



**Universidad Miguel Hernández**  
**Escuela Politécnica Superior de Orihuela**  
**Programa de Doctorado en Recursos y Tecnologías Agrarias,**  
**Agroambientales y Alimentarias**



**Research, Development and  
Innovation of Hydrosustainable  
products based on pomegranate**

**Doctoral Thesis**

**Marina Cano Lamadrid**  
**2020**





# Research, development and innovation of hydrosustainable products based on pomegranate

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## **Marina Cano Lamadrid**

Degree in Food Science and Technology

Master in Agroecology, Rural Development and Agro-tourism

Master in Education

## **Supervisors:**

Dr. Ángel Antonio Carbonell Barrachina

Dr. Aneta Wojdyło

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Thesis for the Degree of Doctor from the  
Miguel Hernández University  
Orihuela, Spain, 2020



# **Research, development and innovation of hydrosustainable products based on pomegranate**

Thesis presented by Marina Cano Lamadrid to qualify for Doctor Degree from Miguel  
Hernández University

**Marina Cano Lamadrid**

Degree in Food Science and Technology

Master in Agro-ecology, Rural Development and Agro-tourism

Master in Education

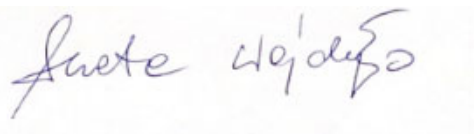
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**Dr. D. Ángel Antonio Carbonell Barrachina**, Catedrático de Universidad y Coordinador del Programa de Doctorado Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche (UMH),

**CERTIFICA:**

Que la Tesis Doctoral titulada "**Research, development and innovation of hydrosustainable products based on pomegranate**" de la que es autora la Máster en Agroecología, Desarrollo Rural y Agroturismo Dña. **Marina Cano Lamadrid**, ha sido dirigida por el Prof. Ángel Antonio Carbonell Barrachina, Catedrático de Universidad del Departamento de Tecnología de los Alimentos de la Universidad Miguel Hernández de Elche y codirigida por la Profa. Aneta Wojdyło (*Wrocław University of Environmental and Life Sciences*), actuando como tutora de la misma la Dr. Francisca Hernández García (UMH). Considero que la tesis es conforme en cuanto a forma y contenido a los requerimientos del Programa de Doctorado ReTos-AAA, por tanto, es apta para su exposición y defensa pública.

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

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*A mi padre, presente en mi mente durante todo este tiempo*

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## Category

This doctoral thesis is classified in a **compendium of publications category** to qualify for Doctor Degree from Miguel Hernández University of Elche.

For that purpose, the 7 selected research articles and their quality, in accordance with the 2018 edition of Journal Citation Reports® (JCR®), are shown:

| Publication 1   |  |                    |
|---|--|--------------------|
| <b>Cano-Lamadrid, M.</b> , Galindo, A., Collado-González, J., Rodríguez, P., Cruz, Z.N., Legua, P., Burló, F., Morales, D., Carbonell-Barrachina, Á.A., and Hernández, F. (2018). Influence of deficit irrigation and crop load on the yield and fruit quality in Wonderful and Mollar de Elche pomegranates. <i>Journal of the Science of Food and Agriculture</i> , 98: 3098-3108. doi: <a href="https://doi.org/10.1002/jsfa.8810">10.1002/jsfa.8810</a> |  |                    |
| Published   | Dec 6 2018   | ISSN 0022-5142     |
| Publisher   | Elsevier Science BV, PO Box 211, 1000 AE Amsterdam, Netherlands                  |                    |
| Research Domain   | Agriculture, Multidisciplinary; Chemistry, Applied;<br>Food Science & Technology |                    |
| JCR® Category   | Agriculture, Multidisciplinary   |                    |
| <b>Quartile</b>   | <b>Q1</b>  | <b>Rank 9/57</b>   |
| Impact Factor (IF) / 5 year IF  | 2.422 / 2.733  |                    |
| Publication 2   |  |                    |
| <b>Cano-Lamadrid, M.</b> , Lipan, L., Calín-Sánchez, Á., Hernández, F., and Carbonell-Barrachina, Á.A. (2017). A comparative study between labeling and reality: The case of phytochemical composition of commercial pomegranate-based products. <i>Journal of Food Science</i> , 82: 1820-1826. doi: <a href="https://doi.org/10.1111/1750-3841.13788">10.1111/1750-3841.13788</a>   |  |                    |
| Published   | Jun 20 2017  | ISSN 0022-1147     |
| Publisher   | Wiley  |                    |
| Research Domain   | Food Science & Technology  |                    |
| JCR® Category   | Food Science & Technology  |                    |
| <b>Quartile</b>   | <b>Q2</b>  | <b>Rank 56/135</b> |
| Impact Factor (IF) / 5 year IF  | 2.081 / 2.416  |                    |

### Publication 3

**Cano-Lamadrid, M.**, Turkiewicz, I.P., Tkacz, K., Sánchez-Rodríguez, L., López-Lluch, D., Wojdyło, A., Sendra, E., and Carbonell-Barrachina, A.A. (2019). A critical overview of labeling information of pomegranate juice-based drinks: Phytochemicals content and health claims. *Journal of Food Science*, 84: 886-894. doi:[10.1111/1750-3841.14497](https://doi.org/10.1111/1750-3841.14497)

|                 |                           |      |           |
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| Published       | Mar 18 2019               | ISSN | 0022-1147 |
| Publisher       | Wiley                     |      |           |
| Research Domain | Food Science & Technology |      |           |
| JCR® Category   | Food Science & Technology |      |           |

|                 |           |             |               |
|-----------------|-----------|-------------|---------------|
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| Impact Factor (IF) / 5 year IF | 2.82/ 2.416 |
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### Publication 4

**Cano-Lamadrid, M.**, Lech, K., Michalska, A., Wasilewska, M., Figiel, A., Wojdyło, A., and Carbonell-Barrachina, A.A. (2017). Influence of osmotic dehydration pre-treatment and combined drying method on physico-chemical and sensory properties of pomegranate arils, cultivar Mollar de Elche. *Food Chemistry*, 232: 306-315. doi:[10.1016/j.foodchem.2017.04.033](https://doi.org/10.1016/j.foodchem.2017.04.033)

|                 |  |      |           |
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| Published       | Oct 1 2017   | ISSN | 0308-8146 |
| Publisher       | Elsevier Science BV, PO Box 211, 1000 AE Amsterdam, Netherlands      |      |           |
| Research Domain | Chemistry, Applied; Food Science & Technology; Nutrition & Dietetics |      |           |
| JCR® Category   | Food Science & Technology  |      |           |

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|-----------------|-----------|-------------|--------------|
| <b>Quartile</b> | <b>Q1</b> | <b>Rank</b> | <b>7/135</b> |
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|--------------------------------|---------------|
| Impact Factor (IF) / 5 year IF | 5.399 (5.488) |
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**Publication 5**

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**Cano-Lamadrid, M.**, Vázquez-Araújo, L., Sánchez-Rodríguez, L., Wojdyło, A., and Carbonell-Barrachina, Á.A. (2018). Consumers' opinion on dried pomegranate arils to determine the best processing conditions. *Journal of Food Science*, 83: 3085-3091. doi:[10.1111/1750-3841.14390](https://doi.org/10.1111/1750-3841.14390)

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**Quartile Q2 Rank 56/135**

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**Publication 6**

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**Cano-Lamadrid, M.**, Nowicka, P., Hernández, F., Carbonell-Barrachina, A.A., and Wojdyło, A. (2018), Phytochemical composition of smoothies combining pomegranate juice (*Punica granatum* L) and Mediterranean minor crop purées (*Ficus carica*, *Cydonia oblonga*, and *Ziziphus jujube*). *Journal of the Science of Food and Agriculture*. 98: 5731-5741. doi:[10.1002/jsfa.9120](https://doi.org/10.1002/jsfa.9120)

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**Cano-Lamadrid, M.**, Hernández, F., Nowicka, P., Carbonell-Barrachina, A.A., and Wojdyło A. (2018). Formulation and storage effects on pomegranate smoothie phenolic composition, antioxidant capacity and color. *LWT- Food Science and Technology*. 96,322-328, doi: [10.1016/j.lwt.2018.05.047](https://doi.org/10.1016/j.lwt.2018.05.047)

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| <b>Quartile</b> | <b>Q1</b> | <b>Rank</b> | <b>23/135</b> |
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# **1. DOCTORAL THESIS STRUCTURE**

This Doctoral Thesis has been structured following Miguel Hernández University internal regulation for the presentation of Doctoral Thesis as Compendium of Publications, this is:

- **Abstract/Resumen**, where the principal objectives and more relevant results obtained have been presented.
- **Introduction**. The state of the art of pomegranate tree production focused on deficit irrigation strategies has been reviewed and studied as well as the current importance of pomegranate-based products. Additionally, the background of the dried pomegranate arils and smoothies based on pomegranate has been researched.
- **Objectives**. The aim and specific objectives have been established in this section.
- **Material and Methods**. The plant material, experimental conditions, treatments and the analytical methods used to carry out the experiments included in this PhD dissertation have been explained and referenced.
- **Publications**. The 7 publications used for this Thesis are presented in 4 blocks:
  - i. **Block I**
    - i.i. Publication 1: The effect of deficit irrigation and crop load on the yield and fruit quality in Wonderful and Mollar de Elche pomegranates has been studied. *Journal of the Science of Food and Agriculture*, 98: 3098-3108. doi:[10.1002/jsfa.8810](https://doi.org/10.1002/jsfa.8810)
  - ii. **Block II**
    - ii.i. Publication 2: The phytochemical composition of commercial pomegranate-based products has been studied. *Journal of Food Science*, 82: 1820-1826. doi:[10.1111/1750-3841.13788](https://doi.org/10.1111/1750-3841.13788)
    - ii.ii. Publication 3: A critical overview of labeling information of pomegranate juice-based drinks has been studied (*Journal of Food*

*Science*, 84: 886-894. doi:[10.1111/1750-3841.14497](https://doi.org/10.1111/1750-3841.14497)

iii. **Block III**

iii.i. Publication 4: The effect of osmotic dehydration pre-treatment and combined drying method on physico-chemical and sensory properties of pomegranate arils, cultivar Mollar de Elche, has been studied. *Food Chemistry*, 232: 306-315. doi: [10.1016/j.foodchem.2017.04.033](https://doi.org/10.1016/j.foodchem.2017.04.033)

iii.ii. Publication 5: Consumers' opinion on dried pomegranate arils to determine the best processing conditions has been studied. *Journal of Food Science*, 83: 3085-3091. doi:[10.1111/1750-3841.14390](https://doi.org/10.1111/1750-3841.14390)

iv. **Block IV**

iv.i. Publication 6: Phytochemical composition of smoothies combining pomegranate juice and *Ficus carica*, *Cydonia oblonga*, and *Ziziphus jujube* purées has been studied. *Journal of the Science of Food and Agriculture*. 98: 5731-5741. doi: [10.1002/jsfa.9120](https://doi.org/10.1002/jsfa.9120)

iv.i. Publication 7: The effect of formulation and storage conditions on pomegranate smoothie phenolic composition, antioxidant capacity and color has been studied. *LWT- Food Science and Technology*. 96: 322-328. doi: [10.1016/j.lwt.2018.05.047](https://doi.org/10.1016/j.lwt.2018.05.047)

- **Results and Discussion.** In this section, the main results obtained in this Doctoral Thesis are explained, discussed and summarized. Four well-differentiated blocks can be observed: i) Block I: Farming practices of hydrosustainable fruits; ii) Block II: Overview of commercial pomegranate-based products (*Study I* and *Study II*); iii) Block III: Dehydrated pomegranate arils (*Study I* and *Study II*); and, IV) Block IV: Smoothies based on pomegranate and minor Mediterranean fruits (*Study I* and *Study II*).

- **Conclusions.** The main conclusions obtained after the achievements obtained and the future research lines opened by this work have been listed.
- **References.** Literature used for writing and justifying this Doctoral Thesis have been referenced following APA 6<sup>th</sup> edition.

## **2. ABBREVIATIONS**

|                      |   |
|----------------------|---|
| A                    | Apple ( <i>Malus domestica</i> Borkh.) 50%                        |
| AP                   | Apple ( <i>Malus domestica</i> Borkh.) 75%                        |
| CD                   | Color Density   |
| Ch                   | Chokeberry ( <i>Aronia melanocarpa</i> L.)                        |
| CPD                  | Convective pre-drying   |
| CPD-VMFD             | Convective pre-drying, followed by vacuum microwave finish drying |
| $\Sigma$             | Electrical conductivity   |
| EA                   | Ellagic Acid  |
| ETo                  | Daily crop reference evapotranspiration                           |
| F                    | Fig   |
| FD                   | Freeze drying   |
| GAE                  | Gallic Acid Equivalent  |
| HPLC                 | High-Performance Liquid Chromatography method                     |
| J                    | Jujube  |
| JAR                  | Just About Right  |
| $\Psi_{\text{stem}}$ | Midday stem water potential                                       |
| $g_{\text{leaf}}$    | Midday leaf conductance   |
| MC                   | Moisture Content  |
| ME                   | Pomegranate, cultivar <i>Mollar de Elche</i>                      |
| Wond                 | Pomegranate, cultivar <i>Wonderful</i>                            |
| OD                   | Osmotic dehydration   |
| OD(A+Ch)             | Osmotic dehydration with Ch and A (50:50)                         |
| OD(AP+Ch)            | Osmotic dehydration with Ch and A (25:75)                         |
| OD(POM+A)            | Osmotic dehydration with POM Wond and A (50:50)                   |
| OD(POM+Ch)           | Osmotic dehydration with POM Wond and Ch (50:50)                  |
| OD(POM)              | Osmotic dehydration with POM Wond 100%                            |

|             |   |
|-------------|---|
| PC          | Polymeric Color                             |
| PCA         | Principal Component Analysis                |
| PLS         | Partial Least-Square regression             |
| Pn          | Punicalagin                                 |
| Q           | Quinices                                    |
| SG          | Solid Gain                                  |
| TA          | Titratable Acidity                          |
| TPC         | Total Polyphenols Content                   |
| UPLC-PDA-FL | Ultra-Performance Liquid Chromatography-PDA |
| VMFD        | Vacuum microwave finish drying              |
| WL          | Water Loss                                  |

### **3. ABSTRACT / RESUMEN**



## Abstract

The aim of this dissertation was to think of, develop and characterize novel pomegranate-based products; the new products must have their own identity/personality including functional and organoleptic properties, and must be based on hydroSOSustainable pomegranate fruits and must be fully adapted to the needs and requirements of European consumers. To reach this general objective, the whole thesis was structured into 4 research-blocks:

- *Block I*, Farming of hydroSOSustainable pomegranate fruits.
- *Block II*, Overview of commercial pomegranate products.
- *Block III*, Preparation of dehydrated pomegranate arils.
- *Block IV*, Preparation of pomegranate-based smoothies.

