996) and Re et al. (1999), respectively, using the same Helios Gamma spectrophotometer named above. The radical scavenging activity was evaluated using the $DPPH^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams et al. (1995). The results were expressed in mM Trolox per 100 g of dw.

Statistical analysis

For physical, chemical and biochemical parameters, a basic descriptive statistical analysis was followed by one-way analysis of variance test (ANOVA) for mean

comparisons. Three analyzes have been carried out: grouping the cultivars by subspecies, grouping cultivars by populations and without grouping. The method used to discriminate among the means (multiple range test) was Fisher's LSD (Least Significant Difference) procedure at a 95.0% confidence level. These analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, USA).

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

Results and discussion

Genetic diversity and hierarchical classification

The 32 *Capparis* cultivars were amplified consistently with 8 of the 18 primers (Table 2 and Fig. 1). The number of products generated per primer was found to range from 5 to 15 of different sizes in the range of 0.23–2.20 kb (Table 3). The primer ISSR15 exhibited the maximum (15) product whereas primer ISSR10 gave the least (5) number of products. A total of 83 amplified products were produced with an average of 10.37 products per primer, of which 81 (97.6%) were polymorphic and 2 (2.4%) products were monomorphic (Table 3). The percentage of polymorphic bands ranged from 80% for primer ISSR10 to 100% for the other primers. These ISSR primers gave a high PIC value of 0.446 for primer ISSR16 and low PIC value of 0.305 for primer UBC825, with an average PIC value of 0.356 per primer. An average RP of 5.56 per primer was obtained with the highest RP value of 7.59 for primer ISSR16, and the lowest value of 2.40 for primer ISSR10 (Table 3). These results are similar with obtained through ISSR by Gristina et al. (2014) in Italy and Al-Safadi et al. (2014) in Syria, lower than those obtained by Tamboli et al. (2018) in India and Rhimi et al. (2019) in Tunisia, while they are clearly superior to those obtained with AFLP by Inocencio et al. (2005) in Spain. All works included several *Capparis* species and/or subspecies.

The dendrogram obtained by the ISSR data appears in Fig. 2. Nei's similarity coefficient ranged from 0.20 to 0.96. In the dendrogram, obtained only with 8 ISSR markers, two thirds of the nodes were supported by bootstrap values high than 25%, which indicates the robustness of the result obtained. All cultivars could be distinguished with the ISSR markers used. Intra-population variability was obtained in all the populations. Subspecie-specific or unique fragments were detected in all the markers (Tables 2 and 3), as in previous works with ISSR (Al-Safadi et al. 2014; Gristina et al. 2014) and AFLP markers (Inocencio et al. 2005).

In the dendrogram (Fig. 2) the cultivars are grouped in two main clusters. Cluster I contains all the TE cultivars with the TE10 cultivars as the most different. Cluster II contains all the rest cultivars, with a good grouping obtained for the others groups: all TS cultivars, except TS7, all TA cultivars, all TALB cultivars except TALB4, and all TALCY cultivars. The clustering of cultivars by subspecie was also found by Gristina et al. (2014) in Italy, as well as by specie by Al-Safadi et al. (2014) in Syria.

Figure 3 shows the PCA obtained with the ISSR results. The first two main principal components (PC1 and PC2) explained the 58.9 and the 7.1% of the variability, respectively. The cultivar distribution was very similar to that of the dendrogram, with two large groups, and the TE10 cultivar in an intermediate position. No cultivar TE has stipules thorny except TE10, which does. Due to these stipules thorny and the results obtained with PCA, it can be thought that TE10 is a hybrid between the *spinosa* and *rupestris* subspecies.

Genetic structure analysis

The identification of genetically similar groups of plants was performed using an admixture model-based clustering analysis implemented in the software Structure. The Evanno's test indicated that the most informative number of subpopulations (K) is 2, suggesting the existence of two major clusters in present *Capparis* cultivars. The inferred population structure is presented in Fig. 4. The groups defined by the Structure's analysis represent statistically different

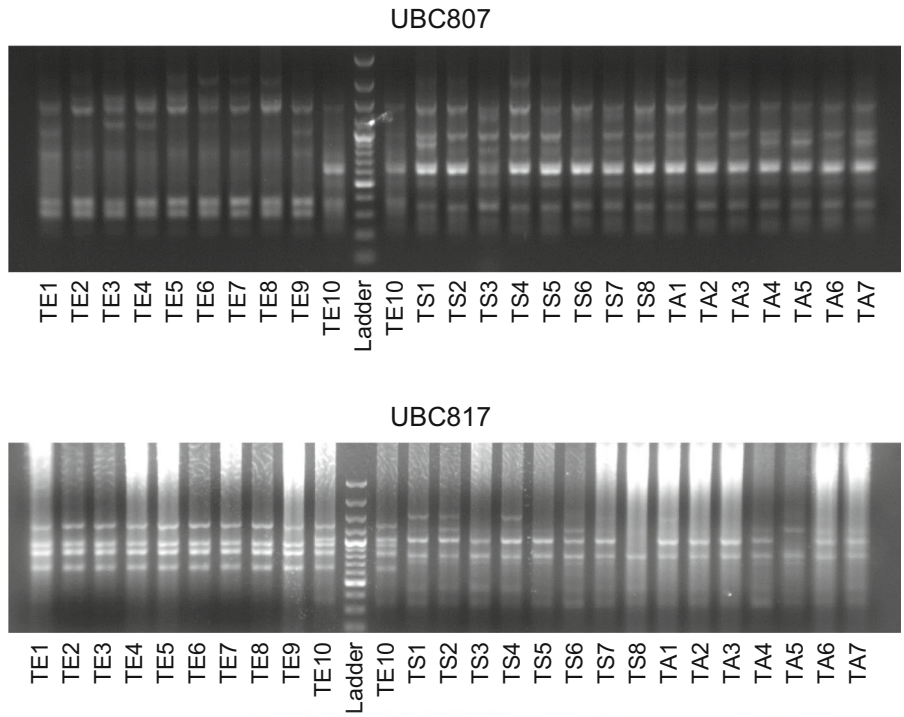

Fig. 1 Banding profiles obtained with the markers UBC807 and UBC817 in part of the studied plants. In each gel, the ladder is GeneRuler100 bp Plus (ThermoFisher)

Table 3 Genetic variation obtained with ISSR markers

Marker	Number of bands	Size range (pb)	MR	PIC	RP	Unique bands (Number, population)
UBC807	12	320–2200	11	0.323	6.00	3, TE; 4, rest
UBC817	11	350–1650	11	0.417	6.69	4, TE; 7, rest
UBC820	7	1000–2200	7	0.349	3.83	2, TE; 1, rest
UBC825	10	460–1750	10	0.305	4.44	1, TE
ISSR10	5	800–1800	4	0.336	2.40	2, TE; 2, rest
ISSR14	12	250–1500	12	0.364	6.57	2, rest
ISSR15	15	230–1750	15	0.312	6.94	4, TE; 3, rest
ISSR16	11	300–1350	11	0.446	7.59	3, TE; 2, rest
Total	83	ND	81	ND	ND	18, TE; 21 rest
Average	10.37	ND	10.12	0.356	5.56	

MR multiplex ratio, *RP* resolving power, *PIC* polymorphic information content, *ND* not determined

subpopulations, as indicated by the evaluation of genetic differentiation. All the cultivars formed two subpopulations: subpopulation 1 consisting of all the TE cultivars (*C. spinosa* subsp. *rupestris*) and subpopulation 2 containing the rest of the cultivars of *C. spinosa* subsp. *spinosa* (TS, TA, TALB and TALCY). Bayesian analysis performed in Structure agrees the results obtained with the UPGMA

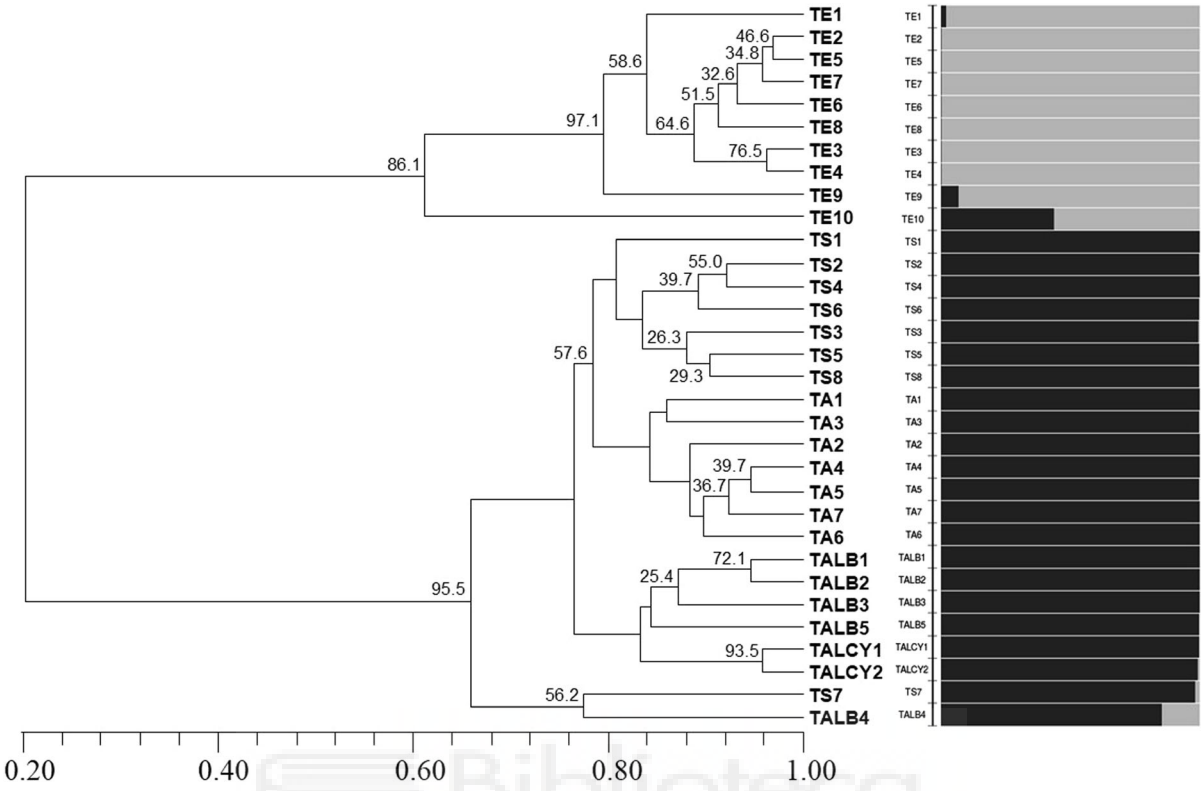

Fig. 2 Genetic relationship among accessions. Left: Dendrogram (UPGMA) of the *Capparis* plants based on genetic distances calculated with the genetic similarity coefficient matrices. The numbers near nodes represent the percentage of time when the node occurred among 1000 bootstraps (only for nodes with bootstrap values > 25% are given). Right: estimated population structure of the pea genotypes for K = 2. Each genotype is represented by a horizontal line, which is partitioned into colored segments that represent the estimated membership fractions in the K clusters

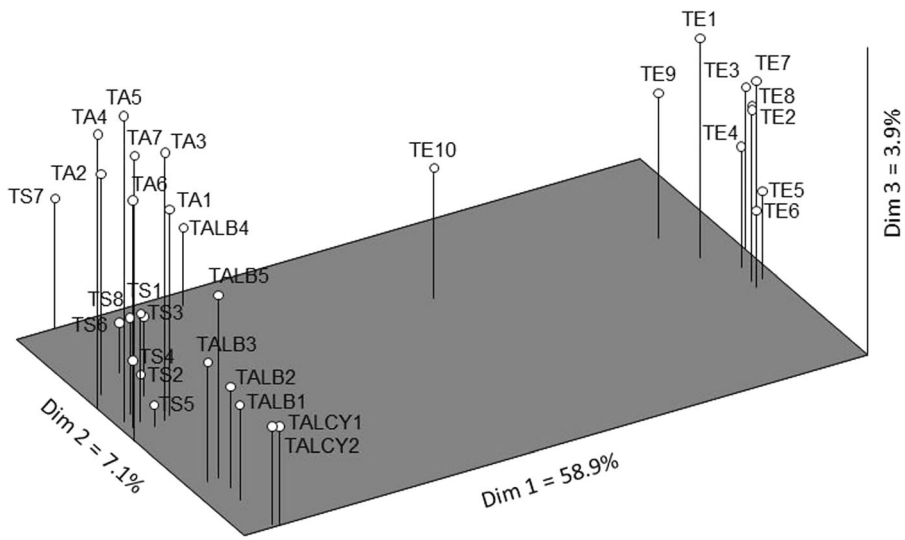

Fig. 3 Principal component analysis (PCA) using the ISSR results

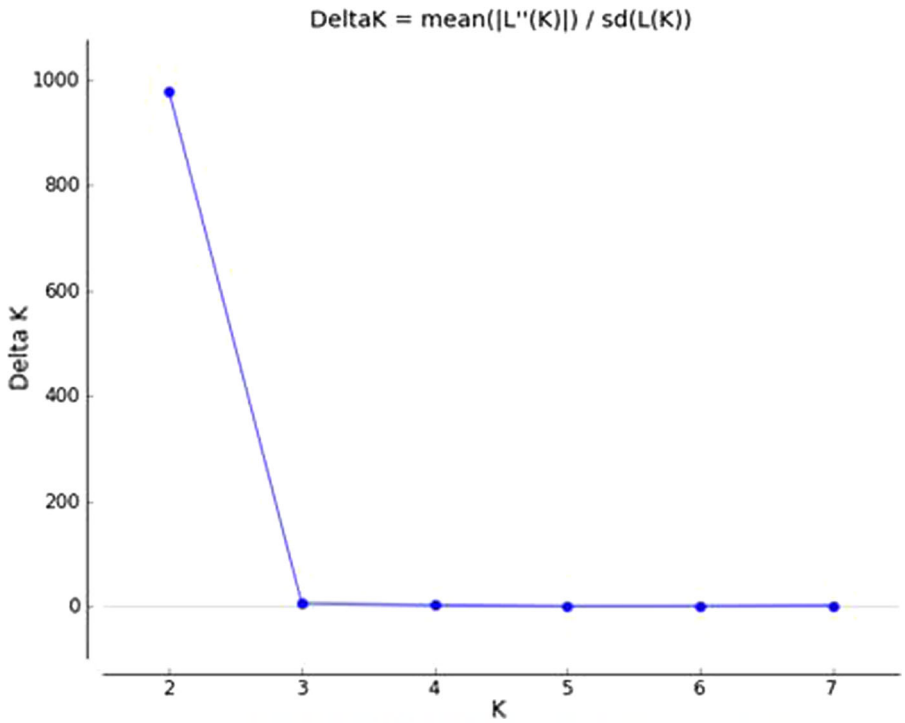

Fig. 4 Estimation of the optimum number of clusters for thepea genotypes according to the Evanno's method. The graph displays the Delta K [mean (lL00(K)l/SD(L(K))] for each K value

dendrogram and the PCA, indicating the robustness of the results obtained.