Block I. Firstly, it is well-known that food production depends more on water availability than on any other environmental resource. Consequently, it is necessary to accept the coexistence of Spanish agriculture simultaneously with an important water shortage. Therefore, a policy change of the sustainable management of this essential resource is needed, and deficit irrigation (DI) strategies (e.g. sustained deficit irrigation, SDI) can be an interesting option. Although pomegranate trees are drought tolerant, it is expected that DI pomegranate farming will create water stress on the trees which will respond increasing their content of bioactive compounds and a more tasteful sensory profile; at the same time, that water-use efficiency will be enhanced. The specific objective of this block was to gather deeper knowledge on the simultaneous effects of SDI (during fruit growth and ripening) and crop load (thinning) on yield and fruit quality of *Mollar de Elche* and *Wonderful* fruits by evaluating fruit (i) physical- and (ii) chemical-characteristics, and (iii) descriptive sensory attributes. Thinning was effective in increasing the size and weight of fruits, but unfortunately neither punicalagin nor total polyphenolic content were positively affected by irrigation and thinning. *Wonderful* fruits under water stress and thinning were characterized by high sugar content (glucose and fructose), together with high fruit size and weight, while *Mollar de Elche* fruits were

characterized by high contents of alcohols and monoterpenoids and key sensory attributes (color, fruity, and fresh pomegranate).

Block II. Secondly, scientific research and food industry are working on developing novel products based on fruits and vegetables due to the preference of consumers on sustainable, healthy and ready-to-eat products. Consumer awareness on the impact of food on health and well-being has been increasing in recent years. Many pomegranate-based products take advantage from the widespread healthy image of this fruit, whereas their real content of bioactive phytochemicals is “unfortunately” low. Commercial pomegranate products, including capsules and supplements, and, juices and nectars were assayed by comparing the labelling information with the real phytochemical contents (punicalagin, ellagic acid and total polyphenolic content) and their associated antioxidant capacity (DPPH\*, ABTS\*+ and FRAP). The experimental results showed a high variability in the content of bioactive compounds and the need to urge food companies to optimize processes and storage conditions, and a labelling “standardization” which includes only data from real analysis and not “theoretically” expected contents.

Block III. Because the daily intake of fruits and vegetables is lower than the recommended dietary intake (RDI), a good option to increase the intake of fruit and vegetables is dried fruit and fruit-based smoothie consumption. Therefore, the improvement of the pomegranate products catalogue will be one of the important objectives of this work: (i) dehydrated arils and (ii) pomegranate smoothies. The aim of the third block was to evaluate the drying kinetics, quality parameters (anthocyanin content, antioxidant capacity, color, rehydration ratio), sensory properties and consumer acceptance of the dried arils (cultivar *Mollar de Elche, ME*) prepared using osmotic dehydration (OD) with selected fruit juice concentrates (apple, *Wonderful* pomegranate and/or chokeberry) and comparing results with those obtained after using a combined drying technique [convective pre-drying

(CPD) and vacuum-microwave finish drying (VMFD)] for dehydration of pomegranate arils cultivar *ME*. The use of OD provided dried arils with characteristic sweetness of *ME* arils, but improved color and aromatic complexity characteristic of *Wonderful* fruits. All the samples prepared using the proposed new drying techniques were more liked than the commercial sample assayed ("liking drivers": esters, fruity and sweet attributes); the best results were obtained for a sample dried using pre-osmotic dehydration in *Wonderful* concentrate pomegranate juice followed by a combined drying technique.

Block IV. Regarding to the last block, the objective was to evaluate the effect of adding fig, jujube, or quince purée to pomegranate juice (cultivars *Wonderful* and *Mollar de Elche*) in preparing smoothies at two ratios purée:juice (40:60 and 60:40) on the composition of chemical and nutritional compounds, antioxidant activity, and polyphenols. Besides, the effect of storage was also evaluated. The factor affecting the most the smoothies composition was the type of fruit purée. Fig smoothies were rich in anthocyanins, jujube ones in flavonols and vitamin C, and finally the quince smoothies in hydroxycinnamic acids. The best formulation and storage conditions were addition of quince purée at a ratio of 40:60 purée:juice to *Wonderful* pomegranate juice stored at 4 °C; this preparation protocol and storage conditions led to the highest contents of anthocyanins, flavanols, flavan-3-ols, polymeric procyanidins and phenolic acids after 6 months of storage.

As an overall conclusion, it has been demonstrated that novel pomegranate-based products prepared using environmental friendly fruits (hydroSOSustainable) should take the attention of both food industry and consumers, because they are high-quality products based on the following facts: (i) they are environmental friendly because are prepared using fruits grown saving irrigation water, (ii) they are social-acceptable food products because use local raw materials, and (iii) they are tasty and rich in bioactive compounds as the result of the stress created by the hydroSOSustainable farming.

## Resumen

El objetivo de esta tesis doctoral fue valorar, desarrollar y caracterizar nuevos productos a base de granada; los nuevos productos deben tener su propia identidad, incluyendo determinadas propiedades funcionales y organolépticas, deben estar basados en granadas hidroSOSostenibles y deben estar totalmente adaptados a las necesidades y requisitos de los consumidores europeos. Para alcanzar este objetivo general, la tesis doctoral se estructuró en 4 bloques de investigación:

- *Bloque I*, Cultivo de granada hidroSOSostenibles.
- *Bloque II*, Visión general de los productos comerciales de granada.
- *Bloque III*, Preparación de arilos de granada deshidratados.
- *Bloque IV*, Preparación de *smoothies* a base de granada.

Bloque I. En primer lugar, es bien conocido que la producción de alimentos depende mayoritariamente de la disponibilidad de agua que de cualquier otro recurso. En consecuencia, es necesario aceptar la coexistencia de la agricultura española con una importante escasez de agua. Por tanto, se necesita un cambio de política en el manejo sostenible de este recurso esencial, y las estrategias de riego deficitario (DI) (por ejemplo, riego deficitario sostenido, SDI) pueden ser una opción interesante. Aunque los granados son tolerantes a la sequía, se espera que el cultivo de granada bajo condiciones DI genere estrés hídrico en los árboles, lo que implicará un aumento en su contenido de compuestos bioactivos y en su perfil sensorial; al mismo tiempo, se mejorará la eficiencia del uso del agua. El objetivo específico de este bloque fue reunir un conocimiento más profundo sobre los efectos simultáneos de SDI (durante el crecimiento y maduración del fruto) y de la carga de la cosecha (aclareo) sobre el rendimiento y la calidad de la granada *Mollar de Elche* y la granada *Wonderful* mediante la evaluación de la fruta: (i) características físicas, (ii) características químicas, y (iii) atributos sensoriales descriptivos. El aclareo fue efectivo para aumentar el tamaño y el peso de las

frutas, pero desafortunadamente ni el contenido en punicalagina ni el contenido polifenólico total se vieron afectados positivamente por el riego y el aclareo. Las granadas *Wonderful* bajo estrés hídrico y aclareo se caracterizaron por un alto contenido de azúcar (glucosa y fructosa), junto con un mayor tamaño y peso, mientras que las granadas *Mollar de Elche* se caracterizaron por un alto contenido de alcoholes y monoterpenoides, y algunos de los atributos sensoriales clave (color, afrutado, y granada fresca).

Bloque II. En segundo lugar, la investigación científica y la industria alimentaria están trabajando en el desarrollo de nuevos productos basados en frutas y verduras debido a la preferencia de los consumidores por productos sostenibles, saludables y listos para el consumo. La conciencia del consumidor sobre el impacto de los alimentos en la salud y el bienestar ha aumentado en los últimos años. Muchos productos a base de granada aprovechan la imagen saludable generalizada de esta fruta, mientras que su contenido real de fitoquímicos bioactivos es "desafortunadamente" bajo. Los productos comerciales disponibles de granada, incluyendo cápsulas y suplementos, y zumos y néctares fueron analizados comparando la información del etiquetado con el contenido real de compuestos bioactivos (punicalagina, ácido elágico y fenoles totales) y con su capacidad antioxidante asociada (DPPH\*, ABTS\*\* y FRAP). Los resultados experimentales mostraron una alta variabilidad en el contenido de compuestos bioactivos y la necesidad de instar a las empresas alimentarias a optimizar los procesos y las condiciones de almacenamiento, y una "estandarización" de etiquetado y no aportar una imagen "teóricamente" esperada.

Bloque III. Debido a que la ingesta diaria de frutas y verduras es inferior a la ingesta dietética recomendada (IDR), una buena opción para aumentar la ingesta de frutas y verduras es el consumo de frutas deshidratadas y *smoothies*. Por lo tanto, la mejora del catálogo de productos de granada en el mercado será uno de los objetivos importantes de esta tesis doctoral: (i) arilos deshidratados y (ii)

*smoothies* de granada. El objetivo del tercer bloque fue evaluar la cinética de secado, los parámetros de calidad (contenido de antocianinas, capacidad antioxidante, color, rehidratación), propiedades sensoriales y aceptación del consumidor de los arilos secos (cultivar *Mollar de Elche*, ME) preparados usando deshidratación osmótica (OD) con concentrados de zumo de frutas seleccionados (manzana, granada *Wonderful* y/o aronia) y comparando los resultados con los obtenidos después de utilizar la técnica de secado combinada [pre-secado por convección (CPD) seguido de un secado a microondas al vacío (VMFD)] de arilos de granada ME. El uso de OD proporcionó arilos deshidratados con la dulzura característica de los arilos ME, mejorando el color y la complejidad aromática característica de la granada *Wonderful*. Todas las muestras preparadas con las nuevas técnicas de secado propuestas fueron más apreciadas por el consumidor que la muestra comercial analizada ("drivers": ésteres, atributos afrutados y dulces); los mejores resultados se obtuvieron para la muestra deshidratada utilizando el zumo de granada *Wonderful* concentrado en la OD seguido de la técnica propuesta de secado combinado.

Bloque IV. Con respecto al último bloque, el objetivo fue evaluar el efecto de la adición de puré de higos, jínjol o membrillo al zumo de granada (cultivares *Wonderful* y *Mollar de Elche*) en la preparación de *smoothies* en dos proporciones diferentes de puré:zumo (40:60 y 60:40) sobre la composición nutricional, la actividad antioxidante y el contenido en polifenoles. Además, también se evaluó el efecto de los compuestos bioactivos durante el almacenamiento. El factor que más afectó a la composición de los *smoothies* fue el tipo de puré de frutas utilizado. Los *smoothies* de higo fueron ricos en antocianinas, los *smoothies* de jínjol en flavonoles y vitamina C, y finalmente los *smoothies* de membrillo en ácidos hidroxicinámicos. Se observó que la adición de puré de membrillo al zumo de granada *Wonderful* en una proporción de 40:60 condujo a un mayor contenido de antocianinas, flavanoles, flavan-3-oles, procianidinas poliméricas y ácidos fenólicos tras 6 meses de almacenamiento a 4°C.

Como conclusión general, se ha demostrado que tanto la industria alimentaria como los consumidores deben presentar especial atención a los nuevos productos a base de granada hidroSOSostenible ya que son productos de alta calidad basados en los siguientes hechos: (i) son respetuosas con el medio ambiente porque se preparan utilizando frutos cultivados bajo estrategias de ahorro de agua de riego, (ii) son productos aceptados socialmente porque usan materias primas locales y (iii) son sabrosos y ricos en compuestos bioactivos como resultado del estrés creado por las estrategias de riego utilizadas (cultivo hidroSOSostenible).