Chlorophyll, carotenoid and protein content in flower buds

The content of chlorophyll *a*, chlorophyll *b* and total chlorophylls is shown in Table 4. Chlorophyll *a* presented values from 9.07 to 21.65 mg 100 g^{-1} dw in cultivars'TA5' and 'TE9', respectively. Chlorophyll *b* content presented its highest value in cultivar 'TE1' with a total of 8.48 mg 100 g^{-1} dw, followed by cultivar 'TE4' with a total of 8.44 mg 100 g^{-1} dw, being cultivar 'TALCY2' the one with the lowest value, with a total of 4.25 mg 100 g^{-1} dw. Regarding the total chlorophylls, the values oscillated from 14.09 to 29.44 mg 100 g^{-1} dw, in cultivars 'TA5' and 'TE9', respectively. As observed in Fig. 5, there was a positive correlation between chlorophylls *a*, chlorophylls *b* and total chlorophylls.

The total content of chlorophylls was influenced by the subsp and population (Table 4). Thus, the *C. spinosa* subsp. *rupestris* showed the highest content of total chlorophylls compared to *C. spinosa* subsp. *spinosa*. Regarding the populations, the highest mean value was observed for the TE, TS and TALB (> 23.3 mg 100^{-1} dw) and the lowest for TA and TALCY (< 17.47 mg 100^{-1} dw).

Therefore, *spinosa* cultivars show a low content of chlorophylls with respect to *rupestris*, although there are some populations of *spinosa* that have not presented significant differences with *rupestris*. Therefore, there are more differences between the *spinosa* populations than the average between *spinosa* and *rupestris*.

The carotenoid content ranged from 20.88 to 59.73 mg β-carotene 100 g^{-1} dw, being the cultivar 'TS2' the one with the highest value, followed by cultivar 'TS1' (56.90 mg β-carotene 100 g^{-1} dw). On the contrary, the cultivar 'TA4' has presented a lower value in carotenoid content. If we relate the results by geographical areas, the TS population presented the highest values of carotenoid content with a mean value of 49.62 mg β-carotene 100 g^{-1} dw, followed by 'TALB', 'TALCY' and 'TE' with similar values without significant differences between them, with means values of 45.91, 44.92 and 42.20 mg β-carotene 100 g^{-1} dw, respectively.

Table 4 Photosynthetic pigments (mg 100^{-1} g dw), carotenoids (mg β-carotene 100 g^{-1} dw) and proteins content (mg g^{-1} dw) of flowers buds

	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids	Protein
Subsp.					
rupestris	18.86 b	7.42 b	26.27 b	42.20	10.47
spinosa	14.79 a	6.28 a	21.07 a	42.50	11.96
Population					
TE	18.86 d	7.42 b	26.27 b	42.20 ab	10.47 a
TS	16.05 bc	7.27 b	23.31 b	49.62 b	11.79 ab
TA	12.36 a	5.11 a	17.47 a	32.86 a	11.84 ab
TALB	17.97 cd	7.25 b	25.21 b	45.91 ab	13.53 b
TALCY	11.13 a	4.25 a	15.38 a	44.92 ab	8.63 a
Cultivar					
'TE 1'	20.90 ± 1.31^{lmn}	8.48 ± 1.17^{g}	29.36 ± 1.07^{j}	52.04 ± 5.89^{ijklm}	9.84 ± 0.64^{bc}
'TE 2'	18.97 ± 1.76^{ijklmn}	8.24 ± 0.59^{g}	27.21 ± 2.28^{ij}	38.40 ± 2.73^{defg}	9.82 ± 0.40^{bcd}
'TE 3'	19.77 ± 1.50^{jklmn}	8.23 ± 0.62^{g}	28.00 ± 1.90^{ij}	33.45 ± 1.47^{bcd}	9.01 ± 0.06^{abcd}
'TE 4'	19.08 ± 0.55^{ijklmn}	8.44 ± 0.49^{g}	27.51 ± 1.00^{ij}	46.83 ± 0.57^{ghijk}	9.47 ± 0.38^{bc}
'TE 5'	19.85 ± 1.20^{ijklmn}	6.82 ± 0.63^{defg}	26.67 ± 1.03^{ij}	34.66 ± 4.91^{defgh}	9.39 ± 0.62^{abcd}
'TE 6'	20.12 ± 0.99^{klmn}	6.21 ± 0.83^{cde}	26.33 ± 0.20^{hij}	49.65 ± 2.73^{hijkl}	11.12 ± 1.09^{bcdef}
'TE 7'	17.24 ± 0.96^{fghij}	7.70 ± 0.45^{efg}	24.93 ± 1.19^{ghi}	46.49 ± 2.09^{ghijk}	11.11 ± 0.57^{bcdef}
'TE 8'	18.50 ± 0.25^{hijklm}	6.63 ± 0.36^{def}	25.12 ± 0.50^{ghi}	50.34 ± 1.05^{hijklm}	11.90 ± 0.32^{cdef}
'TE 9'	21.65 ± 0.97^{n}	7.80 ± 0.46^{fg}	29.44 ± 1.18^{j}	45.42 ± 2.94^{ghij}	11.62 ± 1.04^{bcdef}
'TE 10'	12.48 ± 0.45^{bcd}	5.67 ± 0.51^{abcd}	18.14 ± 0.87^{bcd}	24.72 ± 0.32^{ab}	11.46 ± 0.20^{bcdef}
'TS 1'	14.94 ± 1.20^{cdef}	7.80 ± 0.55^{fg}	22.73 ± 0.65^{efg}	56.90 ± 3.38^{lm}	10.47 ± 0.68^{bcde}
'TS 2'	18.40 ± 1.02^{hijkl}	8.42 ± 0.36^{g}	26.81 ± 0.72^{ij}	59.73 ± 1.64^{m}	13.03 ± 1.48^{ef}
'TS 3'	17.65 ± 0.86^{ghijk}	7.46 ± 0.43^{efg}	25.10 ± 1.12^{ghi}	43.55 ± 1.75^{efghij}	11.58 ± 0.47^{bcdef}
'TS 4'	14.18 ± 0.09^{cde}	5.82 ± 0.43^{bcd}	19.99 ± 0.45^{cdef}	42.93 ± 4.73^{efghi}	12.24 ± 1.54^{def}
'TS 5'	15.06 ± 0.44^{bcdefgh}	6.86 ± 0.04^{cdefg}	21.91 ± 0.41^{efg}	45.02 ± 3.67^{ghij}	11.67 ± 0.95^{cdef}
'TA 1'	12.59 ± 0.77^{bcd}	4.40 ± 0.33^{ab}	16.99 ± 0.46^{abc}	27.17 ± 2.71^{abc}	9.02 ± 1.04^{ab}
'TA 2'	13.30 ± 0.28^{bcd}	5.44 ± 0.26^{abcd}	18.73 ± 0.14^{cde}	32.35 ± 3.12^{bcd}	11.29 ± 1.36^{bcdef}
'TA 3'	11.69 ± 0.20^{abc}	5.79 ± 0.22^{bcd}	17.48 ± 0.35^{bcd}	47.65 ± 5.45^{ghijk}	16.66 ± 1.26^{g}
'TA 4'	15.16 ± 0.40^{defg}	4.92 ± 0.32^{abc}	20.08 ± 0.54^{cdef}	20.88 ± 2.20^{a}	10.79 ± 1.55^{bcdef}
'TA 5'	9.07 ± 0.21^{a}	5.02 ± 0.09^{abc}	14.09 ± 0.29^{a}	36.23 ± 2.18^{cdef}	11.44 ± 0.41^{bcde}
'TALB 1'	16.58 ± 0.49^{efghi}	6.43 ± 0.46^{cdef}	23.01 ± 0.39^{fgh}	53.96 ± 1.90^{klm}	15.71 ± 0.49^{g}
'TALB 2'	21.35 ± 1.53^{mn}	7.93 ± 0.78^{fg}	29.27 ± 2.28^{j}	52.66 ± 0.28^{jklm}	14.42 ± 0.35^{fg}
'TALB 4'	15.97 ± 0.21^{defghij}	7.39 ± 0.51^{defg}	23.36 ± 0.75^{efghi}	31.11 ± 1.61^{bcde}	10.46 ± 1.80^{bcdef}
'TALCY 2'	11.13 ± 2.25^{ab}	4.25 ± 0.47^{a}	15.38 ± 2.72^{ab}	44.92 ± 3.75^{fghijkl}	8.63 ± 1.59^{a}

Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n = 30). TE: cultivar EPSO; TS: cultivar Serón; TA: cultivar Aguilas; TALB: cultivar La Alberca; TALCY: cultivar La Alcayna

On the other hand, the protein content was not affected by subsp. but population affected it. The 'TALB' population presented the highest values (13.53 mg 100 g^{-1} dw), followed by the 'TS' and 'TA' population that presented mean values of 11.79 and 11.84 mg 100 g^{-1} dw, respectively, without significantly differences between them. The lowest mean values were observed for 'TE' and 'ALCY'

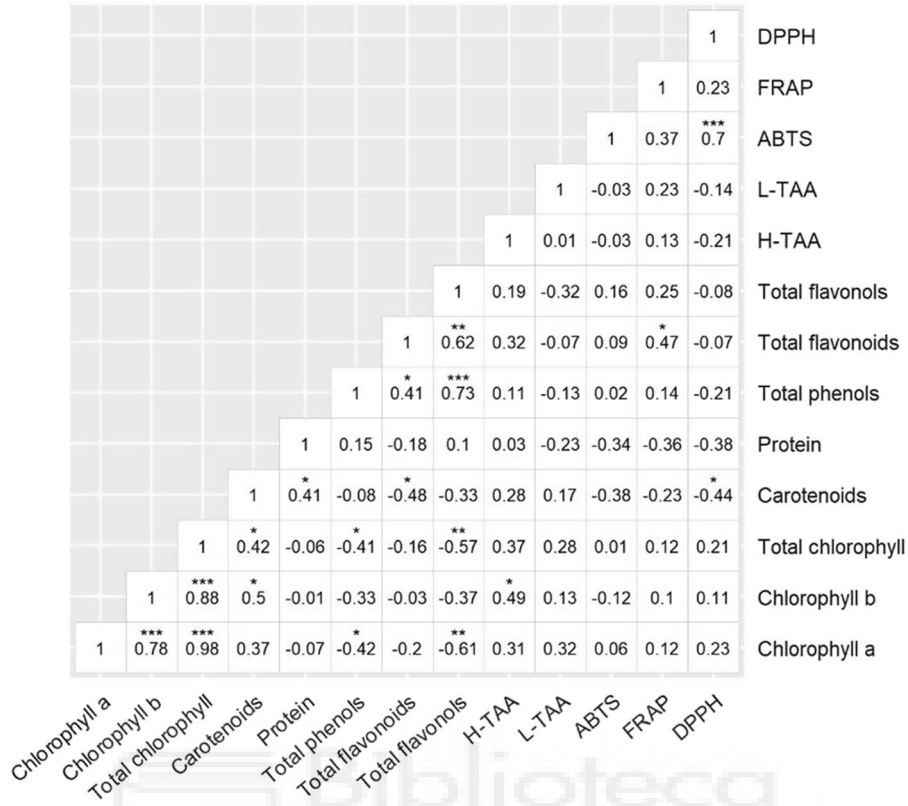

Fig. 5 Pearson coefficient of the correlations between chemical and biochemical parameters. Positive correlations are represented by positive values, and negative correlations by negative values. The significance level of correlations is represented by * (p value < 0.5), ** (p value < 0.01) and *** (p value < 0.001)

(10.47 and 8.63 mg 100 g^{-1} dw, respectively). The protein content was in a range of values between 8.63 and 16.66 mg 100 g^{-1} dw, being the cultivar 'ALCY2' the one that has presented the lowest content and the cultivar 'TA3' the one that has presented the highest value. The protein content is affected by the genetic differences of the cultivars.

However, the protein content did not show significant differences between the *spinosa* and *rupestris* subspecies, finding more differences between the *spinosa* populations than between the average *rupestris* and *spinosa*.

In Fig. 5, a positive correlation is observed between chlorophylls *b* and carotenoids, for this reason it is observed that the cultivar 'TE1' presents the highest value in chlorophylls *b* and carotenoids. In the case of cultivar 'TA4' the same happens it has presented low values in both chlorophylls *b* and carotenoids.

There are not many previous studies on the biochemical properties of *C. spinosa* in Spain. These results did not agree those reported by Grimalt et al. (2019), who observed higher content of total chlorophylls and proteins and lower content of carotenoids compared to ours. This may be due to the fact that the collection dates were different and possibly the climatic effects have generated these differences. Ulukapi et al. (2016) obtained a carotenoid content of 21.24 mg kg^{-1} in capers from Turkey, values lower than those of the present study. Tlili et al. (2009) obtained a carotenoid content in a range between 411.3 and 3452.5 µg g^{-1} fw, so our values are in the same interval. Grimalt et al. (2019), Özcan and Akgül (1998) and Ulukapi et al. (2016) have been obtained higher results in the content of protein that in this study. This is possibly due that they are found in different geographical areas and different cultivars.

Table 5 Total phenols (mg GAE 100 g^{-1} dw), flavonoids and flavonols (mg rutin Eq. 100 g^{-1} dw) of flowers buds

	Total phenols	Total flavonoids	Total flavonols
Subsp.			
rupestris	1571.45 a	2166.70	1426.43 a
spinosa	2183.54 b	2187.79	1810.32 b
Population			
TE	1571.45 a	2166.70 ab	1426.43 a
TS	2445.69 b	2197.61 ab	1854.39 b
TA	2440.54 b	2305.08 b	1993.95 b
TALB	1547.84 a	2046.02 a	1551.37 a
TALCY	1494.86 a	1977.69 a	1448.62 a
Cultivar			
'TE 1'	1694.14 ± 32.87^{bc}	2334.27 ± 55.01^{defgh}	1353.31 ± 49.93^{ab}
'TE 2'	1676.79 ± 43.12^{bc}	2404.08 ± 79.22^{fgh}	1642.30 ± 116.25^{bcdefgh}
'TE 3'	896.25 ± 4 9.26^{a}	2157.95 ± 156.90^{abcdefgh}	1428.35 ± 230.24^{abcd}
'TE 4'	1396.44 ± 97.72^{ab}	2140.19 ± 65.93^{abcdefgh}	1410.55 ± 26.73^{abc}
'TE 5'	2030.31 ± 2.17^{cde}	1954.20 ± 84.26^{abc}	1378.24 ± 50.27^{abc}
'TE 6'	1679.32 ± 28.96^{bc}	2054.93 ± 75.14^{abcde}	1363.02 ± 66.71^{ab}
'TE 7'	1724.51 ± 22.59^{bc}	2043.17 ± 91.89^{abcde}	1449.51 ± 85.33^{abc}
'TE 8'	1491.06 ± 129.54^{b}	2032.83 ± 166.00^{abcd}	1299.80 ± 72.48^{a}
'TE 9'	1633.26 ± 59.60^{bc}	2215.48 ± 81.54^{bcdefgh}	1421.08 ± 54.86^{abcd}
'TE 10'	1492.40 ± 162.13^{b}	2329.86 ± 195.99^{defgh}	1518.16 ± 122.96^{abcde}
'TS 1'	2899.88 ± 66.53 ^{g}	2274.37 ± 82.88^{bcdefgh}	2107.02 ± 82.02^{jk}
'TS 2'	1784.84 ± 289.65^{bcd}	2063.61 ± 60.18^{abcdef}	1669.10 ± 30.01^{cdefg}
'TS 3'	2416.73 ± 249.64^{ef}	2293.41 ± 125.07^{cdefgh}	1897.33 ± 121.84^{fghij}
'TS 4'	2432.23 ± 361.11^{ef}	2099.84 ± 21.05^{abcdefg}	1758.88 ± 146.34^{defghi}
'TS 5'	2694.75 ± 168.28 ^{fg}	2256.83 ± 93.01^{bcdefgh}	1839.64 ± 16437^{fghij}
'TA 1'	2771.26 ± 150.92 ^{fg}	2248.99 ± 155.73^{bcdefgh}	1869.77 ± 170.12^{fghij}
'TA 2'	2772.67 ± 240.67 ^{fg}	2276.86 ± 8.25^{bcdefgh}	1930.43 ± 39.08^{ghijk}
'TA 3'	2787.94 ± 244.86 ^{fg}	2349.45 ± 141.45^{efgh}	1951.59 ± 80.72^{hijk}
'TA 4'	2308.72 ± 43.12^{def}	2495.46 ± 153.91 ^{h}	2227.77 ± 225.85 ^{k}
'TA 5'	1562.10 ± 46.65^{bc}	2154.63 ± 58.86^{abcdefgh}	1990.21 ± 68.06^{ijk}
'TALB 1'	1506.16 ± 181.30^{b}	1849.55 ± 43.59^{a}	1425.30 ± 50.85^{abc}
'TALB 2'	1473.36 ± 73.12^{b}	1995.32 ± 190.12^{abcd}	1453.98 ± 109.27^{abcde}
'TALB 4'	1664.00 ± 69.45^{bc}	2293.18 ± 158.43^{bcdefgh}	1774.82 ± 211.57^{efghi}
'TALCY 2'	1494.86 ± 103.08^{bc}	1977.59 ± 71.00^{ab}	1448.62 ± 57.21^{abc}

Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n = 30). TE: cultivar EPSO; TS: cultivar Serón; TA: cultivar Aguilas; TALB: cultivar La Alberca; TALCY: cultivar La Alcayna

Total phenols content (TPC), flavonoids and flavonols in the flower buds

Table 5 shows the results of total phenols content (TPC), flavonoids and flavonols analyzed in flower buds. The total phenols content and total flavonols were affected by subsp and population, while the total flavonoids was only affected by population.

Regarding the cultivars, higher value of total phenols content was observed in the cultivar 'TS1' with a total of 2899.88 mg GAE 100 g^{-1} dw, followed by the cultivar 'TA2', being on the contrary the cultivars 'TE3' the one who presented the lowest values (896.25 mg GAE 100 g^{-1} dw). On the other hand, significant differences were detected between populations and also among subsp. Flowers buds of 'TS' and 'TA' populations showed the highest total phenols content (> 2.440 mg GAE 100 g^{-1} dw) in opposition to populations 'TE', 'TALB' and 'TALCY'; while between subsp. the flower buds of

subsp. *spinosa* showed the higher total phenols content in comparison with subsp. *rupestris*. The results obtained showed that the total phenols content was influenced by the genotype and environment conditions.

In reference to the total flavonoid content, this was not affected by the subsp.; however, significant differences were reported for populations and among cultivars. Thus, the maximum values were obtained for the flower buds of 'TA', 'TS' and 'TE' populations (> 2160 mg rutin Eq. 100 g^{-1} dw). Regarding among cultivars the total flavonoid content ranged from 1849.55 to 2495.46 mg rutin Eq. 100 g^{-1} dw ('TALB1' and 'TA4', respectively). Finally, the total flavonols content, was also significantly affected by subsp., populations and cultivars. Thus, the flower buds with higher value was observed for subsp. *spinosa* and among populations for 'TS' and 'TA' (> 1854.39 mg rutin Eq. 100 g^{-1} dw); while among cultivars the higher values were observed for cultivar 'TA4' (2227.77 mg GAE 100 g^{-1} dw), while the cultivar 'TE8' presented the lower content compared to the other cultivars.

There is a negative correlation between chlorophylls *a* and total chlorophylls with the total content of total phenols and flavonols, and this is reflected in the results obtained (Fig. 5). Thus, the cultivar 'TE9' has presented a high content of chlorophylls *a* and total with respect to the other cultivars, while in the content of total phenols and flavonols it has presented one of the lowest values.

Aliyazicioglu et al. (2013) obtained a total phenol value of 37.01 mg GAE 100 g^{-1} DW, which is much lower than those obtained in this study. In another study carried out in different regions of Tunisian (Tlili et al. 2010), low values were obtained compared to those obtained in the present work, with a total of 2.74 mg 100 g^{-1} fw. However, Mansour et al. (2016) obtained higher values in *C. spinosa* than those of the present work, with a total of 427.27 mg GAE g^{-1} dw in total phenols and 57.93 mg QE g^{-1} dw in flavonoids. Tesoriere et al. (2007) obtained 48.75 mg GAE 100 g^{-1}. Maldini et al. (2016) obtained values of total phenols between 98 and 149 mg 100 g^{-1} FW, among which flavonoids were the majority with 82–117 mg RE 100 g^{-1} FW. Inocencio et al. (2000) observed a wide variation in the flavonoid contents of capers from different regions and they proposed that environmental and physiological factors could have important effects. Compared with total phenols of other crops such as mango (208 mg 100 g^{-1}), bilberry (525 mg 100 g^{-1}) or raspberry (517 mg 100 g^{-1}), the results in *Capparis spinosa* showed that it is an excellent natural source of these compounds (Tlili et al. 2010).

Total content of antioxidant activity (TAA) in the flower buds

The total antioxidant activity has been quantified by the $ABTS^+$ method in the hydro-soluble (H-TAA) and lipidic-soluble (L-TAA) fractions in the caper flower buds of *C. spinosa* (Table 6). Results from TAA (both H-TAA and L-TAA) showed that genotype plays an important role in determining the antioxidant capacity of each cultivar. H-TAA did not show differences between subspecies, although it did between populations, being the cultivar 'TS' the one that presents the highest values, with an average of 1403.36 mg Trolox Eq. 100 g^{-1} dw, followed by 'TE' cultivars that presented an average of 1036.93 mg Trolox Eq. 100 g^{-1} dw. 'TA', 'TALB' and 'TALCY' presented the lowest values and without significant differences between them. Regarding cultivars, the H-TAA content showed values in a range between 384.99 and 1883.13 mg Trolox Eq. 100 g^{-1} dw in cultivars 'TALB4' and 'TS1', respectively. In the case of the L-TAA content, there were differences between subspecies and population, being the *rupestris* subspecies and the 'TE' population the ones that presented the highest L-TAA. Regarding the cultivars, 'TE6' presented the highest value with 460.00 mg Trolox Eq. 100 g^{-1} dw and the lowest was presented by 'TALB2' with a total of 158.49 mg Trolox Eq. 100 g^{-1} dw. Although the differences were not significant in the mean of the subsp. *rupestris* and *spinosa* for the H-TAA parameter and they have been for the L-TAA parameter, in both cases more differences were found in the contents of both parameters between the populations of *spinosa* than between the means of *spinosa* and *rupestris*.

Antioxidant activity was not significantly different among the subsp. and populations, but yes among cultivars (Table 6). In all cases of TAA, there were more differences between the *spinosa* populations than between *spinosa* and *rupestris* populations. The antioxidant activity by the $ABTS^+$ method presented the highest values in the cultivar 'TE5' with a total of

Table 6 H-TAA and L-TAA and total antioxidant activity by different methods: $ABTS^{+}$, $DPPH^{\bullet}$ and FRAP (mg Trolox Eq. 100 g^{-1} dw) of flower buds

	H-TAA	L-TAA	$ABTS^{+}$	FRAP	$DPPH^{\cdot}$
Subsp.					
rupestris	1036.93	305.85 b	547.00	1799.91	804.46
spinosa	967.56	214.54 a	550.46	1653.09	723.12
Population					
TE	1036.93 ab	305.81 b	547.7	1799.91	804.46
TS	1403.36 b	215.88 a	535.92	1787.52	578.87
TA	821.18 a	246.74 ab	585.28	1720.64	686.29
TALB	654.44 a	168.67 a	481.81	1389.95	986.39
TALCY	459.84 a	184.46 a	655.01	1432.66	838.72
Cultivar					
'TE 1'	1126.15 ± 72.48^{fgh}	279.11 ± 33.75^{fg}	497.07 ± 37.54^{cdef}	2191.79 ± 221.01^{gh}	462.79 ± 22.53^{abcd}
'TE 2'	$1223.37 \pm 32.55^{fghij}$	276.41 ± 83.45^{hi}	578.92 ± 20.02^{fg}	2033.86 ± 114.38^{fgh}	$582.17 \pm 28.28^{cdefgh}$
'TE 3'	$1133.69 \pm 98.56^{efghi}$	279.34 ± 100.66^{hi}	661.02 ± 186.22^{gh}	1259.21 ± 64.07^{bc}	1392.11 ± 93.36^{lm}
'TE 4'	$1046.38 \pm 107.14^{efgh}$	359.80 ± 26.30^{hi}	544.13 ± 27.03^{ef}	1733.26 ± 14.52^{def}	961.86 ± 15.77^{jk}
'TE 5'	562.26 ± 235.40^{ab}	264.66 ± 49.14^{def}	801.68 ± 10.26^{k}	$1751.53 \pm 31.79^{bcddef}$	1939.25 ± 164.69^{n}
'TE 6'	619.55 ± 4.53^{abcd}	460.00 ± 14.15^{j}	460.03 ± 30.03^{bcde}	1857.65 ± 73.84^{fg}	439.51 ± 10.26^{abc}
'TE 7'	1033.70 ± 139.08^{efg}	324.63 ± 13.74^{gh}	515.10 ± 43.05^{def}	1964.28 ± 97.11^{fgh}	645.75 ± 92.86^{efgh}
'TE 8'	929.89 ± 98.90^{def}	361.32 ± 5.71^{hi}	514.35 ± 31.54^{def}	2009.33 ± 43.55^{fgh}	571.41 ± 85.85^{cdefg}
'TE 9'	1548.77 ± 57.88^{jkl}	267.81 ± 21.53^{f}	552.64 ± 63.07^{ef}	1922.23 ± 286.58^{fgh}	$533.12 \pm 42.55^{abcdef}$
'TE 10'	1145.59 ± 34.37^{fghi}	185.01 ± 48.29^{a}	345.15 ± 14.02^{a}	1275.98 ± 174.20^{bc}	516.60 ± 65.33^{abcde}
'TS 1'	1883.13 ± 243.83^{l}	277.90 ± 21.87^{fg}	431.25 ± 31.54^{abcd}	1876.67 ± 38.54^{fg}	503.58 ± 81.34^{abcde}
'TS 2'	$1696.57 \pm 422.64^{ghijk}$	189.55 ± 37.05^{ab}	490.82 ± 12.26^{cdef}	1819.61 ± 121.14^{f}	544.38 ± 48.31^{bcdef}
'TS 3'	$1445.03 \pm 160.89^{hijk}$	171.49 ± 10.12^{abc}	688.30 ± 29.78^{hi}	1797.08 ± 86.85^{efg}	765.89 ± 36.04^{hij}
'TS 4'	$1237.76 \pm 136.07^{fghij}$	235.17 ± 11.6^{def}	653.51 ± 46.30^{gh}	1797.08 ± 116.64^{efg}	698.81 ± 42.30^{fghi}
'TS 5'	754.29 ± 101.82^{bcde}	205.31 ± 5.85^{bcde}	415.73 ± 10.51^{abc}	$1647.16 \pm 196.23^{cdef}$	381.69 ± 39.55^{ab}
'TA 1'	787.52 ± 164.32^{cdef}	241.19 ± 16.70^{ef}	483.06 ± 21.78^{cdef}	1117.54 ± 224.01^{b}	$511.84 \pm 111.88^{abcdef}$
'TA 2'	470.78 ± 176.44^{a}	235.85 ± 12.97^{cdef}	541.63 ± 404.72^{ef}	1910.46 ± 315.87^{fgh}	752.37 ± 131.15^{ghi}
'TA 3'	889.61 ± 148.29^{def}	265.23 ± 5.59^{f}	560.40 ± 287.33^{f}	1259.96 ± 192.47^{b}	459.28 ± 44.80^{abcd}
'TA 4'	$1312.24 \pm 347.10^{ijkl}$	248.72 ± 33.89^{def}	774.40 ± 178.46^{ij}	2974.45 ± 111.63^{h}	1225.92 ± 95.61^{l}
'TA 5'	645.74 ± 101.01^{abcd}	242.69 ± 23.94^{ef}	566.91 ± 193.72^{fg}	1340.80 ± 15.52^{bcd}	482.06 ± 48.81^{abcde}
'TALB 1'	448.45 ± 21.93^{ab}	186.48 ± 9.80^{abcd}	384.95 ± 4.51^{ab}	1069.49 ± 61.07^{b}	371.18 ± 9.76^{a}
'TALB 2'	$1129.87 \pm 114.29^{efghi}$	158.49 ± 27.95^{a}	480.56 ± 52.26^{bcdef}	393.21 ± 10.26^{a}	992.65 ± 78.34^{k}
'TALB 4'	384.99 ± 32.96^{ab}	161.05 ± 18.18^{abcd}	579.92 ± 159.43^{fg}	2707.14 ± 115.88^{h}	1595.35 ± 39.05^{m}
'TALCY 2'	459.84 ± 29.11^{abc}	184.46 ± 6.49^{abcde}	655.01 ± 183.46^{gh}	$1432.66 \pm 108.88^{bcde}$	838.72 ± 28.28^{ijk}