## **4. INTRODUCTION**



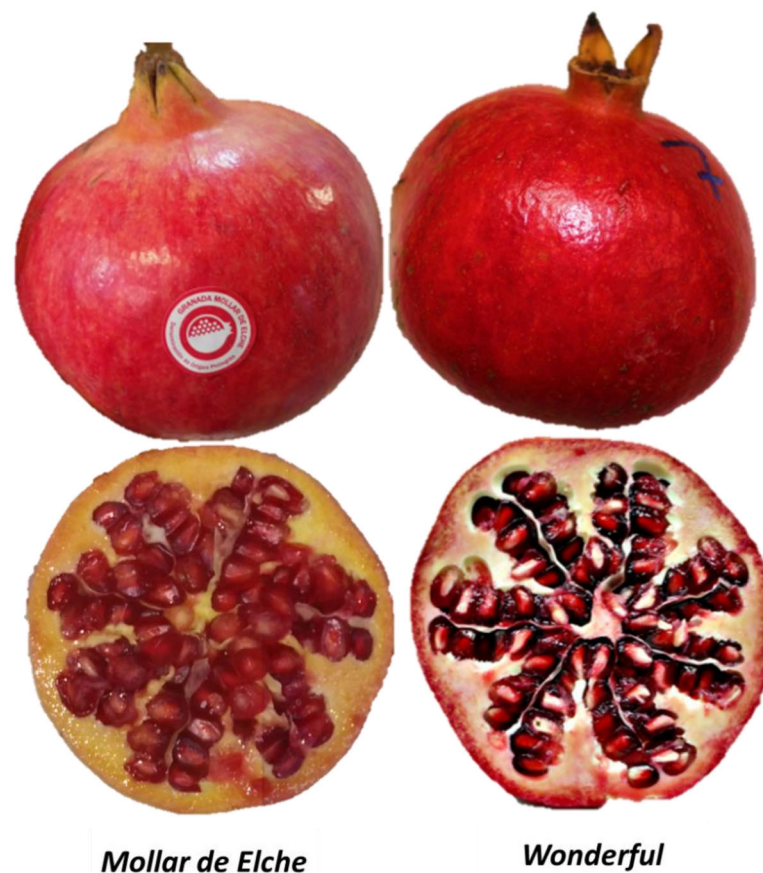
## INTRODUCTION

Scarcity and droughts are progressively frequent and universal phenomena that influence around 100 million people and one third of the European area (European Commission, 2019). Besides, **climate change** is expected to aggravate this situation, especially in Mediterranean countries (Hervás-Gómez *et al.*, 2019). A fact that supports this **water scarcity** is that from 1960 until 2010, there has been a reduction of 24 % in renewable water resources per capita in Europe, particularly in southern Europe (European Environment Agency, 2019). Therefore, the aridity of the climate and low available water resources are the main characteristics of Mediterranean agrosystems, specially Spain (Hervás-Gómez *et al.*, 2019). Among others activities and policies, agriculture/farming could mitigate global climate change by reducing its consumption of water resources improving irrigation management, the most limiting production factor in Mediterranean fruit orchards. It is well-known that food production depends more on water availability than on any other adverse environmental restriction (Grant *et al.*, 2012). Consequently, it is necessary to accept the coexistence of Spanish agriculture with water shortage. Consequently, a policy change of the sustainable management of this essential resource (water) is needed, and **deficit irrigation** (DI) can be an interesting option (Ondrasek, 2019). The objective of DI, defined in terms of water supply in correlation with the maximum crop evapotranspiration (ET<sub>c</sub>), is to increase the reduction of ET<sub>c</sub> minimizing the effect on the production and quality (Ondrasek, 2019). Among DI strategies, regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI) are the main ones, and lead to fruits with a specific identity (higher sensory complexity and richer biochemical compounds) which are branded as "hydroSOSustainable" products (Cano-Lamadrid *et al.*, 2015; Cano-Lamadrid *et al.*, 2016; Galindo *et al.*, 2014; Noguera-Artiaga *et al.*, 2016). RDI reduces irrigation water during a specific phenological fruit growth phase and SDI reduces irrigation during the whole fruit growth season (Ferreles *et al.*, 2012).

Due to the drought tolerance characteristics of **pomegranate tree**, which has a high adaptability to water deficit (Rodríguez *et al.*, 2012), it is expected that pomegranate farming under DI will improve its content of fundamental chemical compounds (bioactive molecules) and its sensory profile at the same time that water-use efficiency will also be enhanced. Several studies about the response of pomegranate fruit to DI concluded that water deficit effects depend on the phenological stage of fruit growth at which DI is applied, and the water deficit level achieved. As to fruit growth phase, it has been recently concluded that pomegranate fruit ripening is a critical period from the yield point of view. But, a short irrigation restriction period (~6 days) at the end of ripening will lead to an enhancement of the appearance attributes and to an improved accumulation of bioactive compounds (Galindo *et al.*, 2017). Regarding the water stress level, a no linear correlation between applied water stress and accumulation of secondary metabolites was lately reported. Moderate water stress may induce a lower plant growth and a larger amount of secondary carbon metabolites, whilst severe water stress may stimulate the synthesis of primary metabolites consequently reducing that of secondary metabolites (Galindo *et al.*, 2017; Mellisho *et al.*, 2012; Mena *et al.*, 2013). But, it is worth highlighting that studies about the effect on quality are still not solid. In this sense, SDI applied throughout the pomegranate season reduces total yield, the number of fruits and their size (Galindo *et al.*, 2014). However, other authors (Peña-Estévez *et al.*, 2015) concluded that pomegranates from SDI trees presented desirable sensory attributes, favorable bioactive compounds, and more resistance to chilling injury, and longer shelf-life than fully irrigated fruits.

**Pomegranate** is an important fruit crop of both tropical and subtropical areas. It is grown in central and South Asia, Middle East, Caucasus region, the Mediterranean region, North and Tropical Africa. Spain is the largest Mediterranean European pomegranate producer, yielding 75,673 t in 2018, with the Valencian Community being the highest producer (78.6 %) followed by Murcia (13.5 %)

(MAPA, 2019); the main cultivars grown in Spain are *Wonderful* (*Wond*) and *Mollar de Elche* (*ME*) (**Figure 1**). The *ME* is the most popular and traditional cultivar in Spain due to its protection through a Protected Designation of Origin (PDO) "*Granada Mollar de Elche*" (R(UE)2016/83, 2016). This cultivar is characterized by its predominant sweetness, the softness of its wooden portion, and its pale pink color, which is the main disadvantage because it easily deteriorates, leading to grey color, after heat treatments. Besides, its sensory characteristics are flat with very weak fruity notes (Vázquez-Araújo *et al.*, 2014).



**Figure 1.** The two most grown pomegranate cultivars in Spain.

Not only pomegranate is consumed in fresh but also as pomegranate-based products, including jams, juices, jellies, wines and dried snacks, among others (Bchir *et al.*, 2012; Berenguer *et al.*, 2016; Calín-Sánchez *et al.*, 2013; Dimitreli *et al.*, 2019; Mantzourani *et al.*, 2020; Trigueros *et al.*, 2014; Ventura *et al.*, 2013).

During the last decades, pomegranate consumption has drastically increased mainly because the expected beneficial effects on different functions of the organism, thus, improving health and human well-being. It has been demonstrated that pomegranate presents a low caloric index and its composition highlights the presence of polyphenols, such as tannins (especially, punicalagin and punicalin) and flavonoids (especially anthocyanins) and also contains vitamin C (Banerjee *et al.*, 2012). Because of the antioxidant capacity is positively correlated with total contents of polyphenols and flavonoids, pomegranate and its based products can be recognized as a functional food (Bchir *et al.*, 2012). Recently, the scientific community has concluded that punicalagins are not directly the compounds which present this antioxidant effect on the human body because they have too high molecular weights. They are not absorbed in the stomach or the small intestine, and reach the colon almost unaltered (García-Villalba *et al.*, 2017); however, they (punicalagins) act as precursor of urolithins, which are the compounds that will finally benefit human health (González-Sarrías *et al.*, 2017). In addition, among several diseases that can be prevented by pomegranate consumption, the most important ones are: i) diabetes; ii) cancer; and, iii) metabolic and cardiovascular diseases (Tomás-Barberán *et al.*, 2014). Apart from these functional compounds, pomegranate juice presents techno-functional properties and is useful as natural colorant. Therefore, scientific research and food industry are currently working on novel products based on pomegranate. It is worth highlighting that the European Food Safety Agency (EFSA) still does not allow health claims for pomegranate product antioxidants, because more clinical trials are needed to establish the connection.

The **current fruit intake** is lower than the recommended one (400 g *per* day) and nowadays consumers demand healthy and ready-to-eat products, novel fruits- and vegetables-based products, such as pomegranate-based products, could be a good alternative to enhance the improvement of health and the prevention of chronic diseases (e.g. diabetes and obesity). Epidemiological studies concluded that

high consumption of fruits is correlated with a risk reduction of chronic diseases (EUFIC, 2012). The difficulty encountered in separating edible arils from pomegranate fruit has several limitations for its direct consumption unlike other fruits such as oranges, banana, and grapes. Plus, separated pomegranate arils are highly perishable and have short shelf-life. Hence, pomegranate-based products have become very popular recently due to their convenience, high value, health benefits and unique sensory characteristics. The improvement of the pomegranate products catalogue will be one of the important objectives of this PhD dissertation and from now on, I will focus on 2 new or improved pomegranate-based products, **(i) dehydrated arils and (ii) pomegranate-based drinks.**

In our opinion, dried pomegranate-based products could be a good option for the pomegranate industry. Consumers are interested in this type of products due to their easy-consumption and the stability of their healthy and sensory properties, including aroma and taste attributes. **Dehydrated arils** are a good source of vitamins, minerals and bioactive compounds (Alaei *et al.*, 2015). The production of dehydrated arils is focused on their consumption as snack. One example of dehydrated pomegranate arils, which are found around world, is *anardana*, which are dried arils of sour-type pomegranates with hard seed and are very popular in southern Asian countries. Sour-pomegranate varieties are suitable for preparation of *anardana* but they will not easily accept by European consumers, especially Spaniards, due to their sourness and hardness of the wooden parts. Dehydrated arils made from sweet or sour-sweet pomegranate cultivars with soft seed, such as *ME*, could be produced generating novel and consumer-adapted dehydrated pomegranate product (Cano-Lamadrid *et al.*, 2018; Szychowski *et al.*, 2015). Another critical point is that commercial available dried pomegranate arils present undesirable appearance (non-enzymatic browning) and off-flavors (e.g. furfural, hydroxyl methyl furfural) due to unsuitable and/or uncontrolled drying process. Currently, various drying techniques are used to prepare dehydrated arils around the world such as sun-drying, convective drying, greenhouse drying, osmotic

drying, freeze drying, vacuum drying, and vacuum freeze drying (Calín-Sánchez *et al.*, 2013; Cano-Lamadrid *et al.*, 2018; Horuz *et al.*, 2015). Various pre-treatments are also used before drying of arils to improve the quality parameters of the final product; these pre-treatments include steam blanching, chemical dips in sodium benzoate and citric acid addition. High quality dried arils could be prepared using appropriate drying methods (combined drying treatment) and well-known pre-treatments (osmotic dehydration, transferring bioactive and organoleptic-active compounds) improving the functionality and appearance of the final dried products (Bchir *et al.*, 2012; Calín-Sánchez *et al.*, 2013; Khoualdia *et al.*, 2020).

On the other hand, among fruit-based drinks, **smoothies** have increased their popularity during last decade. They are a semi-liquid drink, consisting of a blend of juices with the purée from other complementary fruits, plus optionally ice, yoghurt and/or milk (Fernandez *et al.*, 2019; Park *et al.*, 2016; Tiwari, 2018). It seems a great opportunity to promote “ready to drink” fruit drinks with low glycemic index instead of fruit nectars with lots of sugar-added and to enhance the daily fruits intake especially in young consumers (Castillejo *et al.*, 2016). The effect of storage time of non-pasteurized smoothies on color, polyphenolic composition and shelf-life has been previously studied (Castillejo *et al.*, 2016; Nowicka *et al.*, 2016). Therefore, it can be worth to use “moderate” thermal pasteurization or high hydrostatic pressure technologies (Timmermans *et al.*, 2016; Walkling-Ribeiro *et al.*, 2010) to extend their shelf life and to maintain acceptable sensory attributes (color, fruity) and functional properties (polyphenols), guaranteeing food safety (Fernandez *et al.*, 2019; Guazi *et al.*, 2019; Tiwari, 2018). Pineapple, strawberry, mango, passion fruit, beetroot and banana are the main ingredients of the smoothies which are currently available on the market, while there is a lack of smoothies based on traditional, local and minor Mediterranean fruits (Castillejo *et al.*, 2016; Fernandez *et al.*, 2019; Guazi *et al.*, 2019; Nowicka *et al.*, 2016a; Park *et al.*, 2016; Timmermans *et al.*, 2016; Tiwari, 2018; Walkling-Ribeiro *et al.*, 2010). Additionally, it is important to highlight that southeastern Spain (Alicante and

Murcia provinces) is one of the regions with more tradition on Mediterranean agriculture based on cultivation of local and well-adapted cultivars and traditional fruit orchards, including *Mollar de Elche* pomegranate farms. In this same regions some **minor crops** (figs, jujube, quinces, among others), with huge potential due to their pro-healthy properties, are also produced (Almansa *et al.*, 2016; Szychowski *et al.*, 2014; Wojdyło, Nowicka *et al.*, 2016), and can be included in the formulation of new fruit products, such as smoothies.

It is important to be aware of the concept of agrofood quality, its meaning and structure level. In the basis of the quality, safety food products must be available (level 1), followed by tasty and social-acceptable food products (sensory analysis) (level 2). Moreover, the enhancement of their quality is based on using local raw materials (PDO, traditional cultivars) and on their linkage to products maintaining and supporting the local lifestyle and diet (level 3). To achieve the full quality, sustainability during the primary production must be considered, including, among other factors, friendly agricultural water management (level 4). The concept of agrofood quality is well-linked with the 5S criteria (from the Spanish: sano "healthy", seguro "safe", sabroso "tasty", sostenible "sustainable" and socialmente *aceptado* "Socially accepted") recently developed for processed food products (Pérez-Álvarez *et al.*, 2020).

Taking into account all the above mentioned information, novel pomegranate-based products such as osmotic-dehydrated arils and pomegranate-based smoothies blended with minor Mediterranean crops produced by environmental friendly practices (hydroSOS) should encourage both food industry and consumers to be focused on these high-quality healthy, local and sustainable products.