[a]Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n = 30). TE: cultivar EPSO; TS: cultivar Serón; TA: cultivar Aguilas; TALB: cultivar La Alberca; TALCY: cultivar La Alcayna

801.68 mg Trolox Eq. 100 g^{-1} dw, followed by the cultivar 'TA4' with a total of 774.4 mg Trolox Eq. 100 g^{-1} dw, being on the contrary the cultivar 'TE10' the one that presents a low value with a total of 345.15 mg Trolox Eq. 100 g^{-1} dw. On the other hand, the antioxidant activity by the FRAP method presented a range between 393.21 and 2974.45 mg Trolox Eq. 100 g^{-1} dw, in cultivars 'TALB2' and

'TA4' respectively. The cultivar 'TE5' presented the highest value in DPPH method (1939.25 mg Trolox Eq. 100 g^{-1} dw), followed by the cultivar 'TALB4' (1595.35 mg Trolox Eq. 100 g^{-1} dw). On the contrary, the cultivar 'TALB1' was the one that presented the lowest value, with a total of 371.18 mg Trolox Eq. 100 g^{-1} dw.

Grimalt et al. (2019) obtained slightly higher results on the content of H-TAA in flower buds and in L-TAA lower than those of the present work. Tesoriere et al (2007) found lower values of H-TAA and L-TAA in flower buds of Italian cultivars. Aliyazicioglu et al. (2013) obtained lower FRAP values in Turkish cultivars. Mansour et al. (2016) also obtained lower ABTS values in Tunisian cultivars. These differences may be because genotypic and environmental differences.

Correlation between traits and principal component analysis

Those traits that measure chlorophylls content have a very close positive correlation among them. Chlorophyll *a* and total chlorophyll are correlated negatively with total phenols and total flavonols, whereas that chlorophyll *b* is positively correlated with carotenoids and H-TAA. Carotenoids are positively correlated with total chlorophyll, chlorophyll *b* and protein, and negatively with total flavonoids and DPPH. Phenols, flavonoids and flavonols have a positive correlation among them. FRAP and total flavonoids are correlated positively, as well as DPPH and ABTS (Fig. 5).

In order to gain a better understanding of the results and trends of the variables studied (13 traits and 24 *Capparis* cultivars), the main components analysis (PCA) was applied and results are showed in Table 7 and Figs. 6 and 7.

The first three main components accounted for 68.44% of the total variation for the results obtained in the caper buds, and the 52.01% of the variability of the data studied were explained by the first two components. PC1 and PC2 from the PCA explained the 31.35 and the 20.66% of the variability, respectively (Table 7). The 'TE' group (except 'TE10') is mainly defined by the amount of chlorophylls, while the 'TA' and 'TS' groups (except 'TS2'), 'TYALC' and 'TE10' are principally correlated with phenols, flavonols and flavonoids. 'TS2', 'TALB1' and 'TALB2' are defined by the amount of carotenoids, but not 'TALB4'

Table 7 Eigenvectors of each variable in the three first Principal Components obtained with chemical and biochemical parameters

	PC1	PC2	PC3
Eigenvalues	4.08	2.69	2.14
Cumulative variation (%)	31.35	52.01	68.44
Parameters	Eigenvectors		
Chlorophyll a	0.90	0.26	0.13
Chlorophyll b	0.81	0.14	0.40
Total chlorophylls	0.92	0.24	0.22
Carotenoids	0.59	− 0.56	0.25
Protein	0.00	− 0.68	0.17
Phenols	− 0.61	− 0.08	0.50
Flavonoids	− 0.44	0.39	0.63
Flavonols	− 0.78	0.09	0.50
H-TAA	0.26	0.07	0.78
L-TAA	0.36	0.13	0.04
$ABTS^{+}$	− 0.14	0.75	− 0.18
$DPPH^{\bullet}$	0.07	0.75	− 0.40
FRAP	− 0.08	0.67	0.36

(Figs. 6 and 7). The first component (PC1) was positively related to chlorophylls, H-TAA and L-TAA, and negatively related to total phenols, flavonoids and flavonols. The PC2 was positively correlated with ABTS, FRAP and DPPH, and was negatively correlated with proteins (Fig. 6). PCA clearly distinguishes the 'TE 'except 'TE10 ' population on the right of Fig. 3 along with TS2, in positive PC1. The rest of the cultivars are PC1 negative including 'TE10', but not including 'TALB1' and 'TALB2' (Fig. 7).

ISSR markers and chemical and bioactive compounds comparison

Comparing the PCA obtained with chemical and functional compounds and ISSR results (Figs. 3 and 7, respectively), we shall observe that although the cultivar distribution is not identical, figures were very similar. In both cases, 'TE' cultivars were grouped, with the exception of 'TE10' cultivar. In the PCA obtained with chemical and functional compounds the 'TE10' cultivar was grouped with the rest of the cultivars no with the other 'TE' cultivar. In the same

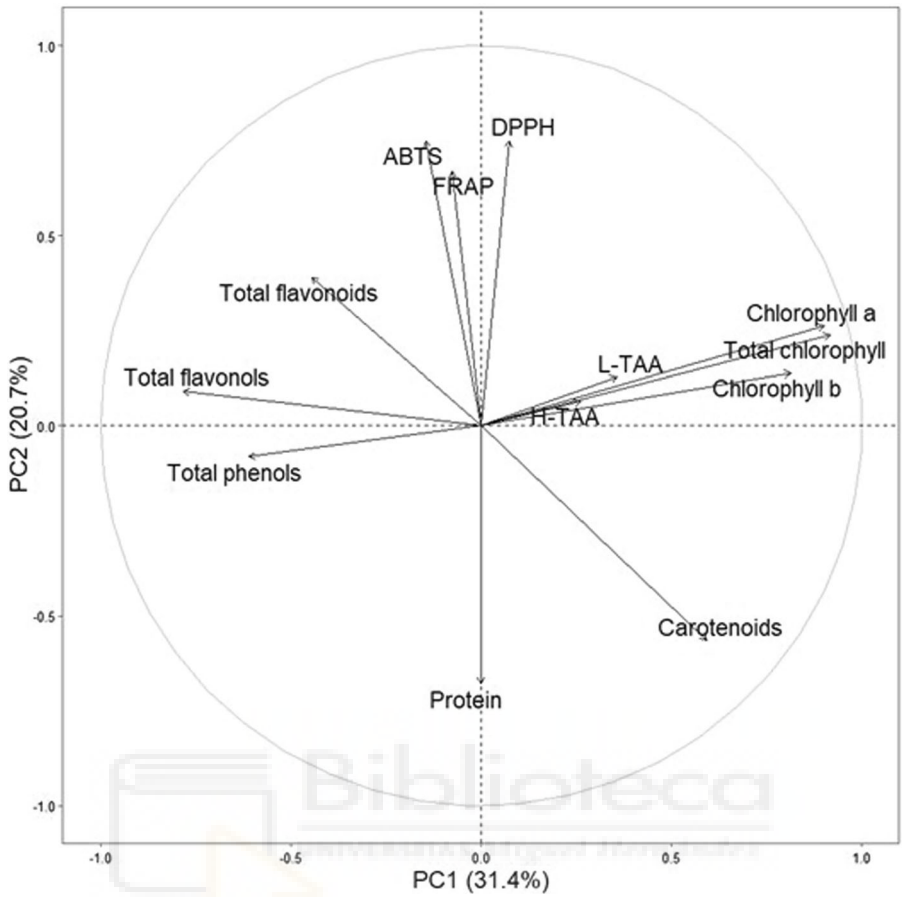

Fig. 6 Variables factor map from principal component analysis using chemical and biochemical parameters

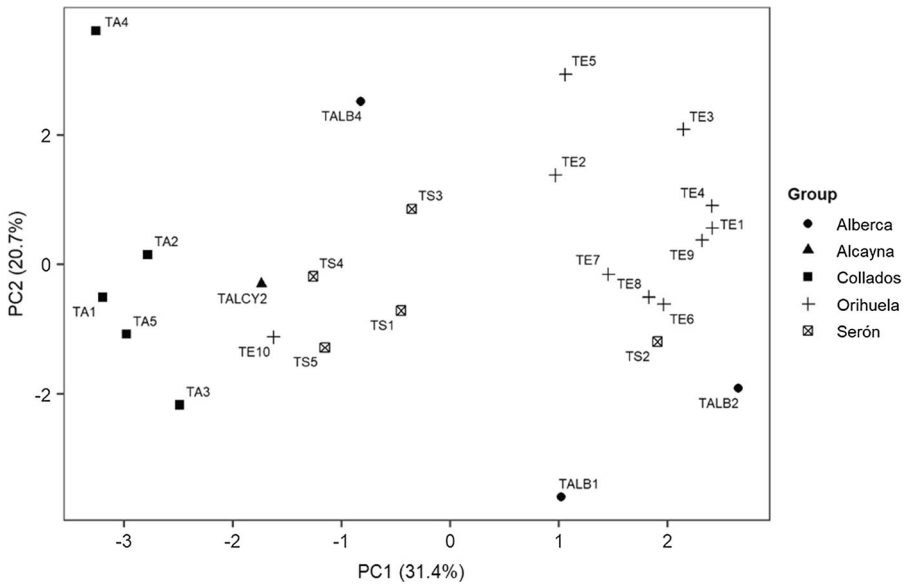

Fig. 7 Principal component analysis using chemical and biochemical parameters

way, 'TALB1', 'TALB2' and 'TS2' cultivars were grouped with 'TE' cultivars.

Conclusions

The PCAs carried out seem to indicate that the 'TE' cultivars, except 'TE10', belong to a subspecies, which, since they do not have stipules thorny, must be the *rupestris* subspecies, while the rest of the populations belong to the *spinosa* subspecies since they do have stipules thorny. According to the genetic PCA, the cultivar 'TE10' seems to be a hybrid between both subspecies, since it is in an intermediate position, or it belongs to the *spinosa* subspecies according to the results of the PCA using biochemical parameters.

In any case, from the results of the biochemical and functional parameters studied, it is clear that there is no difference between the *spinosa* and *rupestis* subspecies, but rather that in all the parameters studied, except chlorophylls, there is more difference between the results of the subspecies *spinosa* among them, than with the subspecies *rupestris*. For all this, for the first time, it can be concluded that the *rupestris* subspecies that does not present stipules thorny, can be cultivated instead of the *spinosa* subspecies that does present them, without losing functional or nutritional characteristics of the flower buds, facilitating agronomic work.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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RESUMEN DE LOS RESULTADOS Y DISCUSIÓN

5. Resumen de los resultados y discusión

A. Publicación I

PUBLICACIÓN I

Physicochemical composition and antioxidant activity of three Spanish caper (*Capparis spinosa* L.) fruit cultivars in three stages of development

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El objetivo de esta investigación fue realizar una evaluación de los parámetros morfológicos (diámetro, peso y color), además de la evaluación de parámetros bioquímicos (clorofilas, AAT, compuestos fenólicos, proteínas y carotenoides) en los tres estados de desarrollo 'Finos', 'Medianos' y 'Gruesos' de los frutos de la alcaparra, con la finalidad de determinar qué estado presenta mejores propiedades para el consumo. El estudio se llevó a cabo en tres cultivares españoles.

Propiedades físicas

Los frutos de alcaparra mostraron diferencias significativas de tamaño entre las tres etapas de desarrollo de cada cultivar. Se observó que los frutos de alcaparra de 'ORI 4' eran significativamente más grandes que los de 'ALB 2' y 'ALC 2'. Estas diferencias de peso dieron lugar a diferencias de diámetro y longitud, siendo el cultivar 'ORI 4' (fruto con más peso) el de mayor longitud entre los cultivares. Sin embargo, el diámetro de 'ORI 4' fue solo mayor que el de los otros dos cultivares en la etapa delgada, pero no en las etapas media o gruesa. Esto se debió a la forma de los alcaparrones de estos tres cultivares, ya que 'ORI 4' en su estado de pleno desarrollo, fue alargado, mientras que 'ALB 2' y 'ALC 2' fueron casi redondeados. Por ello, la relación longitud/diámetro alcanzó valores superiores a 2 en los frutos de alcaparra de 'ORI 4' en estado grueso (2,4), mientras que fue significativamente menor en los cultivares 'ALB 2' y 'ALC con un valor de 1,7.

Propiedades bioquímicas

Los caparrones se caracterizaron por tener un exocarpio externo de color verde en todas las etapas de desarrollo, incluso en su madurez y apertura natural de los frutos. Solo el mesocarpio y el endocarpio pueden cambiar de color, de blanco a rojizo o amarillento, cuando el fruto madura. En este trabajo se ha analizado el color del exocarpio del fruto, de forma que los frutos de alcaparra eran de color verde, con ligeras intensidades de este color en los tres estados de desarrollo estudiados. Por ello, el parámetro *a** de color fue negativo en todas las etapas en los tres cultivares. El parámetro L* no mostró diferencias significativas entre los tres estados de desarrollo estudiados en 'ORI 4' o 'ALB 2', mientras que 'ALC 2' mostró mayor luminosidad cuando aumentó el estado de desarrollo. Este aumento de brillo se debió a un color más amarillento a medida que aumentaba el desarrollo, como se comprobó ya que el parámetro *b**, que cuantifica el color amarillo, aumentó en 'ALC 2' pero no en 'ORI 4' o 'ALB 2'. Los frutos modifican su coloración debido a la síntesis y degradación de pigmentos a lo largo de su desarrollo y maduración (Pék et al., 2010). En este caso, los ligeros cambios de color no se produjeron por un aumento en la síntesis de carotenoides, ya que en los caparrones se mantuvo el contenido de carotenoides, mientras que hubo una degradación de clorofilas (*a*, *b* y total) al incrementar el desarrollo de estos frutos. En los caparrones, no hubo diferencias significativas entre cultivares ni en el contenido de carotenoides ni en las clorofilas totales. Los contenidos de carotenoides fueron bajos en comparación con los obtenidos por Allaith (2016) con alcaparras maduras en Bahrein.

En los tres cultivares estudiados (‘ALB2’; ’ORI4’; ‘ALC2’) hubo una disminución en el contenido de proteína de los frutos de alcaparra, con diferencias significativas entre las etapas inicial y final en todos los casos. El contenido de proteína fue mayor en los cultivares 'ALB2’. Estos contenidos de proteína fueron bajos en comparación con los obtenidos en frutos maduros de alcaparras por Sher y Alyemeni (2010).

Los contenidos de azúcares y ácidos orgánicos de los frutos de alcaparras se analizaron por HPLC y en todos los casos el contenido estaba por debajo del límite de detección del dispositivo, por lo que no se pueden mostrar los resultados.

La actividad antioxidante total (AAT) se cuantificó por tres métodos: $ABTS^{+}$, DPPH· y FRAP. Además, se cuantificó la AAT por el método $ABTS^{+}$ en las fracciones hidrofílica (AAT-H) y lipofílica (AAT-L). Se observó que la AAT-H se mantuvo o incrementó paralelamente a la etapa de desarrollo de los frutos de alcaparra, en los cultivares 'ALB 2' y 'ALC 2' aumentó significativamente con la etapa de desarrollo del fruto. AAT-L fue aproximadamente la mitad que AAT-H en los cultivares 'ORI 4' y 'ALC 2' en las tres etapas de crecimiento del fruto. Sin embargo, el comportamiento de AAT-L fue igual al de AAT-H siendo mayor en el cultivar 'ALB 2' y significativamente menor en los otros dos cultivares, los cuales no mostraron diferencias significativas entre ellos.

La actividad antioxidante por el método $ABTS^{+}$ se mantuvo ('ALB 2') o disminuyó ('ORI 4' y 'ALC 2') al aumentar el estado de desarrollo del fruto entre 8,6 y 1,2 mM de Trolox pf en los tres cultivares.

Prácticamente la misma tendencia se encontró al analizar la actividad antioxidante por el método FRAP, ya que la actividad antioxidante en 'ORI 4' y 'ALC 2' disminuyó significativamente al aumentar el estado de desarrollo del fruto, mientras que el comportamiento del cultivar 'ALB 2' fue, por el contrario, aumentando la actividad antioxidante con el desarrollo del fruto. Sin embargo, la actividad antioxidante cuantificada por el método DPPH· mostró una tendencia diferente, con incrementos significativos en la actividad antioxidante de los frutos de alcaparras paralelos al incremento en el desarrollo del fruto.

El factor de cultivo afectó significativamente los TPC en los frutos de alcaparras. 'ALB 2' fue el cultivar que mostró un contenido significativamente mayor que los otros dos cultivares estudiados. Este cultivar mostró un aumento significativo en TPC paralelo al desarrollo del fruto, mientras que los otros dos cultivares mostraron el mismo contenido de TPC en las tres etapas de desarrollo de los frutos estudiados. Los valores de TPC oscilaron entre 61,5 y 119,2 mg GAE x

100 g^{-1} pf. Este estudio mostró mayores contenidos de TPC en alcaparrones en comparación con otras frutas, como aguacate (21,86 mg GAE x 100 g^{-1} pf), mango (3,037 mg GAE x 100 g^{-1} pf) y manzana (58,12 GAE x 100 g^{-1} pf) (Fu et al., 2011).

En nuestros estudios hemos encontrado que la mayoría de TPCs eran TFCs, alrededor del 60%, dependiendo de la etapa y cultivar. El contenido de TFC prácticamente no varió entre etapas de desarrollo o cultivares.

B. Publicación II

PUBLICACIÓN II

Antioxidant Activity and Bioactive Compounds Contents in Different Stages of Flower Bud Development from Three Spanish Caper (*Capparis spinosa*) Cultivars

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El objetivo de la presente investigación fue realizar una evaluación de los parámetros morfológicos (diámetro, longitud y peso), así como la evaluación de parámetros bioquímicos (clorofilas, AAT, compuestos fenólicos, proteínas, carotenoides y azúcares) en los seis estados de desarrollo 'Nonpareilles', 'Surfines', 'Capucines', 'Capotes', 'Finas' y 'Gruesas' las alcaparras. El estudio se realizó en tres cultivares españoles. La finalidad fue determinar qué estado de desarrollo es el más apropiado para el consumo.

Propiedades físicas

Los pesos de los botones florales de alcaparras presentaron diferencias significativas entre los seis estados de desarrollo, principalmente en el estado “Capucines”. Las alcaparras de 'Orihuela 7' fueron las de más peso, además, éstas presentaban una relación longitud/diámetro que disminuía a medida que evolucionaba su estado de desarrollo, pasando de ser más alargadas a más redondeadas. Las alcaparras de 'Collados 5' mostraron una relación longitud/diámetro muy cercano a 1 en todos los estados de desarrollo, y fueron más redondeadas desde el principio. Las alcaparras de 'Serón 3' tuvieron una menor ratio en todas las etapas.

Propiedades bioquímicas

En el estudio del contenido de carotenoides en botones florales de alcaparras, los resultados fueron muy similares en los tres cultivares, excepto en los estadios “Nonpareilles” y “Surfines” en 'Orihuela 7' donde se reflejaron valores significativamente más elevados. Además, el nivel de carotenoides no cambió durante el desarrollo de las alcaparras, excepto en el estado “Gruesas” de 'Orihuela 7' en el que disminuyó.

En la literatura, los datos sobre los carotenoides de *C. spinosa* son limitados. En alcaparras de diferentes regiones de Túnez (Tlili, et al., 2009), el contenido total de carotenoides obtuvo valores muy superiores a los encontrados en alcaparras de España.

El contenido total de clorofila de las alcaparras fue superior en la mayoría de los estadios del cultivar ‘Collados 5’, excepto en los estadios “Nonpareilles” y “Gruesas” de ‘Orihuela 7’ y ‘Serón 3’, respectivamente. El contenido de clorofila *a* fue superior al de clorofila *b* en los tres cultivares. No hubo una tendencia clara entre las etapas de desarrollo con respecto al contenido de clorofila, pero hubo diferencias significativas entre ellas.

El contenido de proteína fue muy similar en la mayoría de los estados de desarrollo de las alcaparras de los cultivares 'Serón 3' y 'Collados 5' y, en ambos casos, fue muy superior al contenido del cultivar 'Orihuela 7', que presentaba un contenido entre 3,5 y 5 veces inferior. En los tres cultivares, el contenido de proteína mostró una tendencia a disminuir a medida que se desarrollaban las alcaparras.

El contenido de azúcares totales fue muy superior en 'Collados 5' y 'Serón 3' respecto al cultivar 'Orihuela 7'. En la literatura, los datos sobre el contenido de azúcar de *C. spinosa* son muy limitados.

La AAT por el método $ABTS^{+}$ solo mostró diferencias significativas con 'Serón 3' y 'Orihuela 7'. La AAT tendió a disminuir a medida que se desarrollaron las alcaparras en 'Orihuela 7' y 'Collados 5', no así en 'Serón 3', que aumentó hasta la etapa de "Capotes" y luego disminuyó. En el contenido de AAT por el método FRAP no se observaron diferencias estadísticas entre los cultivares. Los valores obtenidos por el método DPPH· fueron superiores y las únicas diferencias significativas fueron entre cultivares en los estadios "Nonpareilles" y "Capucines". La AAT tendió a disminuir a medida que se desarrollaron las alcaparras en los tres cultivares.

La AAT-H disminuyó ligeramente a medida que aumentó el tamaño de la alcaparra, mientras que AAT-L aumentó ligeramente.

Factores como la fuente geográfica y el genotipo, pero principalmente la etapa de madurez en el momento de la cosecha, pueden explicar la divergencia observada. La cuantificación de la actividad antioxidante en muestras biológicas depende en gran medida del método utilizado. La variación en la capacidad antioxidante entre cultivares podría deberse a diferencias en los compuestos antioxidantes y su efectividad (Robards et al., 1999).

El TPC en las alcaparras varió de 849,4 mg GAE x 100 g^{-1} pf ("Nonpareilles") a 525,8 mg GAE x 100 g^{-1} pf ("Grueso"), lo que indica que el contenido de polifenoles disminuyó a medida que se desarrollaban las alcaparras. El contenido de TFC fue de 68 a 95% del contenido de flavonoides (TFoC) en la etapa "Nonpareilles" y aumentó a valores de 83 a 96% en alcaparras en la etapa "Gruesa". Por otro lado, los contenidos de TFC y TFoC fueron significativamente mayores en el estadio "Nonpareilles" de los cultivares 'Collados 5' y en el estadio "Capotes" del cultivar 'Serón 3'.

Inocencio et al. (2000) observaron una amplia variación en el contenido de TFC de alcaparras de diferentes regiones y propusieron que los factores ambientales y fisiológicos podrían tener efectos importantes. El contenido de TFC promedió 6,55 mg x g^{-1} pf, valor similar al obtenido para los cultivares del presente estudio. Tlili et al. (2010) demostraron que las hojas y botones florales de *C. spinosa* de diferentes lugares de Túnez eran muy ricos en TPC, con un promedio de 3643 y 2621 mg x 100 g^{-1} pf, respectivamente. Estos valores fueron mucho más altos que los encontrados en este estudio y mostraron una gran variabilidad según la ubicación. En nuestros cultivares españoles no se encontró variabilidad entre las alcaparras de tres localidades, aunque los valores promedio fueron más altos para 'Collados 5'.

Las alcaparras son ricas en compuestos polifenólicos y flavonoides que a menudo se asocian con la tolerancia a las altas temperaturas (Chedraoui et al., 2017). Las concentraciones de polifenoles

y flavonoides varían según el método de extracción, los factores genéticos y las condiciones climáticas/de cultivo de los diferentes sitios (Tagnaout et al., 2016).

Por tanto, a la vista de los resultados obtenidos en nuestra investigación, se ha demostrado que las alcaparras podrían considerarse una buena fuente de compuestos fenólicos y sería recomendable consumir alcaparras para asegurar un aporte máximo de compuestos fenólicos.

Las correlaciones entre la actividad antioxidante, polifenoles totales, flavonoides, flavonoles y carotenoides en los botones florales de alcaparras en los tres cultivares muestran que en el cultivar 'Collados 5', los polifenoles totales se correlacionaron positivamente con las actividades antioxidantes $ABTS^{+}$, FRAP y DPPH·. Sin embargo, los flavonoides y los flavonoles se correlacionaron con las actividades de $ABTS^{+}$ y DPPH·. En el cultivar 'Orihuela 7', los polifenoles totales se correlacionaron con las actividades $ABTS^{+}$, FRAP y DPPH· y los flavonoides con las actividades $ABTS^{+}$ y DPPH·. Por otro lado, en 'Serón 3', los polifenoles totales se correlacionaron con las actividades $ABTS^{+}$ y DPPH·, mientras que los flavonoides sólo se correlacionaron con la actividad $ABTS^{+}$. Los polifenoles, flavonoides y flavonoles totales son compuestos bioactivos importantes que generalmente contribuyen a la actividad antioxidante hidrófila (AAT-H). Estos compuestos se correlacionaron positivamente con AAT-H en el cultivar 'Collados 5'. Sin embargo, los flavonoides y flavonoles se correlacionaron negativamente en el cultivar 'Serón 3'. Finalmente, sólo se encontró una correlación negativa entre AAT-L y el contenido de carotenoides en 'Orihuela 7'. Considerando los resultados de correlación, se encontró una alta correlación entre polifenoles totales y/o flavonoides con actividades $ABTS^{+}$, FRAP y/o DPPH· en los tres cultivares.

Yadav y Malpathak (2016) no encontraron correlación entre las actividades antioxidantes y los polifenoles totales y el contenido de flavonoides de los extractos de tallo y hojas de *Capparis moonii* de la India. Es bien sabido que los polifenoles totales, los flavonoides y los flavonoles son compuestos bioactivos importantes que generalmente contribuyen a la actividad antioxidante hidrófila (AAT-H). Estos compuestos se correlacionaron positivamente con AAT-H en el cultivar 'Collados 5', pero los flavonoides y flavonoles se correlacionaron negativamente en 'Serón 3'. En este estudio, los contenidos de AAT-L y carotenoides se correlacionaron negativamente en el cultivar 'Orihuela 7'. Ulukapi et al. (2016) observaron que el contenido de carotenoides totales y polifenoles totales se correlacionó positivamente (0,983) en yemas de *Capparis spinosa*.

C. Publicación III

PUBLICACIÓN III

Antioxidant activity and the physicochemical composition of young caper shoots (*Capparis spinosa* L.) of different Spanish cultivars

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El objetivo de la presente investigación consistió en realizar una evaluación de los parámetros fisicoquímicos de los brotes jóvenes (compuestos fenólicos, proteínas y clorofilas) y actividad antioxidante ($ABTS^+$, FRAP, DPPH y actividad antioxidante de la fracción hidro y liposoluble) en los brotes jóvenes de las alcaparras.

Los brotes tiernos presentaron valores más elevados en carotenoides en comparación con los botones florales y los frutos.

En los brotes tiernos se observaron diferencias significativas en el contenido de clorofilas *a*, *b* y totales, así como en el contenido de carotenoides entre los distintos cultivares, presentando el cultivar 'ORI 5' los valores más elevados en el contenido de clorofilas *a*, *b* y totales.