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number: FPU15/02158) from the Spanish Ministry of Education. From this project, this Doctoral thesis was focused on the innovation, the development and the characterization of new products based on hydroSOSustainable pomegranate. Keeping this aim in mind, agronomic practices (e.g. deficit irrigation), food unit operations (e.g. dehydration), and morphological-, nutritional-, functional- and sensory-properties have been studied to be able to generate high quality products based on pomegranate (dehydrated pomegranate arils and pomegranate smoothies).

Additionally, other research articles linked with this PhD dissertation topic are shown:

1. Nuncio-Jáuregui, N.P., **Cano-Lamadrid, M.**, Hernández, F., **Carbonell-Barrachina, Á.A.**, Calín A. (2015). Comparison of fresh and commercial pomegranate juices from *Mollar de Elche* cultivar grown under conventional or organic farming practices. *Beverages*, 1 – 2: 34-44.
2. **Cano-Lamadrid M.**, Marhuenda-Egea, F.C., Hernández, F., Rosas-Burgos, E.C., Burgos-Hernández, A., **Carbonell Barrachina, Á.A.** (2016). Biological activity of conventional and organic pomegranate juices: antioxidant and antimutagenic potential. *Plant Foods for Human Nutrition*, 71(4):375-380.
3. Galindo, A., Rodríguez, P., Collado-González, J., Calín, Á., Cruz, Z.N., Corell, M., Moriana, A., **Cano-Lamadrid, M.**, Medina, S., Gil-Izquierdo, A., **Carbonell-Barrachina, Á.A.**, Hernández, F., Torrecillas, A. (2016). El granado. Un cultivo resistente a la sequía y fuente natural de salud. *Fruticultura*, 46.
4. **Cano-Lamadrid, M.**, Calín-Sánchez, Á., Rosas-Burgos, E.C., Krzysztof, K., Figiel, A., **Wojdylo, A.**, Wasilewska, M., and **Carbonell-Barrachina Á.A.** (2018). Quality of pomegranate pomace as affected by drying method. *Journal of Food Science and Technology*, 55 (3): 1074–1082.
5. **Cano-Lamadrid, M.**, Trigeros, L., **Wojdylo, A.**, **Carbonell-Barrachina, Á.A.**, Sendra-Nadal, E. (2018). Anthocyanins decay in pomegranate enriched fermented milks as a function of bacterial strain and processing conditions. *LWT-*



*Food Science and Technology*, 80: 193-199.

6. **Cano-Lamadrid, M.**, Navarro-Martinez, P., Lopez-Miranda, S., **Wojdyło, A.**, **Carbonell-Barrachina, Á.A.**, Perez-Lopez, A.J. (2018). Chapter 8. Pomegranate: Cultivation, Antioxidant Properties, Health Benefits and Effects of Industrial Processing. Pomegranate: Cultivation, Composition, Antioxidant Properties, and Health Benefits. Nova Science Publishers, ISBN: 978-1-53614-119-1
7. **Cano-Lamadrid, M.**, Calín-Sánchez, A., Clemente-Villalba, J., Hernández, F., **Carbonell-Barrachina, Á.A.**, Sendra, E., **Wojdyło, A.** (2020). Quality parameters and consumer acceptance of jelly candies based on pomegranate juice "Mollar de Elche", *Foods*, 9(4): 516.
8. **Cano-Lamadrid, M.**, Viuda-Martos, M., García-Garvía, J., Clemente-Villalba, J., **Carbonell-Barrachina, Á. A.**, & Sendra, E. (2020). Polyphenolic Profile and Antimicrobial Potential of Peel Extracts Obtained from Organic Pomegranate (*Punica granatum* L.) Variety *Mollar De Elche*, *Acta Horticulturae et Regiotecturae*, 23(1), 1-4.

## **5. AIM AND OBJECTIVES**

The **overall aim** of this PhD dissertation was to innovate, to develop and to characterize novel products based on pomegranate with an identity that includes the functional and organoleptic properties of hydrosustainable products which are adapted to the needs and requirements of European consumers (**Figure 1**).

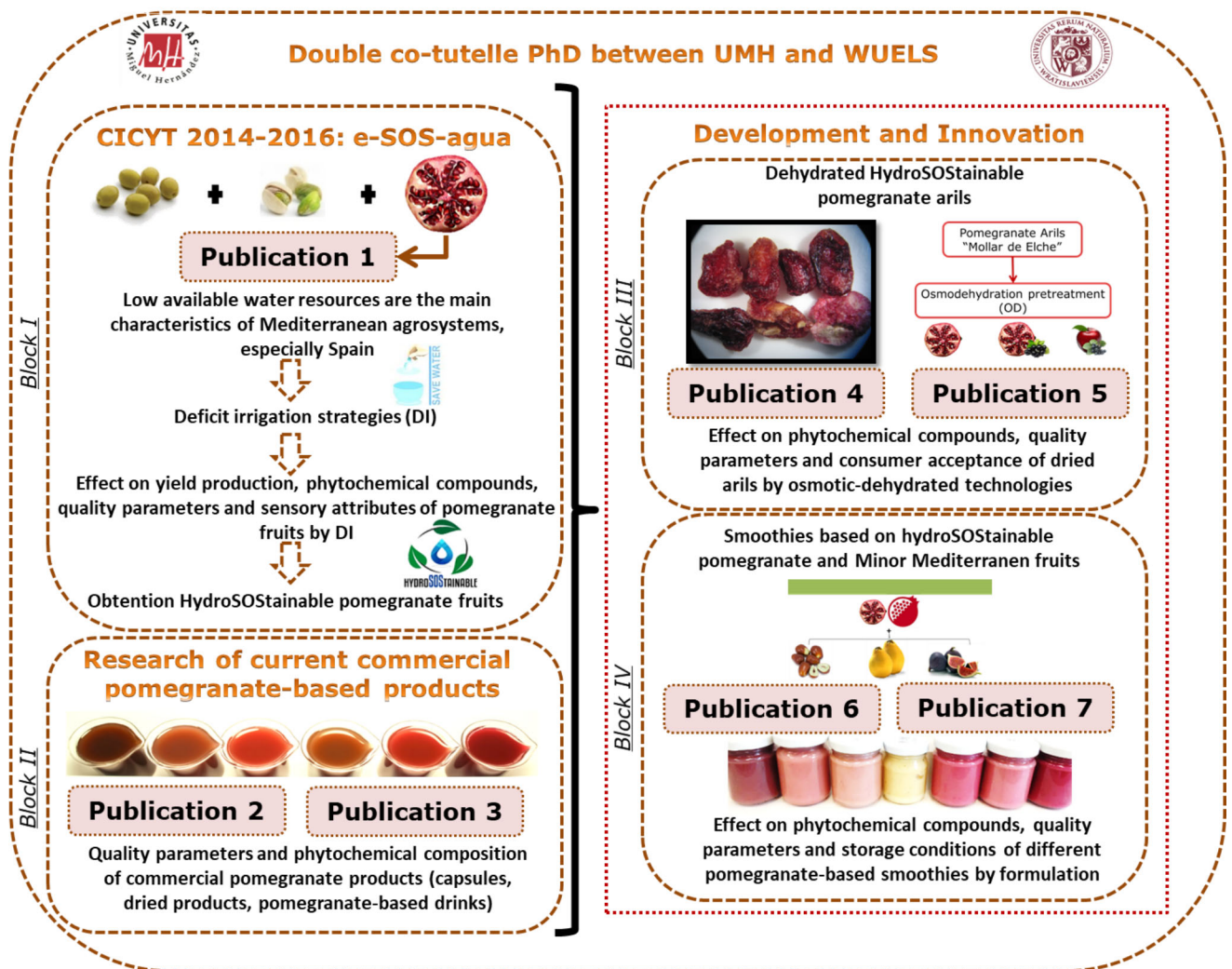
To reach this aim, more **specific objectives** were established and are listed here:

- Objective 1: To look the simultaneous effects of deficit irrigation (during fruit growth and ripening) and crop load on yield and fruit quality in the two most popular pomegranate cultivars in Spain, *Mollar de Elche* and *Wonderful* (Block I: Publication 1).
- Objective 2: To research and to compare the phytochemical content and antioxidant capacity of different commercial pomegranate based products available on the international market (Block II: Publication 2 & 3).
- Objective 3: To develop novel products based on hydrosustainable pomegranate fruits: dehydrated arils and smoothies (Block III and Block IV: Publication 4, 5, 6 & 7).
- Objective 4: To evaluate the physico-chemical of the hydrosustainable pomegranate dried arils prepared using first osmotic dehydration (OD), with selected fruit juice concentrates, and later a combined drying technique for dehydration of pomegranate arils cultivar *Mollar de Elche* (Block III: Publication 4).
- Objective 5: To determine consumer insights about dried hydrosustainable pomegranate arils using different technologies and to link the consumer data with descriptive and volatile composition data (Block III: Publication 5).
- Objective 6: To determine the effect on color, minerals, pectin, vitamin C, sugars, organic acids, phenolic compounds and antioxidant capacity of pomegranate based smoothies by the addition of (i) different minor crops

(fig, jujube and quinces) purée, (ii) pomegranate cultivar and (iii) ratio purée:juice (*Block IV*: Publication 6).

- Objective 7: To study how storage conditions (6 months at 4 or 20 °C) affected color, polyphenolic profile, and antioxidant activity of hydrosustainable pomegranate smoothies (*Block IV*: Publication 7).

**Figure 1.** Diagram showing the structure and aim/objectives of this doctoral thesis.



## **6. MATERIALS AND METHODS**

*In this section the main characteristics of the experimental conditions, irrigation treatments, processing treatments, analytical methodologies, statistical design and analyses used in this thesis are included. For more detailed aspects the papers that constitute this thesis can be consulted.*

## **6.1. Block I: Farming of hydrosustainable pomegranate fruits** (publication 1)

### ***Plant material, experimental conditions and irrigation treatments***

The experiment was carried out in 2016 in a pomegranate orchard, *Punica granatum* L. cultivar *Wonderful* (*Wond*) and *Mollar de Elche* (*ME*), at the CEBAS-CSIC experimental station in Santomera (Murcia, Spain) (38°11', 1°03'). The trees were own-rooted 6-year old *Wond* and *ME* with only one trunk and spaced at 3 m × 5 m. The design of the field experiment was completely randomized with 4 replications; each replication consisted of 3 adjacent tree rows, each with 7 trees.

There were two *irrigation treatments*, T0 and T1, to study the effects on the plant water status:

- i) T0: control pomegranate trees, from day of the year 109 (DOY 109) to DOY 279, both cultivars were irrigated daily above the estimated crop evapotranspiration (120 % E<sub>Tc</sub>) to obtain non-limiting soil water conditions.
- ii) T1: deficit irrigated plants of both cultivars were irrigated at 120 % E<sub>Tc</sub> from the beginning of the experiment to fruit setting (DOY 168), and at 60 % E<sub>Tc</sub> from then to harvest (fruit growth and ripening).

Crop irrigation requirements were determined using the daily crop reference evapotranspiration (E<sub>To</sub>), calculated using the Penman–Monteith equation (FAO method) (Allen *et al.*, 1992), and a crop factor based on the time of the year (Bhantana *et al.*, 2010), and the percentage of ground area shaded by the tree canopy (Ferreles *et al.*, 1990). The total irrigation water amounts, measured with

in-line water meters, applied to each treatment were 516 mm (T0) and 317 mm (T1) in *Wond* trees, and 537 mm (T0) and 327 mm (T1) in *ME* trees.

There were two *thinning* treatments to study the effects on the crop load:

- i) A0: pomegranate fruits were not thinned.
- ii) A1 trees were manually thinned, leaving 20-25 cm between fruits and avoiding the presence of "double fruits" (two fruits fused together).

### ***Plant water status***

The water relations of the leaves were measured at midday (12 h solar time). Fully expanded leaves from the south-facing side and middle height of four trees per treatment were selected for measurements. Midday leaf conductance ( $g_{\text{leaf}}$ ) was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of 2 leaves *per* tree. Midday stem water potential ( $\Psi_{\text{stem}}$ ) was measured in 2 leaves similar to those used for  $g_{\text{leaf}}$ , which were enclosed in a small black plastic bag covered with aluminum foil for at least 2 h before measurements were made using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA)(Galindo *et al.*, 2014).

### **6.2. Block II: Overview of commercial pomegranate products** (publications 2 & 3)

Firstly, samples (n=22) from 2 commercial pomegranates (*Punica granatum* L.) based products groups were analyzed: (i) capsules and supplements (n=11), and (ii) juices and concentrated juices (n=11). Products were purchased from pharmacy chains, through internet (on-line shops), or by mail order (**Table 1**).

Secondly, samples (n=30) from 2 groups were analyzed: (i) 100 % pomegranate juices (n=12), and (ii) <100 % pomegranate drinks (n=18). Products were purchased at local stores and supermarkets from Alicante province (**Table 2**).