El contenido de proteína obtenido de los brotes jóvenes de alcaparra fue más elevado que los obtenidos en los frutos de alcaparra, observándose que el contenido de proteína era mayor en los brotes que en otras partes aéreas del alcaparro.

En los tallos tiernos se encontraron valores de AAT por el método $ABTS^{+}$ en un rango entre 1399,64 y 1816,63 mg Trolox x 100 g^{-1} ps siendo el valor más elevado el del cultivar 'ORI 4'. Por otra parte, la actividad antioxidante total determinada por el método DPPH· mostró los valores más elevados para el cultivar 'ALB 2'. En la AAT obtenida por el método FRAP, el cultivar con el valor más elevado ha sido 'ORI 10'. Las determinaciones de la AAT-H y AAT-L mostraron valores muy diferentes según el cultivar, siendo el cultivar 'ORI 8' el que ha presentado valores más elevados para la AATT-H, mientras que la AAT-L ha presentado los valores más elevados en el cultivar 'ORI 5'.

En los tallos tiernos se observaron diferencias significativas en el contenido total de polifenoles entre los diferentes cultivares analizados. Se obtuvo valores en un rango entre 3483,01 y 2200,77 mg GAE x 100 g^{-1} ps en los cultivares 'ORI 1' y 'ALB 2', respectivamente. Los resultados obtenidos en el presente trabajo fueron menores que en otras investigaciones realizadas por Tlili et al. (2010) donde se obtuvieron valores de 3643.29 mg x 100 g^{-1} pf.

El contenido en flavonoides presentó los valores más elevados en el cultivar 'ORI 1'. En otras investigaciones realizadas por Gull et al. (2018) se obtuvieron valores más bajos que los obtenidos en el presente trabajo, con un total de 215,3 mg x 100 g^{-1} ps. En cuanto al contenido total de flavonoles presentó valores en un rango entre 1685,50 y 719,87 mg RE x 100 g^{-1} ps en los cultivares 'ORI 1' y 'ALC 1', respectivamente. No existen estudios previos sobre el contenido en flavonoles en los tallos tiernos de las alcaparras, pero según los estudios realizados en la presente investigación se ha observado que fueron superiores en las alcaparras (691,7 mg RE x 100 g^{-1} pf), mientras que en los frutos se obtuvieron valores inferiores a los tallos tiernos y botones florales.

D. Publicación IV

PUBLICACIÓN IV

Polyphenol Compounds and Biological Activity of Caper (*Capparis spinosa* L.) Flowers Buds

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El objetivo de la presente investigación consistió en realizar la caracterización química, bioquímica y funcional de los botones florales, mediante la determinación de los compuestos polifenólicos y la actividad biológica.

Compuestos polifenólicos

Glucósidos de flavonol

Las alcaparras contenían un total de 34 compuestos de flavonol diferentes, incluidos los derivados de aglicona de quercetina (*m/z* 301), kaempferol (*m/z* 285), isorhamnetina (*m/z* 315) y miricetina (*m/z* 317). Un análisis de la estructura química de las agliconas individuales ([Aglc-H]$^-$) mostró un límite de azúcar en *m/z* 162 (glucosa o galactosa), *m/z* 146 (ramnósido) y *m/z* 308 (ramnohexosil como rutinósido). No observamos azúcares sustituidos por *p*-cumarato, acetato de malonato u otros compuestos. Algunos espectros UV de los compuestos 12, 13, 14, 22, 23, 34, 37 y 42 fueron indetectables debido a su baja abundancia, especialmente de flavonoides-O-tri-o-tetraglucósidos. Por lo tanto, su identificación se basó en un ion molecular desprotonado exhibido de un flavonoide y/o datos de la literatura.

Identificamos dos agliconas en alcaparras como kaempferol (43; *m/z* 285.95) y quercetina (35; Rt: 11.71 min; UV 256, 268sh, 296sh, 374 nm: *m/z* 301.04). También fueron detectados en alcaparras de Cerdeña por Maldini et al. (2016).

Inocencio et al. (2000) encontraron sólo quercetina y kaempferol-3-O-rutinósido en diferentes alcaparras en vinagre comerciales producidas en países mediterráneos.

El segundo grupo más grande de sustancias químicas con una estructura característica de flavonoide-O-triglicósido identificado en las alcaparras incluía los compuestos 11, 15, 16, 17, 19, 22-24, 26, 27, 32 y 37. Los compuestos clasificados como flavonoides-triglicósidos que después de la desprotonación produjeron el ion aglicona como pico base resultaron ser quercetina (11, 15, 16, 22, 24), kaempferol (17, 26, 32, 37) e isorhamnetina (19, 23, 27).

Flavan-3-oles

Nuestras muestras de alcaparras produjeron cinco compuestos en forma de monómero, dímero y trímero, pertenecientes a los flavan-3-oles. La identificación de los compuestos 45, 46, 47 y 48 se logró comparándolos con estándares auténticos. Su *m/z* 289 fue exactamente igual a la de los compuestos comerciales, permitiéndonos identificar los compuestos 45 y 46 como (+) - catequina y (-) - epicatequina, respectivamente. Los compuestos 47 y 49 son un dímero y un trímero de procianidinas con características m/z 577 y 865, respectivamente. La procianidina polimérica se caracterizó como un dímero de procianidina (49) con m/z 577, y después de MS/MS con m/z 289 y 245. Previamente, sólo Jiménez-López et al. (2018) identificaron dos dímeros y dos trímeros de procianidinas y (-) - epicatequina.

Derivados del ácido hidroxicinámico

Los derivados del ácido hidroxicinámico fueron el segundo grupo después de los glucósidos de flavonol que contribuyeron a la concentración final de polifenoles de alcaparras. Los compuestos 1-10 se identificaron sobre la base de su masa y espectros UV característicos de los derivados del ácido hidroxicinámico. Sólo unos pocos ácidos hidroxibenzoicos (ácido cinámico, ácido p-hidroxibenzoico, ácido protocatecúrico y ácido vanílico) y sólo unos pocos ácidos hidroxicinámicos (ácido clorogénico, ácido ferúlico, glucósido cumárico, ácido 4-feruloilquínico y ácido sinápico) fueron identificados previamente (Parveen et al., 2011) en diferentes trabajos y en diferentes partes de *C. spinosa*, como tallo, hojas, flores, raíces o bayas (Jiménez-López et al., 2018; Maldini et al., 2016 y Siracusa et al., 2011).

Se observaron fragmentos de iones después de MS/MS en *m/z* 191 ([M - H] −— ácido quínico) y 173 ([M - H] −— ácido ferúlico) en tres compuestos (6–8), indicándolos como derivados de ácidos quínico y ferúlico con un ión pseudomolecular característico en m/z 367.

Perfil cuantitativo de compuestos fenólicos

Hasta el momento no se ha reconocido la composición fenólica de las alcaparras en sus diferentes estados de crecimiento. Los compuestos fenólicos totales en las alcaparras investigadas oscilaron entre 10720 y 3256 mg x 100 g^{-1} de ps, y dependieron del genotipo y la etapa de crecimiento.

El contenido de fenoles en la muestra de Orihuela 7 (ORI.7) fue significativamente mayor que en Orihuela 10 (ORI.10). Entre seis etapas de crecimiento diferentes de las alcaparras, "Nonpareilles" se caracterizó por un contenido de polifenoles significativamente más alto que las otras etapas. ORI.7 en esta etapa mostró un contenido de compuestos fenólicos tres veces mayor que las alcaparras "Finas" o "Gruesas", pero en ORI.10 las diferencias fueron menores. El contenido de compuestos fenólicos en las alcaparras es mayor que en algunas frutas y verduras exóticas y comunes. Los consumidores valoran los alimentos con un alto nivel de sustancias bioactivas, como los polifenoles, ya que se sabe que son los antioxidantes más abundantes en nuestra dieta. Nuestros análisis de compuestos fenólicos de alcaparras identificaron a los flavonoles como el principal grupo polifenólico, que representa en promedio del 80 % al 95 % de todos los compuestos fenólicos, independientemente de la etapa de crecimiento. Los flavan-3-oles (con una abundancia de 3% a 14%) ocuparon el segundo lugar y los ácidos fenólicos (1% a 5%) fueron terceros. Estos resultados corroboran los de informes anteriores (Francesca et al., 2016; Sharaf et al., 2000 y Pérez et al., 2005), pero nuestro trabajo se centró principalmente en

el contenido de flavonoles. El alto contenido de flavonoles puede reflejar la respuesta de la planta a estreses bióticos y abióticos o aclimatación a estreses ambientales como calor, frío, radiación ultravioleta, sequía, salinidad o un ataque de herbívoros o patógenos (Wang y Zheng et al., 2001). Varios estudios demostraron el efecto de la temperatura y la luz solar sobre la acumulación de flavonoides en la piel de la flor y la baya o el fruto. Wang y Zheng (2001) encontraron que las fresas cultivadas a 18/12 ºC generalmente tenían el contenido más bajo de antocianina, flavonol (quercetina-3-O-glucósido y quercetina-3-O-glucurónido) y ácido fenólico, mientras que a 30/ 22 ºC su contenido fue más alto.

Estos resultados confirman que las alcaparras son una fuente muy rica de compuestos fenólicos, especialmente flavonoles, flavan-3-oles y ácido hidroxicinámico. Los flavonoles totales en las alcaparras comprenden una mezcla de diferentes derivados de quercetina glicosilada, isorhamnetina, kaempferol y miricetina.

Los derivados de quercetina, kaempferol, miricetina e isorhamnetina representaron, respectivamente, 38%-67%, 15%-36%, 4%-7% y 0,85-3% del total de flavonoles en alcaparras, pero su contenido dependía en gran medida del estadio de crecimiento, siendo "Nonpareilles" y "Surfines" más ricos en flavonoles que “Finos” y “Gruesos”. Las diferencias más pequeñas entre etapas individuales se observaron para "Nonpareilles" y "Surfines" o para "Capucines" y "Capotes". Las alcaparras menores de 7-8 mm fueron más ricas en flavonoles que las más grandes. Se observó un efecto similar para las bayas de madreselva y el contenido de antocianinas (Wojdyło et al., 2013).

Las procianidinas poliméricas clasificadas como flavan-3-oles se determinaron mediante métodos de floroglucinol. Demostraron que las procianidinas poliméricas en las alcaparras consistían principalmente en unidades poliméricas de (-) - epicatequina en lugar de (+) - catequina. En nuestro estudio, las procianidinas poliméricas en el grupo fenólico total representaron no más del 14 %, y su concentración osciló entre 200 mg y 700 mg/100 g. Jiménez-López et al. (2018) postularon que las alcaparras fermentadas todavía contenían (-) - epicatequina y (epi) catequina dímero y trímero en la cantidad de 160 mg/100 g. En cuanto a los flavonoles, las "Nonpareilles" tenían un contenido de procianidinas poliméricas similar al de las "Gruesas". "Capucines", "Capotes" y "Finos"; primero hibridaron niveles más altos de procianidina polimérica que las alcaparras en otras etapas de crecimiento. Zhang et al. (2013) informaron que la acumulación de flavan-3-oles en la uva Cabernet Sauvignon dependía de una etapa de desarrollo y correspondía a la luz ultravioleta suplementaria.

El análisis cuantitativo de las procianidinas poliméricas reveló su mayor contenido en las alcaparras ORI.7 en comparación con la muestra ORI.10.

Los ácidos hidroxicinámicos pertenecían a un grupo menor de polifenoles de las alcaparras. Su concentración osciló entre 178,6 y 71,8 mg x 100 g^{-1} para ORI.7 y entre 283,4 y 44,8 mg x 100 g^{-1} para ORI.10. Asimismo, el contenido de ácidos hidroxicinámicos en las "Nonpareilles" fue mayor que en las "Gruesas". Los ácidos hidroxicinámicos predominantes en las alcaparras fueron el ácido feruloilquínico y el ácido cafeoilquínico. Los ácidos restantes estaban presentes en bajas concentraciones, es decir, el ácido sinápico representaba menos del 2% al 14%. Los datos de la literatura sobre ácidos hidroxicinámicos son escasos. Siracusa et al. (2011) identificaron los ácidos 5- y 4-cafeoilquínicos, los ácidos 5-p-cumaroilquínico y 4-feruloilquínico, y postularon los ácidos 5-cafeoilquínico y 4-feruloilquínico como los compuestos principales.

Los ácidos hidroxicinámicos como los ácidos cafeoilquínicos son buenas fuentes de antioxidantes *in vitro* que protegen a las lipoproteínas de baja densidad (LDL) de la oxidación y, por lo tanto, supuestamente previenen varias enfermedades relacionadas con la edad. El ácido sinápico presente en las alcaparras da su sabor amargo y astringente, similar a los productos de colza.

Potencial biológico de las alcaparras

Evaluamos el potencial biológico de las alcaparras en diferentes etapas de crecimiento en función de su actividad antioxidante ($ABTS^{+}$, FRAP, ORAC), actividad antidiabética (α-amilasa y α-glucosidasa) e inhibición de la colinesterasa (acetilcolinesterasa (AChE) y butirilcolinesterasa (BuChE)). Las alcaparras “Nonpareilles” de ORI.7 exhibieron mayor actividad antioxidante ORAC (27,7 mM Trolox x 100 g^{-1}) que las “Nonpareilles” ORI.10 (Trolox 19,3 mM x 100 g^{-1}). Las alcaparras en estado “Gruesas” presentaron la mayor actividad antioxidante de 16,8 y Trolox 10,7 mM x 100 g^{-1} para ORI.7 y OR I.10, respectivamente. Los ensayos ABTS y FRAP mostraron la misma tendencia que los ensayos ORAC.

Los extractos de alcaparra inhibieron las enzimas α-glucosidasa y α-amilasa, con valores de IC_{50} que oscilaron entre 3,74 y 0,93 mg x mL^{-1} para la primera y entre 3,68 y 1,52 mg x mL^{-1} para la segunda. Se descubrió que el extracto "Nonpareilles" de ORI.7 es un inhibidor eficaz potencial de la α-glucosidasa y la α-amilasa. Este no fue el caso de la muestra ORI.10, donde el extracto de “Gruesas” inhibía mucho más eficazmente la α-amilasa y el extracto de “Capotes” mostró un alto potencial inhibidor de la α-glucosidasa. Nuestros resultados son comparables a los

informados por Mollica et al. (2017), donde los extractos acuosos de *C. spinosa* exhibieron una potente actividad antihiperglucémica en ratas diabéticas.