**Table 1.** Main labelling information in the studied pomegranate samples.

| <b>Sample</b>  | <b>Main ingredients</b>   |
|--|---|
| <b>Group 1: CAPSULES AND SUPPLEMENTS</b>                   |   |
| 1A   | Pomegranate extract 833 mg. Bulking agent silicone dioxide 10 mg, magnesium stearate 25 mg  |
| 1B   | Pomegranate extract 250 mg. Rice flour, gelatin. Contains < 2 % of: silica, vegetable cellulose, vegetable magnesium stearate   |
| 1C   | Pomegranate extract 200 mg. Capsule: gelatin, rice flour, bulking agent: magnesium stearate and silica  |
| 1D   | Pomegranate extract (77 %). Gelatin, ascorbate, bulking agent (corn starch), anti-adherent agent (magnesium stearate)   |
| 1E   | Powdered pomegranate extract from peel  |
| 1F   | 70 % concentrate powder of pomegranate juice (pomegranate juice, cornstarch), 10 % pomegranate extract, granulated sugar and palm oil   |
| 1G   | Pomegranate 83 % (of which 84 % pomegranate juice and 16 % pomegranate juice extract) vegetable capsule (hydroxypropylmethylcellulose), bulking agent (maltodextrin), grape extract, anti-adherent agent (silica) |
| 1H   | 350 mg pomegranate dry extract. Bulking agent (microcrystalline cellulose), anti-adherent agent (magnesium stearate and silicone dioxide)   |
| 1I   | 100 % pomegranate kernel flour  |
| 1J   | Pomegranate juice (30 mL) from concentrated   |
| 1K   | Pomegranate powder made from the fleshy seeds of ripe pomegranate   |
| <b>Sample</b>  | <b>Main ingredients</b>   |
| <b>Group 2: POMEGRANATE JUICES AND CONCENTRATED DRINKS</b> |   |
| 2A   | Pomegranate concentrate, water. Preservatives: potassium sorbate E-211 and sodium benzoate E-202  |
| 2B   | Pomegranate juice made from pomegranate concentrate   |
| 2C   | Pomegranate juice squeezed  |
| 2D   | Dehydrated pomegranate juice and maltodextrins  |
| 2E   | Direct pomegranate juice  |
| 2F   | Pomegranate juice based on concentrate (93.7 %, agave syrup (6.1%), preservatives (potassium sorbate and sodium benzoate)   |
| 2G   | Pomegranate, 100 % pure concentrated juice. Preservatives: potassium sorbate and sodium benzoate  |
| 2H   | 100 % pomegranate juice   |
| 2I   | Pomegranate (95 % juice and pulp concentrate partly live fermented), elderberry juice concentrate, potassium lactate, natural flavor (vanilla extract), setting agent: pectin and spices (0.1 %)                  |
| 2J   | Pomegranate juice. 100 % fruit content without sugar or preservatives   |
| 2K   | Concentrated double strength pomegranate fruit juice, ascorbic acid (vitamin C), potassium sorbate and sodium benzoate (preservative)   |



**Table 2.** List of pomegranate juices, codes, and main claims on the labels of pomegranate juices and pomegranate juice based drinks.

| Code   | % Pom | Ingredients  |
|--|-------|--|
| <b>Group 1: 100 % pomegranate juice (pom)</b>  |       |  |
| 1A   | 100   | Squeezed pomegranate juice   |
| 1B   | 100   | Pomegranate juice  |
| 1C   | 100   | 100 % squeezed <i>Mollar de Elche</i> pomegranate juice  |
| 1D   | 100   | Pomegranate juice drink made from concentrated pomegranate juice and enriched with extract from the skin of the pomegranate in powder form, and pomegranate seed extract   |
| 1E   | 100   | Organic pomegranate juice  |
| 1F   | 100   | Squeezed pomegranate juice   |
| 1G   | 100   | Pomegranate juice  |
| 1H   | 100   | 100 % pomegranate <i>Mollar</i> juice  |
| 1I   | 100   | Pomegranate juice  |
| 1J   | 100   | 100 % pomegranate juice  |
| 1K   | 100   | 100 % pomegranate juice  |
| 1L   | 100   | Pomegranate juice  |
| <b>Group 2: Pomegranate juice based drinks</b> |       |  |
| 2A   | 10    | Water, concentrated apple juice (12 %), concentrated pom juice (10 %), sugar, apple purée (5 %), concentrated lemon juice, concentrated elderberry juice, Vit E  |
| 2B   | 50    | Pom juice (50 %) from concentrate, water, sodium cyclamate, sodium saccharin   |
| 2C   | 30    | Water, pom juice squeezed (30.0 %), concentrated lemon (0.2 %), pom aroma (0.2 %)  |
| 2D   | 50    | Water, pom (50 %), panela, black currant, mangostan  |
| 2E   | 15    | Water, pom juice from concentrate (15 %), red grape juice from concentrate (10 %), white grape juice from concentrate (10%), citric acid, E-955, ascorbic acid and aromas  |
| 2F   | 27.5  | White tea, pom juice (27.5 %), concentrated agave juice (7 %), Ch juice (6 %), pear puree (4 %)  |
| 2G   | 30    | Pom concentrate, water, sugar, citric acid: acidulant, ascorbic acid: antioxidant  |
| 2H   | 24    | Water, pom juice (24 %): from concentrate, goji juice (1 %), acidity regulator: citric acid and sweetener: steviol glycosides  |
| 2I   | 70    | Pom juice (70 %) and red grape juice (30 %)  |
| 2J   | 50    | Pom juice (50 %), grape juice (50 %)   |
| 2K   | 20    | Fruit juices partially from concentrates (60 %) (apple and pom 20 %), water, sugar, acidity regulator: citric acid, colorant: anthocyanins, natural aroma  |
| 2L   | 2.5   | Red grape juice (92.5 %), raspberry puree (2.5 %), blackberry puree (2.5 %), pom juice (2.5 %)   |
| 2M   | 22    | Water, multi fruit juice from concentrate (22 % pom, 4 % apple, 3 % chokeberry, 1 % elderberry), sugar, citric acid; Vit C, aroma  |
| 2N   | 8     | Water, pom juice (8 %), raspberry puree, orange fibre, herbal extracts: rice, apple, cherry, radish and hibiscus, citruline, hyaluronic acid, F&V MacroAntioxidants® (pom peel extract), sea collagen (fish), pom punicalagins extract, hydroxytyrosol (natural olive fruit extract), fruit pectin, selenium salt, Vit C and E, sodium benzoate and potassium sorbate, citric acid, sucralose, flavors |
| 2Ñ   | 55    | Juices of red grapes and Pom from concentrate (46 %), water, glucose-fructose syrup, raspberry puree (4%), sugar, acidity regulator: citric acid, vitamin C  |
| 2O   | 25    | Red grape juice (65 %), pom juice (25 %) and red currant juice (10 %)  |
| 2P   | 65    | Pom juice (65 %), apple, grape and quince juice  |
| 2Q   | 30    | Pom (30 %) and apple (30 %) juices, water, natural pom extract of punicalagins, natural extract of rooibos, vitamin C, sweetener: sucralose  |

**6.3. Block III: Preparation of dehydrated pomegranate arils** (publications 4 & 5)

**6.3.1. Study I: Drying processes and osmotic dehydration pre-treatment**

Freeze drying (FD) was carried out in freeze dryer OE-950 (Hungary) at a reduced pressure of 65 Pa for 24 h. The freeze-dried arils, without osmotic treatment, were considered as the control sample. FD conditions for pomegranate arils were previously optimized by Calín-Sánchez *et al.*, (2013).

The process of combined drying (CPD-VMFD) consisted of convective pre-drying (CPD) at temperature 60 °C for 2 h with an air velocity of 0.6 m s<sup>-1</sup> performed in the drier designed and built at the Institute of Agriculture Engineering (Wrocław, Poland), and followed by vacuum microwave finish drying (VMFD) with microwave power reduced from the initial 360 W to 120 W to avoid overheating of the dried material. A similar combined procedure, CPD-VMFD, was also used during chokeberry drying (Calín-Sánchez *et al.*, 2015).

The VMFD process of the CPD dried samples was performed in a SM 200 dryer (Plazmatronika, Wrocław, Poland) connected to a vacuum system consisting of a vacuum pump BL 30 P (Tepro, Koszalin, Poland), a vacuum gauge MP 211 (Elvac, Bobolice, Poland), and a compensation reservoir of 0.15 m<sup>3</sup>. A control sample of *ME* pomegranate arils dried by CPD-VMFD, with no osmotic pre-treatment, was prepared using the above equipment for 240 min at 60 °C.

*ME* arils were osmotically dehydrated at 45 °C for 90 min in different combinations and ratios of hydroSOS *Wond* pomegranate (*Punica granatum* L. cultivar *Wond*, POM), Chokeberry (*Aronia melanocarpa* L., Ch) and Apple (*Pyrus malus* L., A) concentrated juices (40 °Brix):

- i. OD(POM): 100 % POM.
- ii. OD(POM+Ch): 50 % *Wond* and 50 % Ch.
- iii. OD(POM+A): 50 % pomegranate *Wond* and 50 % apple, A.
- iv. OD(A+Ch): 50 % A and 50 % Ch.

- v. OD(AP+Ch): 75 % A and 25 % Ch.

Ch and A concentrated juices were commercial products, whereas POM was prepared by pressing, using a domestic citrus juicer (Braun, model CJ 3053, Barcelona, Spain). The ratio of osmotic solution to pomegranate arils was maintained at 200 mL to 100 g of fresh matter and the mixture was manually agitated every 5 min (Lech *et al.*, 2015).

### 6.3.2. Study II: Consumer drivers

The selection of the samples was based in the previous study conducted by Cano-Lamadrid *et al.*, (2017), which concluded that the technology used in some of the samples enhanced the functionality and therefore the healthy characteristics of the product. The present study was conducted using the 4 following samples:

- i. Commercial sample (unknown variety and drying conditions).
- ii. CPD-VMFD.
- vi. OD(POM).
- vii. OD(POM+Ch).

## 6.4. **Block IV: Preparation of pomegranate-based smoothies** (publications 6 & 7)

### 6.4.1. Study I and Study II

Pomegranate shrubs (*Punica granatum* L., cultivars *ME* and *Wond*) were cultivated in a farm located in Murcia (Spain) under RDI (Cano-Lamadrid *et al.*, 2018). Pomegranate cultivars *ME* and *Wond*, figs (*Ficus carica*, cultivar "Colar"), jujubes (*Ziziphus jujube*, cultivar "Grandes de Albaterra") and quinces (*Cydonia oblonga*, cultivar "Gigante de Vranja") were hand-harvested in between mid-August and mid-October 2016 at a commercial maturity stage, and immediately posted to Poland. The different stages of the smoothie preparation process were (**Table 3**):

- i) Purée preparation: the figs (F), jujubes (J), or quinces (Q) were peeled, ground, and heated at 80 °C in a Thermomix device (Vorwerk, Wuppertal, Germany); 10 mL of rhubarb juice per 1 Kg of fruit were added to prevent enzymatic browning of the fruit. After, the particle size of the mixture was reduced in a blender (Symbio, Zelmer, Rzeszów, Poland) until getting a thin purée. Then, the purées were cooled to room temperature.
- ii) Pomegranate juice preparation: the pomegranate fruits (*ME* and *Wond*) were cut in halves, and arils were manually separated from the husk and ground in a Thermomix to obtain the pomegranate juices.
- iii) Obtaining of semi-products in appropriate proportions: purée and juices samples, immediately after their preparation, were mixed in the proportions 40/60 and 60/40, respectively, obtaining 12 samples. Then, the products were heated to 100 °C and put into glass jars (130 mL) and pasteurized (10 min at 90 °C).

**Table 3.** Formulation of smoothies consisting of pomegranate juice and figs, jujubes or quince purées.

| <b>Code</b> | <b>Formulation</b>        |
|-------------|---------------------------|
| F1Mo        | 40 % F + 60 % <i>ME</i>   |
| F1W         | 40 % F + 60 % <i>Wond</i> |
| F2Mo        | 60 % F + 40 % <i>ME</i>   |
| F2W         | 60 % F + 40 % <i>Wond</i> |
| J1Mo        | 40 % J + 60 % <i>ME</i>   |
| J1W         | 40 % J + 60 % <i>Wond</i> |
| J2Mo        | 60 % J + 40 % <i>ME</i>   |
| J2W         | 60 % J + 40 % <i>Wond</i> |
| Q1Mo        | 40 % Q + 60 % <i>ME</i>   |
| Q1W         | 40 % Q + 60 % <i>Wond</i> |
| Q2Mo        | 60 % Q + 40 % <i>ME</i>   |
| Q2W         | 60 % Q + 40 % <i>Wond</i> |

## **6.5. Measurements**

### ***Morphological, physical, and chemical parameters***

The fruit diameter was measured with an electronic digital slide gauge (Mitutoyo). Ten fruits were carefully cut at the equatorial zone with a sharp knife, and then arils were manually extracted to obtain the fresh pomegranate juice. The color density (CD) and the percentage of polymeric color (PC) were using a previous method proposed by Giusti *et al.*, (1999). Titratable acidity (TA) was determined by titrating sample aliquots using 0.1 N NaOH until pH 8.1 using an automatic pH titration system (Schott Titroline 7500 KF Volumetric KFTitrator; Mainz, Germany) and results were expressed as g of malic acid in 100 g. The total soluble solids, TSS (°Brix) were measured using a refractometer PAL-88S (Atago Co., Tokyo, Japan). Color coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ) were determined by reflectance measurement with a Color Quest XE Hunter Lab colorimeter (Illuminant D65 and 10° observer angle). All analyses were run in triplicate. Moisture content (MC), water loss (WL) and solid gain (SG) were determined according to Nowicka *et al.*, (2015).

### ***Extraction and chromatographic analysis of volatile compounds***

The volatile composition of the samples was determined using headspace solid phase micro-extraction. Approximately 5 g of sample, ultrapure water (10 mL), NaCl (15 % w/v, weight/volume), and 1-octanol (10  $\mu$ L of 1000 mg L<sup>-1</sup>, internal standard) were placed into a 50 mL vial with polypropylene caps and PTFE/silicone septa. The vial was placed in a water bath with controlled temperature (40 °C) and automatic stirring. After 5 min of equilibration time, a 50/30  $\mu$ m DVB/CAR/PDMS fiber was exposed to the sample headspace for 50 min at 40 °C.

The chromatographic set up and conditions were the same as reported by Cano-Lamadrid *et al.*, (2018), and the column used was a Restek Rxi-1301 Sil MS (Restek Corporation, Palo Alto, USA) of 30 m  $\times$  0.25 mm i.d, 0.25  $\mu$ m film thickness. The identification of compounds was conducted by Gas Chromatography

(Shimadzu GC-17A), coupled with a Mass Spectrometer detector (Shimadzu GCMS QP-5050A; Shimadzu Corporation, Kyoto, Japan), and the semi-quantification of compounds was conducted using the same conditions in and GC coupled to a Flame Ionization Detector (GC-FID Shimadzu GC17; Shimadzu Corporation, Kyoto, Japan). The analyses were run in triplicate, and results were expressed as percentage of the total area.

### ***Mineral content***

Approximately 1 g was digested using a START D Medium Microwave Digestion (SK-10) with 8 mL of concentrated HNO<sub>3</sub> and 2 mL H<sub>2</sub>O<sub>2</sub> (30 %). Determination of macro-nutrients micro-nutrients in the previously mineralized samples was performed using a Unicam Solaar 969 atomic absorption-emission spectrometer (Unicam Ltd., Cambridge, UK). All minerals were analyzed using atomic absorption, except K that was measured using atomic emission. Calibration curves were prepared for the quantification of minerals and showed high linearity ( $R^2 \geq 0.999$ ).

### ***Pectin and Vitamin C content***

Pectin content was analyzed according to the Morris method as previously described (Nowicka *et al.*, 2016a). Vitamin C was analyzed using high-performance liquid chromatography method (HPLC) as described by Wojdyło *et al.*, (2013).

### ***Sugar and organic acid content***

Each sample was mixed with distilled water (50 mL) and boiled 30 min, then centrifuged at 20,000×g for 10 min. The supernatant was filtered by Sep-Pak C18 (1 g, Waters, Milford, USA). The HPLC-ELSD (HPLC with evaporative light scattering detection) conditions used in the analysis of the sugars and organic acids were the same reported by Nowicka *et al.*, (2016a). The sugar identification was done by comparison with the standards of fructose, sorbitol, glucose, and sucrose. The organic acids were identified by comparison with standards of oxalic, malic, citric,

tartaric, quinic, shikimic, succinic, and formic acids. The calibration curves were prepared in the range 0.5 to 5 mg mL<sup>-1</sup> and from 0.05 to 0.5 mg mL<sup>-1</sup>, respectively ( $R^2 \geq 0.9998$ ).

### ***Antioxidant capacity and total polyphenols content (TPC)***

A methanol extract was obtained from each product, and analyzed for AOC using the DPPH<sup>•</sup>, ABTS<sup>•+</sup>, and FRAP methods. A known amount of product was mixed with 10 mL of acidified MeOH/H<sub>2</sub>O (80:20 v/v, 1 % HCl), sonicated at 25 °C for 15 min, and left for 24 h at 4 °C. The extract was again sonicated for 15 min, and centrifuged until the separation of the supernatant.

The radical scavenging activity was evaluated using the DPPH<sup>•</sup> radical method, as described by previous research (Brand-Williams *et al.*, 1995), allowing a reaction time of 15 min. The ABTS<sup>•+</sup> and FRAP methods were also used to evaluate the AOC, according to previous methods (Benzie *et al.*, 1999; Re *et al.*, 1999). The ORAC assay was prepared as described by Ou *et al.*, (2001), and a RF-5301 PC spectrofluorometer (Shimadzu, Kyoto, Japan) was used. Calibration curves within the range 0.50–5.00 mmol Trolox kg<sup>-1</sup> were used for the quantification of the AOC in the three methods; these calibration curves showed good linearity ( $R^2 \geq 0.998$ ). Results were expressed in mmol Trolox kg<sup>-1</sup>.

TPC was quantified using Folin–Ciocalteu colorimetric method as previously described by Cano-Lamadrid *et al.*, (2015). The absorbance was measured at 765 nm using an UV-Visible spectrophotometer (Helios Gamma model, UVG 1002E, Mercers Row, Cambridge, UK). Quantification was done with respect to a standard curve of gallic acid. The results were expressed as mg gallic acid equivalents (GAE) g<sup>-1</sup>.

### **Identification and quantification of Punicalagin and Ellagic acid**

Punicalagin ( $\alpha$  and  $\beta$  isomers) (Pn) and ellagic acid (EA) contents were determined in all studied samples. A known amount of sample was diluted with 5 mL of acidified MeOH/water (80:20 v/v, 1 % HCl), and centrifuged. Supernatants were filtered through a 0.45  $\mu\text{m}$  Millipore filter, and injected into a Hewlett-Packard-series 1200 high performance liquid chromatograph, HPLC (Woldbronn, Germany), equipped with a LiChroCART 100 RP-18 reversed-phased column (250  $\times$  4 mm, particle size, 5  $\mu\text{m}$ ; Merck, Darmstadt, Germany), and a pre-column C18 (LiChrospher 100 RP-18, 5  $\mu\text{m}$ ; Merck, Darmstadt, Germany). Eluents were analyzed using a UV-Vis Diode Array detector. The mobile phase consisted of: i) solvent A (1 % acetic acid in Milli-Q water); and ii) solvent B (1 % acetic acid in MeOH). Flow rate was 1 mL  $\text{min}^{-1}$  using a gradient starting with 1 % B for 5 min, and increasing it to 60 % B at 40 min. The compounds were quantified using calibration curves of the standard compounds ( $\alpha$ - and  $\beta$ -Pn, and EA); all analyses were run in triplicate.

### **Polyphenols and procyanidins**

The extraction of polyphenols was conducted using the conditions reported by Wojdyło *et al.*, (2013). The compound identification was done using fast liquid chromatography quadrupole time-of-flight mass spectrometry (LC/MS QTof), and the compound quantification was done using ultra-performance liquid chromatography-PDA (UPLC-PDA-FL). The chromatographic conditions for the identification and quantification of anthocyanin, flavan-3-ols, flavonol, and hydroxycinnamic acids have been previously reported by Wojdyło *et al.*, (2014). The analysis of polymeric procyanidins by phloroglucinol method was performed according to the protocol described previously by Kennedy *et al.*, (2001).



### ***Sensory analysis by trained panel***

Eight trained panelists (30 to 55 years; 4 females and 4 males) with more than 500 h of experience from the Agro-Food Technology Department (UMH) participated in this study. The panel was selected and trained following the ISO standard 8586-1 (1993), and it is specialized in descriptive sensory evaluation of fruits and vegetables, including pomegranate products. For the present study, the panel worked during 2 orientation sessions (90 min each one) discussing about the main appearance, flavor and texture characteristics of dried pomegranate arils. The lexicon used for describing the flavor attributes was developed by Koppel *et al.*, (2010). The lexicon used for describing the texture attributes was reported by Vázquez-Araújo *et al.*, (2014). Both lexicons were adapted for dried arils during the orientation sessions. Samples were served into odor-free, disposable 90 mL covered plastic cups at room temperature, and coded using 3 digit numbers. Unsalted crackers and distilled water were provided to panelists to clean their palates between samples. The sample serving and analysis procedure (using the suitable lexicon and reference products) were conducted according to Vázquez-Araújo *et al.*, (2014). A scale from 0 to 10, with 0.5 increments, was used, where 0 represented no intensity, and 10 represented extremely strong intensity.

### ***Consumer study***

A consumer panel of 100 volunteers recruited from SensoFood Solution, the consumer database at Department of Agrofood Technology (UMH), evaluated the samples. The consumer study was carried out in the testing room of UMH (individual booths, controlled temperature:  $21 \pm 1$  °C; combined natural/artificial light), and each consumer evaluated all four samples in a single session. The presentation order of the samples was randomized to avoid biases. A 9-point hedonic intensity scale was used to determine liking, and 9-point Likert scale was used for the Just About Right (JAR) questions to determine possible improvements of the attributes: color tone, color homogeneity, appearance, fruity flavor,

pomegranate flavor, sweetness, sourness, astringency, and adhesiveness. Unsalted crackers and distilled water were served in-between samples. A demographic questionnaire was presented at the end of sensory test, and a gift was given to encourage participation.

### ***Statistical analysis***

Data was subjected to analysis of variance (ANOVA) and later to Tukey's multiple-range test to compare the means. Differences were considered statistically significant at  $p < 0.05$ . All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD). In addition, the relationship among variables was determined using the Pearson correlation coefficient. Instrumental parameters correlated with sensory descriptors were used to conduct a Principal Component Analysis (PCA regression map) using XLSTAT Premium 2016 (Microsoft Corporation, Redmond, WA, USA). On the other hand, Partial least-square regression (PLS) analysis was carried out to study the relationship of the volatile composition and the descriptive analysis with the overall liking data. Also, penalty analysis was conducted to provide extra information about the possible improvements of some samples.

## **7. PUBLICATIONS**

## **7.1 Publication 1**

### **Publication 1 (Literal transcription):**

**Influence of deficit irrigation and crop load on the yield and fruit quality in *Wonderful* and *Mollar de Elche* pomegranates**

**Cano-Lamadrid, M.**, Galindo, A., Collado-González, J., Rodríguez, P., Cruz, Z.N., Legua, P., Burló, F., Morales, D., Carbonell-Barrachina, Á.A., and Hernández, F.

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# Influence of deficit irrigation and crop load on the yield and fruit quality in *Wonderful* and *Mollar de Elche* pomegranates

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## Abstract

**BACKGROUND:** The working hypothesis of the present study was that, by proper simultaneous control of irrigation (hydroSOSustainable products) and crop load (thinning), it is possible to promote the accumulation of bioactive compounds and improve fruit appearance (size and weight). The effects of (i) irrigation status [T0, 120% ETc (estimated crop evapotranspiration); T1, 60% ETc during fruit growth and ripening] and (ii) crop load (A0, no thinning; A1, thinning) on yield and fruit quality were evaluated in two pomegranate cultivars (*Wonderful*, *Wond* and *Mollar de Elche*, *ME*).

**RESULTS:** Thinning was effective in increasing the size and weight of fruits. Unfortunately, neither punicalagin, nor total polyphenolic content were positively affected by irrigation and thinning. T1A1 *Wond* fruits were characterized by high sugar content (glucose and fructose), together with high fruit size and weight. Furthermore, T1A1 *ME* fruits were characterized by high contents of alcohols and monoterpenoids (providing vegetal and citric flavor notes) and key sensory attributes (color, fruity and fresh pomegranate).

**CONCLUSION:** The final recommendation was to use the treatment T1A1 [simultaneous combination of deficit irrigation during fruit growth and ripening (T1) and thinning (A1)], although the positive results were cultivar-dependent.

**Keywords:** fruit thinning; hydroSOSustainable fruits; *Punica granatum* L.; water deficit

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## INTRODUCTION

Pomegranate (*Punica granatum* L.) is a very interesting crop because its fruits are a source of valuable compounds, such as hydrolysable tannins (punicalagins), anthocyanins (ACNs) and phenolic acids (ellagic acid).<sup>1</sup> These compounds have a major impact on (i) fruit quality and (ii) antioxidant activity, and have been linked to its health promoting properties basically related to the prevention of oxidative stress.<sup>2</sup> Moreover, pomegranate tree exhibits high adaptability to water deficit in arid and semiarid areas because it possesses drought tolerance characteristics.<sup>3</sup> Nonetheless, to reach optimal vegetative growth, yield and fruit size, the crop requires regular irrigation throughout the dry season.

Spain is the largest European pomegranate producer, yielding 56 185 tons in 2015<sup>4</sup>; the main cultivars being farmed are *Mollar de Elche* (*ME*) and *Wonderful* (*Wond*). The major criteria for the commercial quality of pomegranate fruits are fruit size, external color and shape. Fruit size is mainly affected by crop load and plant water status, which must be controlled to obtain large fruits. It is important to consider that the amount of fresh water available for agricultural use worldwide is decreasing; thus, pomegranate farming must adopt the use of deficit irrigation (DI) strategies, leading to an

improved water-use efficiency. Only a few studies have evaluated the response of pomegranate fruit to DI and their conclusions are

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not unanimous, even though they agree that water deficit effects depend on the stage of fruit growth at which DI is applied, as well as the water deficit level achieved. In this sense, sustained deficit irrigation (SDI) applied throughout the pomegranate season reduces total yield per tree, the number of fruits per tree and the size of the fruits.<sup>5</sup> Furthermore, DI can advance the optimal harvest time by approximately 7 – 8 days, which can be of interest for the pomegranate industry in early ripening cultivars together with the fact that they have high contents of bioactive compounds. In a similar way, SDI under moderate water stress showed changes in color and chemical characteristics, related to earlier ripening.<sup>6</sup> However, pomegranate juice obtained from SDI trees, under severe water stress, was of lower quality and less healthy than that from fully irrigated trees.<sup>7</sup> Recently, however, other studies<sup>8</sup> concluded that pomegranates from SDI trees had good sensory quality, a higher content of most of the bioactive compounds, and suffered less chilling injury during cold storage, and also had a longer shelf-life than fully irrigated fruits. Moreover, it was shown that pomegranates from SDI trees, submitted to mild water stress during flowering and fruit set and more severe water stress during the linear phase of fruit growth and ripening, had a redder peel and juice with a higher level of total soluble solids.<sup>9</sup>

On the other hand, previous studies with fruits such as pistachios<sup>10,11</sup> and table olives,<sup>12,13</sup> grown under deficit irrigation strategies were shown to have a proper and specific identity and were termed 'hydroSOSustainable' products; they have a special sensory profile and chemical composition. In this way, it is expected that pomegranate fruits could also be improved on some key chemical parameters and sensory attributes by DI.

Considering all of the previously reported information, the present study aimed to develop knowledge on the simultaneous effects of deficit irrigation (during fruit growth and ripening) and crop load on yield and fruit quality in the two most popular pomegranate cultivars in Spain: *ME* and *Wond*. The quality was studied by evaluating (i) physical characteristics, (ii) chemical characteristics and (iii) descriptive sensory attributes of fruit.