Hasta donde sabemos, este es el primer artículo sobre la inhibición de la colinesterasa, tanto AChE como BuChE por las alcaparras. En todos los casos, la inhibición fue altamente dependiente de la etapa de crecimiento y la variedad. En la muestra ORI.7, la inhibición máxima de AChE y BuChE alcanzó el 18,3 % y el 31,0 %, respectivamente, y en la muestra ORI.10 fue del 28,1 % y el 33,8 %, respectivamente. Las alcaparras "Nonpareilles" resultaron ser los inhibidores más potentes. Además, la supresión de BuChE fue más efectiva que la de AChE. Esto es deseable ya que la actividad peptidasa de la butirilcolinesterasa controla el desarrollo y la progresión de la enfermedad de Alzheimer. Los extractos capaces de inhibir BuChE también pueden prevenir la progresión de la enfermedad causada por la acumulación de proteína β-amiloide, ya que ayudan a propagar las placas de β-amiloide (Brimijoin et al., 2016). La enfermedad de Alzheimer es la causa más común de demencia en los ancianos y se caracteriza por la degeneración de neuronas colinérgicas en áreas específicas del cerebro asociadas con funciones intelectuales superiores, memoria y conciencia (Ferreres et al., 2012).

También se estudió el coeficiente de correlación de Pearson para el contenido de polifenoles y actividad biológica. Así, se puede observar una correlación fuerte y significativa entre ORAC y flavonoles (suma de derivados de quercetina, kaempferol, isorhamnetina y miricetina; arriba de r = 0,684) y ácido fenólico (suma de ácido cafeico, ácido p-cumaroilquínico y ácido feruloilquínico). También encontramos una correlación positiva entre los compuestos fenólicos y la inhibición de la colinesterasa (AChE, BuChE) y la actividad antidiabética (α-amilasa y α-glucosidasa). Sin embargo, sólo observamos una correlación negativa entre las procianidinas poliméricas y la prueba de actividad biológica, con la excepción de BuChE, donde no fue significativa.

Los mono y diflavonoles desempeñaron un papel más importante en la actividad biológica (especialmente la actividad antioxidante ORAC, la α-amilasa y la inhibición de BuChE) que la aglicona o los tetraflavonoides. Además, los ácidos fenólicos mostraron mayor potencial antidiabético y actividad anticolinesterasa que la actividad antioxidante (FRAP>ABTS>ORAC). Estos resultados mostraron que no debemos ignorar el papel de los componentes no fenólicos de las alcaparras, ya que pueden presentar efectos sinérgicos o antagónicos aún desconocidos.

E. Publicación V

PUBLICACIÓN V

Volatile Profile in Different Aerial Parts of Two Caper Cultivars (*Capparis spinosa* L.)

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El objetivo de la presente investigación consistió en realizar el perfil volátil de las distintas partes aéreas de la alcaparra, flores, tallos y hojas, frutos y tápenas, en distintos cultivares españoles ubicados en zonas geográficas distintas.

Se han identificado 43 compuestos volátiles diferentes en las cuatro partes (flores, hojas, alcaparras y alcaparrones) del alcaparro. Los resultados indicaron que el componente volátil predominante en todas las partes aéreas del alcaparro fue el isocianato de metilo (familia química: isocianato). Este compuesto es responsable del olor acre de la planta de alcaparras. En las flores, hojas, tallos y botones florales, hubo diferencias significativas en el contenido de isotiocianato de metilo entre los cultivares "ORI3" y "ORI7" (710 y 2184 mg x kg^{-1} peso fresco (pf), 2654 y 1277 mg x kg^{-1} pf, y 6882 y 667 mg x kg^{-1} pf, respectivamente). En el estudio sobre la composición volátil de *Capparis spinosa* L. de Afsharypuor et al. (1998) usando hojas, frutos y raíces, el compuesto predominante también fue el isotiocianato de metilo, lo cual fue consistente con los resultados actuales. Los compuestos de azufre presentaron la mayor cantidad en estos cultivares, siendo el isotiocianato de metilo el componente principal. Los isotiocianatos derivan de los glucosinolatos por hidrólisis enzimática y se encuentran principalmente en Brassicaceae, aunque también se identifican en otras familias, como Capparaceae y Caricaceae (Condurso et al., 2016). Los glucosinolatos forman parte de las defensas antiherbívoras de las plantas, y el isotiocianato de metilo se puede considerar como el compuesto más representativo de la fracción aromática de la planta de las alcaparras y se deriva de la degradación de la glucocapparina (Gigot et al., 2010).

Otro compuesto de alto contenido fue el nonanal; en general, los aldehídos aportan notas aceitosas y de sabor verde a las matrices vegetales. El nonanal es uno de los compuestos volátiles responsables del característico "aroma verde fresco" de frutas y verduras. Normalmente es el aroma característico de frutas y verduras y también se sintetizan en los órganos verdes de las plantas en respuesta a una lesión. El compuesto nonanal obtenido de los aldehídos C6 y C9 derivados del hidroperóxido liasa convierte ambos tipos de derivados hidroperóxido de ácidos grasos en aldehídos que a menudo se reducen a alcoholes por alcohol deshidrogenasas (Gigot et al., 2010). Estos aldehídos y alcoholes C6/C9 tienen su origen en la biosíntesis de ácidos grasos, la cual se basa en un conjunto de acetil-CoA generado a partir del piruvato, producto final de la glucólisis (Duradeva et al., 2012). Las tendencias para nonanal fueron diferentes según el cultivar; “ORI7” tiene más contenidos que “ORI3” en flores y frutos de alcaparras (3,5 y 1,7 mg x kg^{-1} pf y 29,2 y 1,32 mg x kg^{-1} pf, respectivamente), y en el caso de hojas y alcaparras, “ORI3” presentó contenidos superiores a “ORI7” (4,63 y 2,05 mg x kg^{-1} pf y 772,5 y 2,57, respectivamente). Sin embargo, es importante resaltar que factores como el tipo de suelo, las condiciones climáticas, la etapa de crecimiento y otros factores pueden influir en la composición volátil de las plantas, y se deben realizar análisis específicos para confirmar los resultados mostrados en este estudio.

Los alcoholes y los aldehídos, derivados de la degradación oxidativa de los ácidos grasos, forman el complejo volátil de la mayoría de las plantas de hoja verde, como citan Romeo et al. (2007). Según la bibliografía consultada, existen pocos estudios que evalúen la composición volátil de las partes aéreas del alcaparro (flores, hojas, alcaparras y frutos). En cuanto a las flores, no existen muchos estudios previos de flores enteras. Existen estudios como los realizados por Ascrizzi et al. (2016), los cuales determinaron el contenido de compuestos volátiles en las diferentes partes de la flor, como sépalos, pétalos y estambres, así como flores completas, hojas y botones florales. Los resultados fueron expresados en porcentaje; por lo tanto, no son completamente comparables con los resultados mostrados en la presente investigación.

De todos modos, estos autores reportaron que el compuesto predominante en las flores fue el benzoato de metilo, frente al isotiocianato de metilo (26882 mg x kg^{-1} pf en "ORI3" de botones florales). Esto puede deberse a factores como el cultivo, las prácticas agrícolas, el tipo de suelo y el método de aislamiento de los volátiles. Se identificaron y cuantificaron 32 compuestos volátiles en las flores. El nerolidol fue el compuesto predominante después del isotiocianato de metilo. Se encontraron diferencias significativas entre los cultivares "ORI3" y "ORI7" con un contenido de nerolidol de 144 y 194 mg x kg^{-1} pf, respectivamente. Los descriptores sensoriales de este compuesto son madera, verde, manzana, cítrico o rosa y pueden ser atractivos para los consumidores. En el presente estudio también se encontró un contenido importante de terpenos en flores como limoneno, β-farneseno, α-farneseno (isómeros 1 y 2) y nerolidol, que tienen notas aromáticas afrutadas como limón, naranja, dulce, lima, pera o pomelo. Uno de los usos importantes de las alcaparras a lo largo de la historia ha sido su uso medicinal, y su actividad antimicrobiana (Beir et al., 1992). En cuanto al contenido total de terpenos, "ORI3" tuvo 174,9 mg x kg^{-1} frente a 223,8 mg x kg^{-1} del cultivar "ORI7".

También se encontró un alto contenido de isotiocianato de bencilo (familia química: isocianato). Aunque hubo diferencias significativas entre ambos cultivares, el cultivar "ORI7" presentó una mayor concentración (98,1 mg x kg^{-1} pf) en comparación con el cultivar "ORI3" (16,9 mg x kg^{-1} pf). Este compuesto da un ligero olor a col a las alcaparras. En esta investigación se observó que la familia química predominante presente en las flores fueron los isocianatos. Además, el 3-hexanol (familia química: alcohol) también presentó contenidos elevados, con diferencias significativas entre los cultivares "ORI7" y "ORI3". Por otro lado, el β-farneseno (familia química: terpeno) también tuvo un contenido alto, nuevamente con diferencias entre los cultivares "ORI7" y "ORI3". Se identificaron un total de 10 compuestos volátiles en hojas de alcaparras. Los compuestos trans-2-hexenal (aldehído) tuvieron un alto contenido (después del isotiocianato de metilo). Este compuesto tiene olor a almendra, manzana, verde, vegetal, ciruela y dulce.

También mostró un contenido significativo de isocianuro de bencilo (familia química: isocianato), teniendo "ORI3" un contenido mayor que "ORI7". No hubo estudios previos sobre la composición volátil en hojas de alcaparras. En el estudio de El-Ghorab et al. (2007) con *Capparis ovata* Desf. variedad *canescens* cultivada en Turquía, se publicó que el compuesto más abundante en los brotes de alcaparras era el alcohol bencílico (20,4 %) y el isotiocianato de metilo en las hojas (20,0 %). En este artículo, se han cuantificado nuevos compuestos.

En los botones florales se identificaron 18 compuestos volátiles, predominando el trans-2-hexenal (aldehído) en el cultivar “ORI3”. Este aldehído tiene un aroma similar a vegetal, verde, ciruela, manzana, almendra y dulce. El segundo compuesto relevante en las alcaparras fue el nonanal (aldehído). Este compuesto tiene un aroma similar a verde, limón, lima, carnoso, graso y rosado. El bencenoacetaldehído presentó contenidos elevados en el cultivar “ORI3”. La presencia en alimentos de aldehídos alifáticos, ácidos y alcoholes como trans-2-hexenal, hexanal, ácido nonanoico y 1-octanol está relacionada con reacciones de oxidación de grasas. Estas sustancias se han encontrado en una variedad de productos alimenticios, incluidos carnes, tanto frescas como procesadas, frutas, así como productos lácteos y cereales. Más específicamente se han asociado con aromáticos verdes/herbáceos/vegetales en frutas y verduras (Condurso et al., 2016).

En frutos sólo se encontraron 6 compuestos volátiles. La mayoría de estos compuestos no presentaron diferencias significativas entre los dos cultivares en estudio, salvo los casos del trans-2-hexenal, limoneno, y nonanal. El isotiocianato de metilo fue identificado como el compuesto volátil predominante en el estudio de Afsharypuor et al. (1998), encontrado en caparrones.

F. Publicación VI

PUBLICACIÓN VI

Relationships between chemical composition, antioxidant activity and genetic analysis with ISSR markers in flower buds of caper plants (*Capparis spinosa* L.) of two subspecies *spinosa* and *rupestris* of Spanish cultivars

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El objetivo de la presente investigación consistió en relacionar el contenido químico, y actividad antioxidante con el análisis genético con marcadores moleculares ISSR en botones florales de plantas de Capparis spinosa L. en dos subespecies spinosa y rupestris en distintos cultivares de España.

Diversidad genética y clasificación jerárquica

Los 32 cultivares de *Capparis* se amplificaron consistentemente con 8 de los 18 cebadores. Se encontró que la cantidad de productos generados por cebador oscilaba entre 5 y 15 de diferentes tamaños en el rango de 0,23 a 2,20 kb. El cebador ISSR15 exhibió el máximo (15 productos), mientras que el cebador ISSR10 dio el menor número de productos (5). Se produjeron un total de 83 productos amplificados con un promedio de 10,37 productos por primer, de los cuales el 97,6% fueron polimórficos y el 2,4% de los productos monomórficos. El porcentaje de bandas polimórficas osciló entre el 80 % para el primer ISSR10 y el 100 % para los otros *primers*.

En el dendrograma realizado los cultivares se agruparon en dos grupos principales. El grupo I contiene todos los cultivares TE con el cultivar TE10 como el más diferente. El clúster II contiene todos los demás cultivares, con una buena agrupación obtenida para los otros grupos: todos los cultivares TS (excepto TS7), TA, TALB (excepto TALB4) y TALCY. La agrupación de cultivares por subespecies también fue encontrada por Gristina et al. (2014) en Italia, así como por especie de Al-Safadi et al. (2014) en Siria.

En la PCA obtenida con los resultados de ISSR, los dos primeros componentes principales (PC1 y PC2) explicaron el 58,9 y el 7,1% de la variabilidad, respectivamente. La distribución de cultivares fue muy similar a la del dendrograma, con dos grandes grupos y el cultivar TE10 en una posición intermedia. Ningún cultivar TE tiene estípulas espinosas excepto TE10. Debido a estas estípulas espinosas y a los resultados obtenidos con PCA, se puede pensar que TE10 es un híbrido entre las subespecies *spinosa* y *rupestris*.

Análisis de la estructura genética

La identificación de grupos de plantas genéticamente similares se realizó mediante un análisis de agrupamiento basado en un modelo de mezcla implementado en el software Structure. La prueba de Evanno indicó que el número más informativo de subpoblaciones (K) es 2, lo que sugiere la existencia de dos grupos principales en los cultivares actuales de *Capparis*. Los grupos definidos por el análisis de la estructura representan estadísticamente diferentes subpoblaciones, según lo indicado por la evaluación de la diferenciación genética. Todos los cultivares formaron dos subpoblaciones: la subpoblación 1 que consta de todos los cultivares TE (*C. spinosa* subsp. *rupestris*) y subpoblación 2 que contiene el resto de los cultivares de *C. spinosa* subsp. *spinosa* (TS, TA, TALB y TALCY).

Determinación de parámetros bioquímicos

La clorofila *a* presentó valores de 9,07 a 21,65 mg 100 g^{-1} ps en los cultivares 'TA5' y 'TE9', respectivamente. El contenido de clorofila *b* presentó su mayor valor en el cultivar 'TE1' con un total de 8,48 mg 100 g^{-1} ps. En cuanto a las clorofilas totales, los valores oscilaron entre 14,09 y 29,44 mg 100 g^{-1} ps, en los cultivares 'TA5' y 'TE9', respectivamente. Hubo una correlación positiva entre las clorofilas *a*, las clorofilas *b* y las clorofilas totales.

Los cultivares de *spinosa* muestran un bajo contenido de clorofilas con respecto a *rupestris*, aunque existen algunas poblaciones de *spinosa* que no han presentado diferencias significativas con *rupestris*. Por tanto, hay más diferencias entre las poblaciones de *spinosa* que la media entre *spinosa* y *rupestris*.

El contenido de carotenoides presentó el valor más elevado en el cultivar 'TS2'. Por el contrario, el cultivar 'TA4' ha presentado un menor valor en contenido de carotenoides. Si relacionamos los resultados por zonas geográficas, la población TS presentó los valores más altos de contenido de carotenoides, seguida de 'TALB', 'TALCY' y 'TE' con valores similares.

Por otro lado, el contenido de proteína no se vio afectado por la subespecie, pero la población lo afectó. La población 'TALB' presentó los valores más altos, seguida por la 'TS' y 'TA', sin diferencias significativas entre ellos. Los valores medios más bajos se observaron para 'TE' y 'ALCY'. El contenido de proteína estuvo en un rango de valores entre 8,63 y 16,66 mg x 100 g^{-1} ps, siendo el cultivar ’ALCY2’ el que ha presentado menor contenido y el cultivar ’TA3’ el que ha presentado mayor valor. El contenido en proteína no mostró diferencias significativas entre las subespecies de *spinosa* y *rupestris*, encontrando más diferencias entre las poblaciones de *spinosa* que entre el promedio de *rupestris* y *spinosa*.

Se observó una correlación positiva entre clorofilas *b* y carotenoides, por tal motivo, se observa que el cultivar 'TE1' presenta el mayor valor en clorofilas *b* y carotenoides. En el caso del cultivar 'TA4' sucede lo mismo, ha presentado valores bajos tanto en clorofilas *b* como en carotenoides.

No existen muchos estudios previos sobre las propiedades bioquímicas de *C. spinosa* en España. Ulukapi et al. (2016) obtuvieron un contenido de carotenoides de 21,24 mg x kg^{-1} en alcaparras de Turquía.

El contenido total de fenoles y flavonoles totales se vieron afectados por la subespecie y la población, mientras que los flavonoides totales solo se vieron afectados por la población.

En cuanto a los cultivares, se observó mayor valor de contenido de fenoles totales en el cultivar 'TS1'. Se detectaron diferencias significativas entre poblaciones y también entre subespecie. Los botones florales de las poblaciones 'TS' y 'TA' mostraron el mayor contenido de fenoles totales en oposición a las poblaciones 'TE', 'TALB' y 'TALCY'; mientras que entre subespecie las tápenas de la subesp. *spinosa* mostraron el mayor contenido de fenoles totales en comparación con la subsp. *rupestris*. Los resultados obtenidos mostraron que el contenido de fenoles totales estuvo influenciado por el genotipo y las condiciones ambientales.

En referencia al contenido total de flavonoides, este no se vio afectado por la subsp.; sin embargo, se reportaron diferencias significativas para poblaciones y entre cultivares. Así, los valores máximos se obtuvieron para las tápenas de las poblaciones 'TA', 'TS' y 'TE'. En cuanto a los cultivares, el contenido total de flavonoides osciló entre 1849,55 y 2495,46 mg de rutina eq. 100 g^{-1} ps ('TALB1' y 'TA4', respectivamente). Las tápenas con mayor valor de flavonoides se observaron para la subsp. *spinosa* y entre poblaciones para 'TS' y 'TA'; mientras que entre los cultivares los valores más altos se observaron para el cultivar 'TA4'.

Existe una correlación negativa entre clorofilas *a* y clorofilas totales con el contenido total de fenoles y flavonoles totales. Así, el cultivar 'TE9' ha presentado un alto contenido de clorofilas *a* y totales con respecto a los demás cultivares, mientras que en el contenido de fenoles totales y flavonoles ha presentado uno de los valores más bajos.

Aliyazicioglu et al. (2013) obtuvieron un valor de fenoles totales muy por debajo de los obtenidos en este estudio. Comparado con los fenoles totales de otros cultivos como mango (208 mg 100 g^{-1}), arándano (525 mg 100 g^{-1}) o frambuesa (517 mg 100 g^{-1}), los resultados en *Capparis spinosa* demostraron que es una excelente fuente natural de estos compuestos (Tlili et al., 2010).

Los resultados de AAT (tanto AAT-H como AAT-L) mostraron que el genotipo juega un papel importante en la determinación de la capacidad antioxidante de cada cultivar. La AAT-H no mostró diferencias entre subespecies, aunque sí entre poblaciones, siendo el cultivar 'TS' el que presentó los valores más altos, con un promedio de 1403,36 mg Trolox Eq. 100 g^{-1} ps. En cuanto a los cultivares, el contenido de AAT-H mostró valores en un rango entre 384,99 y 1883,13 mg Trolox Eq. 100 g^{-1} ps en los cultivares 'TALB4' y 'TS1', respectivamente. En el caso del contenido de AAT-L, hubo diferencias entre subespecies y población, siendo la subespecie *rupestris* y la población 'TE' las que presentaron mayor AAT-L. En cuanto a los cultivares, 'TE6' presentó el mayor valor y el más bajo lo presentó 'TALB2'. Se encontraron más diferencias en los contenidos de ambos parámetros entre las poblaciones de *spinosa* que entre las medias de *spinosa* y *rupestris*.

La actividad antioxidante no fue significativamente diferente entre la subsp. y poblaciones, pero sí entre cultivares. En todos los casos de AAT, hubo más diferencias entre las poblaciones de *spinosa* que entre las poblaciones de *spinosa* y *rupestris*. La actividad antioxidante por el método $ABTS^{+}$ presentó los valores más altos en el cultivar 'TE5'. Por otro lado, la actividad antioxidante por el método FRAP presentó los valores más elevados en los cultivares 'TALB2' y 'TA4'.

El cultivar 'TE5' presentó el mayor valor en el método DPPH·. Por el contrario, el cultivar 'TALB1' fue el que presentó el valor más bajo.

Tesoriere et al. (2007) encontraron valores más bajos de AAT-H y AAT-L en tápenas de cultivares italianos. Aliyazicioglu et al. (2013) obtuvieron valores inferiores de FRAP en cultivares turcos. Estas diferencias pueden deberse a diferencias genotípicas y ambientales.

Aquellos rasgos que miden el contenido de clorofilas tienen una correlación positiva muy estrecha entre ellos. La clorofila *a* y la clorofila total se correlacionan negativamente con los fenoles totales y los flavonoles totales, mientras que la clorofila *b* se correlaciona positivamente con los carotenoides y AAT-H. Los carotenoides se correlacionan positivamente con la clorofila total, la clorofila *b* y las proteínas, y negativamente con los flavonoides totales y el DPPH·. Fenoles, flavonoides y flavonoles tienen una correlación positiva entre ellos. FRAP y los flavonoides totales se correlacionan positivamente, así como DPPH· y $ABTS^{+}$.

Para lograr una mejor comprensión de los resultados y tendencias de las variables estudiadas (13 rasgos y 24 cultivares de *Capparis*), se aplicó el análisis de componentes principales (PCA).

El primer componente (PC1) se relacionó positivamente con clorofilas, AAT-H y AAT-L, y negativamente con fenoles totales, flavonoides y flavonoles. El PC2 se correlacionó positivamente con $ABTS^{+}$, FRAP y DPPH·, y negativamente con las proteínas.

Comparando los resultados de la PCA obtenidos con compuestos químicos y funcionales e ISSR, observamos que, aunque la distribución de cultivares no es idéntica, las cifras son muy similares. En ambos casos, se agruparon los cultivares 'TE', con excepción del cultivar 'TE10'. Así, los cultivares 'TALB1', 'TALB2' y 'TS2' se agruparon entre sí distanciándose de los cultivares 'TE'.

CONCLUSIONES GENERALES Y FUTURAS INVESTIGACIONES

6. Conclusiones Generales y Futuras Investigaciones

6.1. Conclusiones generales

Por primera vez se presenta el estudio morfológico, bioquímico, funcional y compuestos volátiles de las diferentes partes aéreas de *Capparis spinosa* L. caparrones, tápenas, tallos tiernos y flores. Asimismo, se ha realizado un estudio comparativo entre la composición química, actividad antioxidante y análisis genético de dos subespecies *spinosa* y *rupestris* de cultivares españoles. El estudio se llevó a cabo en diferentes cultivares ubicados en diferentes áreas geográficas del sureste de España. Las conclusiones generales son las siguientes:

1. Los frutos de *C. spinosa* L. se caracterizaron por presentar el exocarpio verde en todos los estados de desarrollo, con cambios muy leves que no se produjeron por un aumento en la síntesis de carotenoides sino por la degradación parcial de las clorofilas *a* y *b*. También se ha demostrado una buena capacidad antioxidante, debido a la presencia de fenoles, flavonoides y flavonoles totales. De las tres etapas de desarrollo estudiadas, “Fino”, “Medio” y “Grueso”, el estado “Grueso” es el más interesante por su mayor contenido en compuestos bioactivos. Se estudiaron diferentes cultivares, pero el de mayor contenido en AAT y contenido total de fenoles y flavonoides fue 'ALB 2'.

2. Las tápenas presentaron un contenido total de polifenoles, flavonoides y flavonoles muy alto en todas las etapas de desarrollo y muy similar entre los diferentes cultivares estudiados. Los resultados observados llaman la atención sobre el potencial antioxidante de las alcaparras, especialmente cuanto más pequeñas eran, mejores propiedades antioxidantes y nutricionales presentaban. Por lo tanto, la etapa de desarrollo de “Nompareilles” fue la que presentó mayor contenido de flavonoides, flavonoles, proteínas, azúcares y AAT por los métodos DPPH· y AAT-H. El cultivar que ha presentado valores más interesantes ha sido 'Collados 5'.

3. Se ha determinado que la parte aérea con una mayor cantidad de actividad antioxidante total han sido los tallos tiernos. Con lo cual estos podrían desempeñar un papel muy importante en la nutrición humana. De los diferentes cultivares estudiados los que ha presentado unos mejores resultados de AAT han sido ‘ORI 4’, ‘ORI 10’ y ‘ALB 2’.

4. El perfil fenólico de las alcaparras incluía flavonoles (quercetina, kaempferol, miricetina e isorhamnetina), ácidos fenólicos y flavan-3-oles. Nueve compuestos fueron descritos por primera vez en alcaparras, que nunca antes habían sido identificados y cuantificados

en esta especie. De las diferentes etapas de desarrollo que tienen las alcaparras, la etapa "Nompareilles" acumuló las cantidades más altas de compuestos bioactivos que se correlacionaron con propiedades antioxidantes y antidiabéticas, y fueron BuChE más potentes que los inhibidores de AChE. Los dos cultivares estudiados 'ORI 7' y 'ORI 10' presentaron valores interesantes, aunque deben ser mejorados constantemente a través de programas de mejora para refinar sus propiedades funcionales.

5. Las flores de alcaparra presentaron un perfil volátil complejo con 32 compuestos volátiles aislados, identificados y cuantificados. Las tápenas presentaron un perfil con 18 compuestos volátiles y la familia química predominante ha sido la de los isocianatos, principalmente el isotiocianato de metilo, que proporciona el sabor picante característico de las alcaparras. Los tallos y las hojas tienen un total de 10 compuestos volátiles aislados, identificados y cuantificados. Los alcaparrones presentaron perfiles con 6 compuestos volátiles, los cuales no tienen un aroma fuerte. Los resultados mostraron que los perfiles volátiles permitían distinguir los cultivares de alcaparras, siendo el cultivar 'ORI 7' el que presentó mejores resultados. Asimismo, las flores de alcaparra mostraron un perfil volátil complejo y rico, así como una apariencia atractiva que las hará muy útiles en la cocina moderna.

6. En cuanto a las propiedades bioquímicas y actividad antioxidante entre las dos subespecies estudiadas, *rupestris* y *spinosa,* se ha observado que no hay diferencias significativas entre subespecies para los parámetros estudiados. Se observaron más diferencias entre los cultivares de la subespecie *spinosa* entre sí, que con los de la subespecie *rupestris*. Por lo que podemos concluir que la subespecie *rupestris*, que no presenta espinas, podría ser cultivada en lugar de la subespecie *spinosa* que sí las presenta, sin que esto conllevase una pérdida de las propiedades funcionales de las tápenas.

7. Las propiedades observadas a lo largo de este estudio podrían justificar el amplio uso de esta planta como alimento y en medicina tradicional, y como planta para la obtención de compuestos bioactivos para la industria alimentaria, pudiendo ser plantada en lugares cada vez más secos, como la cuenca mediterránea, ante la amenaza del calentamiento global.

6.2. Futuras investigaciones

En base a los resultados obtenidos en esta Tesis Doctoral, con el fin de profundizar más sobre el cultivo de *Capparis spinosa* L., se propone:

1. La determinación del perfil de ácidos grasos en los botones florales y frutos de la alcaparra en los diferentes estados de desarrollo y en diferentes cultivares españoles.
2. El estudio de reproducción y mejora de los cultivares para mejorar el rendimiento agronómico, así como las propiedades físico-químicas de los nuevos cultivares obtenidos.
3. El estudio del gran número de las propiedades funcionales de la alcaparra y otras partes aéreas sobre la salud humana.
4. Estudio de las propiedades funcionales de alcaparras y otras partes aéreas, en vinagre o salmuera, sobre la salud humana.
5. Uso de los marcadores ISSR utilizados para programas de mejora genética.
6. Mejora de los cultivos de frutales frente al cambio climático al cultivar alcaparras entre las líneas de frutales e incremento de valor añadido.

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Información adicional.

**Todas las Imágenes e Ilustraciones que aparecen en la presente Tesis Doctoral son de elaboración propia.*