## MATERIALS AND METHODS

### Plant material, experimental conditions and treatments

The experiment was carried out in 2016 in a pomegranate (*Punica granatum* L.) orchard at the CEBAS-CSIC experimental station in Santomera (Murcia, Spain) (38°11', 1°03'). The trees were own-rooted 6-year-old *Wond* and *ME* with only one trunk and spaced at 3 × 5 m. Yearly, trees were lightly pruned to encourage fruit production. Sprouts and suckers were removed as they appeared and dead and damaged wood was removed in late winter. The soil is a paralithic mollic-calciorthid very stony (33%, w/w) and shallow with a clay-loam texture. Micrometeorological data (air temperature, solar radiation, air relative humidity, rainfall and wind speed 2 m above the soil surface) was collected by an automatic weather station located at the experimental farm; the station has been operating for more than 20 years and it is located on a soil with grass cover.

The design of the field experiment was completely randomized with four replications, with each replication consisting of three adjacent tree rows, each with seven trees. Samples for the morphological, physical, chemical and sensory analyses were taken on the inner tree of the central row of each replicate, which were very similar in appearance (leaf area, trunk cross-sectional area, height, ground shaded area, etc.), whereas the other trees served as border trees. Each plot had a separate irrigation system and a meter

to measure the volume of water applied; the plots were separated by the rows of border trees.

Two irrigation treatments (T0 and T1) were used to study the effects on the plant water status. From 19 April 2016 [day of the year (DOY) 109] to 6 October (DOY 279), control pomegranate trees (T0) of both cultivars were irrigated daily above the estimated crop evapotranspiration (120% ETC) to obtain non-limiting soil water conditions. Deficit irrigated plants of both cultivars (T1) were irrigated at 120% ETC from the beginning of the experiment to fruit setting (DOY 168) and at 60% ETC from then to harvest (fruit growth and ripening). Crop irrigation requirements were determined using the daily crop reference evapotranspiration (ET<sub>c</sub>), as calculated using the Penman–Monteith equation (FAO method),<sup>14</sup> and a crop factor based on the time of the year<sup>15</sup> and also the percentage of ground area shaded by the tree canopy.<sup>16</sup> Irrigation was carried out during the night using a drip irrigation system, with one lateral pipe per tree row and four emitters (spaced 75 cm and each delivering 4 L h<sup>-1</sup>) per plant, and adjusting the irrigation hours. The total irrigation water amounts, measured with in-line water meters, applied to each treatment were 516 mm (T0) and 317 mm (T1) in *Wond* trees and 537 mm (T0) and 327 mm (T1) in *ME* trees.

There were two thinning treatments (A0 and A1) used to study the effects on the crop load. Pomegranate A0 trees were not thinned, and A1 trees were manually thinned, leaving 20 – 25 cm between fruits and avoiding the presence of 'double fruits' (two fruits fused together). Pomegranate fruits from each treatment and cultivar ( $n = 20$  fruits per tree) were manually harvested on DOY 280, when commercial maturity was reached (*Wond* 15 °Brix and *ME* 12 °Brix), with exact values being: (i) *Wond*: 16.8 °Brix, 0.781 g 100 mL<sup>-1</sup>, maturity index (MI) [TSS (°Brix)/TA (g 100 mL<sup>-1</sup>)] 21.5 ± 6.3 and (ii) *ME*: °Brix, 0.140 g 100 mL<sup>-1</sup>, MI 90.8 ± 11.4. Fruits were immediately transported under ventilated conditions to the laboratory and stored under controlled conditions (5 °C and 90% relative humidity) for less than 1 week, until the analysis.

The marketable yield was calculated by weighting all fruits from the three central trees of the central row (three rows per field plot) of each one of the four replications of this experiment (3 trees × 4 replications, giving a total of 12 trees per treatment), and after removing fruits not reaching commercial size, those affected by pest attack and having physiopathies. Then, 20 fruits per tree were used for the morphological, physical, chemical and sensory analyses.

### Plant water status

The water relationships of the leaves were measured at midday (12 h solar time). Fully expanded leaves from the south-facing side and middle height of four trees per treatment were selected for measurements. Midday leaf conductance ( $g_{leaf}$ ) was measured with a porometer (Delta T AP4; Delta-T Devices, Cambridge, UK) on the abaxial surface of two leaves per tree. Midday stem water potential ( $\Psi_{stem}$ ) was measured in two leaves similar to those used for  $g_{leaf}$ , which were enclosed in a small black plastic bag covered with aluminum foil for at least 2 h before the measurements were made using a pressure chamber (PMS 600-EXP; PMS Instruments Company, Albany, OR, USA).<sup>5</sup>

### Morphological, physical and chemical parameters

The moisture ( $M$ ) percentage of arils was determined in accordance with Alcaraz-Mármol *et al.*<sup>17</sup> Fruit diameter was measured

with an electronic digital slide gauge (Mitutoyo, Kawasaki, Japan). Ten fruits were carefully cut at the equatorial zone with a sharp knife, and then arils were manually extracted to obtain the fresh pomegranate juice. The color density (CD) and the percentage of polymeric color (PC) were using a previous method proposed by Giusti and Wrolstad.<sup>18</sup> All analyses were run in four replications.

From the 20 fruits taken, three batches of six fruits were randomly prepared and all arils from each batch were manually extracted and mixed to prepare the juices; thus, three juices were prepared for each of the four field replications per treatment. Then, a total of 12 measurements per treatment were made for all the analyses described below, although the mean values represent four replications per treatment.

### Total polyphenol content (TPC) and punicalagin content (Pn)

Methanol extract was prepared as follows: pomegranate juices (1 mL) were mixed with 10 mL of MeOH/water (80:20, v/v) + 1% HCl, sonicated at 20 °C for 15 min, and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged at 15 000 × *g* for 10 min. TPC was quantified using Folin–Ciocalteu reagent.<sup>19</sup> Absorption was measured using a UV–Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). The extraction and quantification of the Pn content (sum of  $\alpha$  and  $\beta$  isomers) in the pomegranate juices were analyzed using the method proposed by Cano-Lamadrid *et al.*<sup>20</sup>

### Sugar and organic acid content

The extraction and quantification of sugar and organic acids in pomegranate juices were conducted as described for a recent study.<sup>17</sup> Standards of sugars (glucose and fructose) and organic acids (citric and ascorbic) were obtained from Sigma (St Louis, MO, USA) and calibration curves were prepared and showed good linearity ( $r^2 \geq 0.999$ ).

### Extraction and chromatographic analysis of volatile compounds

Headspace solid phase micro-extraction comprised the isolation technique used to study the volatile composition of the pomegranate juice. Pomegranate juice (5 mL), ultrapure water (10 mL), 1-octanol (10  $\mu$ L of 1000 mg L<sup>-1</sup>, internal standard) and NaCl (15% w/v, weight/volume) were placed into a 50-mL vial with polypropylene caps and polytetrafluoroethylene/silicone septa. The vial was placed in a water bath at controlled temperature (40 °C) and automatic stirring. After allowing time for equilibration, a 50/30  $\mu$ m divinylbenzene/carboxen/polydimethylsiloxane fiber was exposed to the sample headspace for 50 min at 40 °C.

The chromatographic set up and conditions were identical to those reported recently,<sup>21</sup> with the only exception that the column used was a Restek Rxi-1301 Sil MS (Restek Corporation, Palo Alto, CA, USA) with an internal diameter of 30 m × 0.25 mm and a film thickness of 0.25  $\mu$ m.

The identification and semi-quantification of volatiles was conducted using gas chromatography–mass spectroscopy and gas chromatography–flame ionization detection, respectively. The volatile composition analysis was run in four replications for each treatment and the results are expressed as a percentage of the total area represented by each one of the volatile compounds.

### Sensory analysis by trained panel

Eight trained panelists (aged 30 – 55 years; four females and four males) with more than 500 h of training in sensory testing from

the department of Agro-Food Technology (UMH) participated in the study. The sample serving and analysis procedures (using the suitable lexicon and reference products) were conducted.<sup>22</sup> A scale from 0 to 10, with increments of 0.5, was used, where 0 represented no intensity and 10 represented extremely strong intensity. Sensory analysis was run in four replications per treatment (four sessions).

### Statistical design and analysis

Data were analyzed using StatGraphics Plus, version 5.0 (Manugistics, Inc., Rockville, MD, USA). A two-way analysis of variance (irrigation treatment and crop load as factors) was performed and means values were compared by Tukey's multiple range test.  $\Psi_{\text{stem}}$  and  $g_{\text{leaf}}$  values for each replicate were averaged before the mean  $\pm$  SE of each treatment were calculated. Percentage values were arcsin-transformed before statistical analysis. Instrumental parameters correlated with sensory descriptors were used for establishing a principal component analysis (PCA regression map) using XLSTAT Premium 2016 (Microsoft Corp., Redmond, WA, USA); only those parameters showing significant differences in any of the two factors under study were included in the PCA.

## RESULTS AND DISCUSSION

### Climate and plant water status

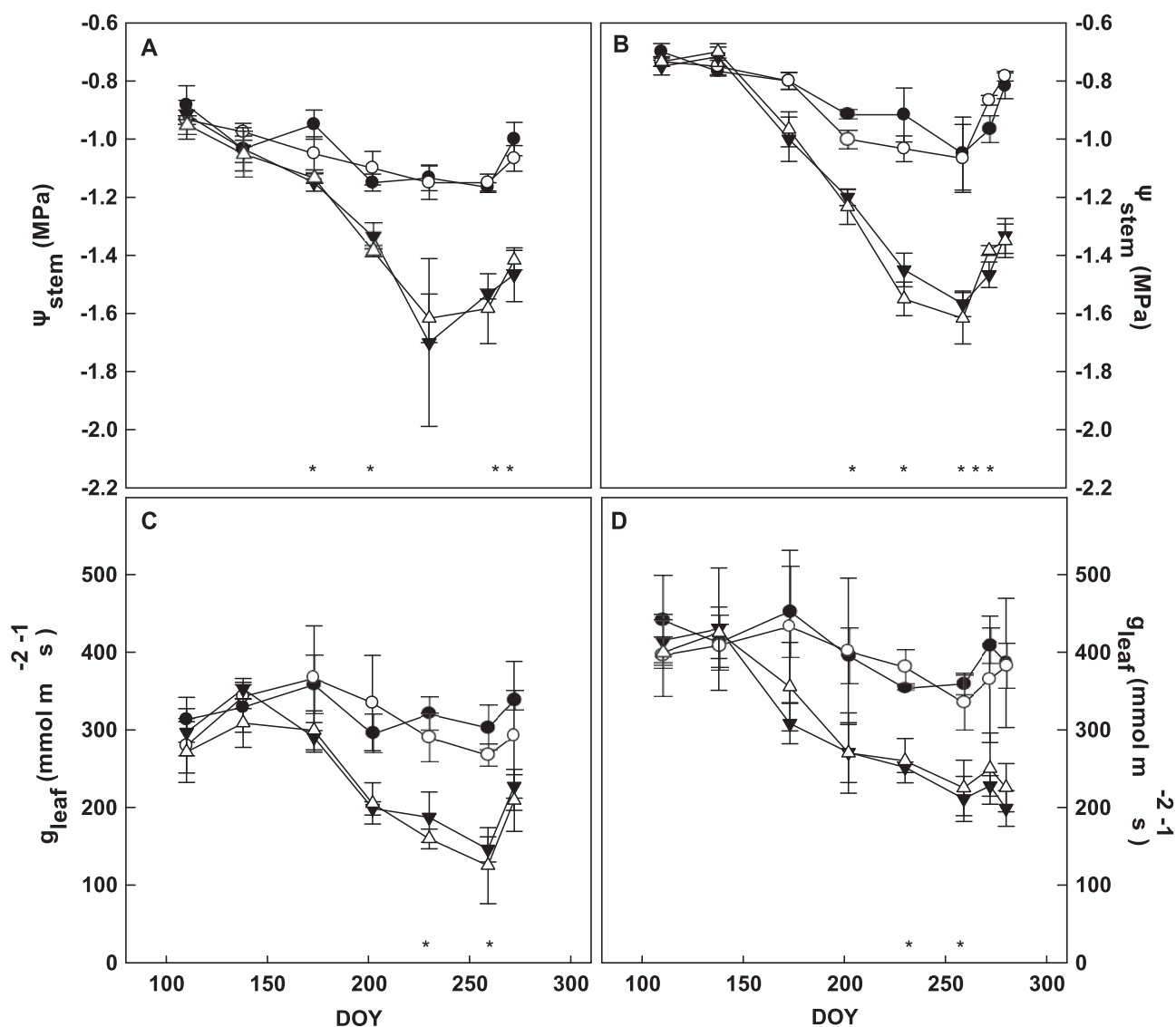
During the experiment, the average daily maximum and minimum air temperatures were 29.4 and 17.8 °C, respectively, and the average mean relative humidity was 60.9%. The total rainfall was extremely low, and reached a total added value of 15 mm, after 4 days of rain, and the total ETo reached 773 mm (DOY 109–279).

$\Psi_{\text{stem}}$  and  $g_{\text{leaf}}$  values for T0 plants in both cultivars were high and almost constant during the experimental period (Fig. 1), implying that the irrigation applied to these treatments in both cultivars was sufficient to avoid any water deficit during the measurement period. The differences in  $\Psi_{\text{stem}}$  and  $g_{\text{leaf}}$  values between T0 and T1 plants were characterized by the gradual decrease of their values in T1 plants from the beginning of the experiment (DOY 109), reaching minimum values near the end of the measurement period, DOY 230 – 279 (Fig. 1), clearly indicated a significant water deficit situation in T1 plants.

Regarding crop load, it has been indicated that high crop load may increase transpiration rates,<sup>23</sup> stomatal conductance,<sup>24</sup> leaf photosynthesis<sup>25</sup> and tree water use.<sup>26</sup> However, studies also reported a reduction in water uptake as a result of a high crop load.<sup>27</sup> Finally, further studies concluded that the effect of crop load on tree water status is not obvious<sup>28</sup> or is apparent only under severe deficit irrigation conditions.<sup>28</sup> In this sense, the results from the current experiment indicate that  $\Psi_{\text{stem}}$  and  $g_{\text{leaf}}$  values in A0 plants were very similar to those of A1 plants not only in full irrigated plants (T0), but also in deficit irrigated plants (T1). Thus, it can be concluded that pomegranate plant water status was not influenced by crop load under these particular experimental conditions.

### Marketable yield and fruit morphology

It is important to note that the marketable yield only includes fruits with a commercial size and with no pest-attack or physiopathies. Both cultivars showed similar response of the marketable yield (production) and fruit morphology to deficit irrigation and thinning (Table 1). Fruit thinning decreased the marketable yield in both cultivars, although the fruits remaining on the tree showed



**Figure 1.** Midday stem water potential ( $\Psi_{\text{stem}}$ ) and leaf conductance ( $g_{\text{leaf}}$ ) values in control (T0, circles) and deficit irrigated (T1, triangles) *Wond* (A, C) and *ME* (B, D) pomegranate trees, which were hand-thinned (A1, open symbols) or non-thinned (A0, closed symbols), during the experimental period (DOY: day of the year). Each value is the mean of four measurements. Asterisks indicate significant differences among treatments according to Tukey's test ( $P < 0.05$ ).

higher weight and size. Thus, it is expected that a higher price can be obtained for these final commercial fruits. However, DI reduced significantly the marketable yield but not the weight and size.

As expected, marketable yield of pomegranates decreased significantly by the plant water deficit effect (Fig. 1), as has been shown previously.<sup>5,6</sup> However, water deficit did not affect fruit weight and size. In this respect, and taking into consideration the results shown previously,<sup>5</sup> probably the maximum water deficit achieved in the current experiment was lower than that necessary to produce fruit turgor loss and, consequently, to decrease fruit growth.

#### Color density, polymeric color and phytochemical compounds

The importance of the copigmentation in pomegranate juice is a result of the importance of the color with respect to determining consumer acceptance of pomegranates and pomegranate-based products (Table 1). Copigmentation is the reaction among ACNs

(responsible of color to pomegranate juices) with copigments (e.g. phenolic acids), producing a hyperchromic effect in the absorption spectrum.

CD, which has a high correlation with catechin-phlorogucinol and monomeric ACNs,<sup>29</sup> was affected by irrigation and thinning in the two pomegranate cultivars. The value of CD in *Wond* fruits (2.69–5.89) was higher than that in *ME* fruits (2.18–4.08) because of the higher content of monomeric ACNs, especially cyanidin-3-glucoside.<sup>30</sup> Color density was reduced (increasing polymerization) when water deficit was applied (T1) in *Wond*; however, the trend was the opposite in *ME* fruits. Crop load affected CD only in *Wond* fruits, by reducing polymerization in remaining fruits; no effect was observed in the *ME* cultivar.

In general, the percentage of polymeric color, PC (high values indicate a high degree of ACN polymerization, leading to a less intense red color) of fresh pomegranate juices should be less than 10%.<sup>18</sup> In the present study, the values were higher (16.4 – 85.9%) as a result of different factors, including the use



**Table 1.** Production (yield), morphology, total polyphenolic content (TPC, mg GAE kg<sup>-1</sup> FW), and, punicalagin (Pn, mg mL<sup>-1</sup> FW) of *Wonderful* and *Mollar de Elche* pomegranates as affected by deficit irrigation and thinning treatments

| Treatment                   | Production (kg) | Fruit weight (g) | Diameter (mm) | Color density, CD | Polymeric color, PC (%) | TPC (mmol L <sup>-1</sup> Trolox FW) | Pn (mg mL <sup>-1</sup> FW) |
|-----------------------------|-----------------|------------------|---------------|-------------------|-------------------------|--------------------------------------|-----------------------------|
| <i>WONDERFUL</i>            |                 |                  |               |                   |                         |                                      |                             |
| ANOVA test                  |                 |                  |               |                   |                         |                                      |                             |
| Irrigation                  | *               | NS               | NS            | *                 | **                      | NS                                   | NS                          |
| Thinning                    | *               | **               | ***           | *                 | NS                      | **                                   | NS                          |
| Irrigation x Thinning       | *               | **               | NS            | **                | ***                     | **                                   | NS                          |
| Tukey's multiple range test |                 |                  |               |                   |                         |                                      |                             |
| Irrigation                  |                 |                  |               |                   |                         |                                      |                             |
| T0                          | 49.7 a          | 387              | 91.4          | 4.45 a            | 22.4 b                  | 704                                  | 3.14                        |
| T1                          | 29.3 b          | 372              | 90.1          | 3.11 b            | 34.8 a                  | 714                                  | 2.96                        |
| Thinning                    |                 |                  |               |                   |                         |                                      |                             |
| A0                          | 42.3 a          | 360 b            | 88.5 b        | 3.10 b            | 31.4                    | 728 a                                | 2.98                        |
| A1                          | 36.7 b          | 400 a            | 92.9 a        | 4.46 a            | 25.9                    | 689 b                                | 3.11                        |
| Irrigation × Thinning       |                 |                  |               |                   |                         |                                      |                             |
| T0A0                        | 53.9 a          | 387 ab           | 90.3          | 3.51 ab           | 25.4 c                  | 754 a                                | 3.15                        |
| T0A1                        | 45.5 b          | 387 ab           | 92.4          | 5.89 a            | 16.4 d                  | 654 c                                | 3.03                        |
| T1A0                        | 30.8 c          | 333 b            | 86.7          | 2.69 b            | 37.6 a                  | 704 b                                | 2.99                        |
| T1A1                        | 27.9 c          | 412 a            | 93.5          | 3.53 ab           | 32.3 b                  | 724 ab                               | 2.80                        |
| <i>MOLLAR DE ELCHE</i>      |                 |                  |               |                   |                         |                                      |                             |
| ANOVA Test                  |                 |                  |               |                   |                         |                                      |                             |
| Irrigation                  | *               | NS               | NS            | **                | **                      | NS                                   | NS                          |
| Thinning                    | *               | *                | *             | NS                | NS                      | ***                                  | NS                          |
| Irrigation × Thinning       | *               | *                | NS            | ***               | ***                     | ***                                  | NS                          |
| Tukey's multiple range test |                 |                  |               |                   |                         |                                      |                             |
| Irrigation                  |                 |                  |               |                   |                         |                                      |                             |
| T0                          | 39.0 a          | 424              | 93.8          | 2.21 b            | 50.2 b                  | 676                                  | 3.04                        |
| T1                          | 29.6 b          | 398              | 92.2          | 3.47 a            | 74.1 a                  | 661                                  | 3.23                        |
| Thinning                    |                 |                  |               |                   |                         |                                      |                             |
| A0                          | 37.1 a          | 392 b            | 91.7 b        | 3.17              | 67.9                    | 692 a                                | 2.98                        |
| A1                          | 31.5 b          | 431 a            | 94.3 a        | 2.52              | 56.4                    | 644 b                                | 3.29                        |
| Irrigation × Thinning       |                 |                  |               |                   |                         |                                      |                             |
| T0A0                        | 39.9 a          | 405 ab           | 92.6          | 2.26 b            | 50.0 c                  | 724 a                                | 3.04                        |
| T0A1                        | 38.1 a          | 443 a            | 95.0          | 2.18 b            | 50.4 c                  | 628 c                                | 2.77                        |
| T1A0                        | 34.4 a          | 378 b            | 90.8          | 4.08 a            | 85.9 a                  | 662 b                                | 2.92                        |
| T1A1                        | 24.9 b          | 418 ab           | 93.6          | 2.86 ab           | 62.4 b                  | 659 b                                | 3.80                        |

NS, not significant at  $P < 0.05$ ; significant at \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , respectively.

Values followed by the same lowercase letter, within the same column and factor, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test.

Values for punicalagin (Pn) content are the sum of  $\alpha$  and  $\beta$  punicalagin isomers.

FW, fresh weight.

of different cultivars, and probably also because pomegranate juices were stored at  $-18^{\circ}\text{C}$  during 2 weeks and then defrosted at  $4^{\circ}\text{C}$ . Previous studies indicated that a prolonged storage at  $4^{\circ}\text{C}$ , or even at  $-18^{\circ}\text{C}$ , produced the polymerization of ACNs with other compounds, mainly condensed tannins.<sup>29,31</sup> The values of PC of *Wond* (16.4 – 37.6%) were significantly lower than those of *ME* (50.0 – 85.9%), being attributed to the difference in phenolic compounds and the stability between cultivars (ACNs from *Wond* juices were more stable than those from *ME*).<sup>17</sup> Water stress increased the value of PC in *Wond* and *ME* cultivars, allowing polymerization and thus deterioration of the red color.

Regarding TPC, the experimental results demonstrated that thinning had no positive effect in the remaining fruits, and even led to slight but significant reductions (6% and 8% in *Wond* and

*ME*, respectively) (Table 1). Moreover, juices from *Wond* cultivar presented higher values of TPC than *ME* because of differences in the polyphenol profile.<sup>32</sup> On the other hand, water deficit did not affect either TPC or Pn content, in contrast to that reported previously in pomegranate fruits under moderate and severe SDI<sup>30</sup>; probably, the level of water stress reached in the previous experiment was higher than that reached in the present study.

No correlation was found between TPC and Pn, in contrast to previously reported data,<sup>20</sup> implying that Pn (isomers  $\alpha$  and  $\beta$ ) was not the only compound implied in the total polyphenolic content; other compounds behind this experimental trend could be anthocyanins and other ellagitannins. Only trace levels of ellagic acid were found in the present study, and cannot account for this lack of correlation.

**Table 2.** Sugars and organic acid profiles of *Wonderful* and *Mollar de Elche* pomegranates as affected by deficit irrigation and thinning treatments

| Treatment                   | Sugars                 |          | Organic acids |               |
|-----------------------------|------------------------|----------|---------------|---------------|
|                             | Glucose                | Fructose | Citric acid   | Ascorbic acid |
|                             | (g L <sup>-1</sup> FW) |          |               |               |
| <i>WONDERFUL</i>            |                        |          |               |               |
| ANOVA test                  |                        |          |               |               |
| Irrigation                  | *                      | *        | NS            | NS            |
| Thinning                    | *                      | *        | NS            | NS            |
| Irrigation x Thinning       | ***                    | ***      | NS            | NS            |
| Tukey's multiple range test |                        |          |               |               |
| Irrigation                  |                        |          |               |               |
| T0                          | 121 b                  | 142 b    | 1.47          | 0.32          |
| T1                          | 143 a                  | 171 a    | 1.17          | 0.20          |
| Thinning                    |                        |          |               |               |
| A0                          | 141a                   | 143 b    | 1.23          | 0.20          |
| A1                          | 123 b                  | 169 a    | 1.40          | 0.32          |
| Irrigation x Thinning       |                        |          |               |               |
| T0A0                        | 119 b                  | 138 c    | 1.45          | 0.21          |
| T0A1                        | 123 b                  | 146 b    | 1.47          | 0.23          |
| T1A0                        | 126 b                  | 149 b    | 1.04          | 0.23          |
| T1A1                        | 159 a                  | 192 a    | 1.33          | 0.44          |
| <i>MOLLAR DE ELCHE</i>      |                        |          |               |               |
| ANOVA test                  |                        |          |               |               |
| Irrigation                  | NS                     | NS       | NS            | NS            |
| Thinning                    | NS                     | NS       | NS            | NS            |
| Irrigation x Thinning       | NS                     | NS       | ***           | NS            |
| Tukey's multiple range test |                        |          |               |               |
| Irrigation                  |                        |          |               |               |
| T0                          | 119                    | 151      | 0.93          | 0.43          |
| T1                          | 120                    | 142      | 0.32          | 0.47          |
| Thinning                    |                        |          |               |               |
| A0                          | 120                    | 148      | 0.93          | 0.44          |
| A1                          | 118                    | 144      | 0.32          | 0.47          |
| Irrigation x Thinning       |                        |          |               |               |
| T0A0                        | 118                    | 151      | 1.54 a        | 0.40          |
| T0A1                        | 120                    | 151      | 0.32 b        | 0.46          |
| T1A0                        | 122                    | 145      | 0.32 b        | 0.47          |
| T1A1                        | 117                    | 138      | 0.32 b        | 0.47          |

NS, not significant at  $P < 0.05$ ; significant at \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , respectively.

Values (mean of three replications) followed by the same lowercase letter, within the same column and factor, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test.

FW, fresh weight.

### Sugars and organic acids

In accordance with previous data,<sup>17,32</sup> two sugars (glucose and fructose) and two organic acids (citric and ascorbic) were identified in the *Wonderful* and *ME* pomegranate juices (Table 2).

In terms of sugar profile, the glucose/fructose ratios were 0.84 and 0.82 in *Wonderful* and *ME*, respectively; similar results were reported previously.<sup>17,32</sup> An increase of glucose and fructose in *Wonderful* cultivar under water stress (T1) was found. The level of water stress played a role in fruit maturity; an accelerated early fruit maturity can be hypothesized based on the stimulation of the conversion of sucrose (disaccharide combining glucose and fructose) to

the noted monosaccharides.<sup>33</sup> The interaction on SDI and thinning (T1A1) gave rise to the highest increase in glucose and fructose in the *Wonderful* cultivar. By contrast, sugars from *ME* cultivar were not affected, probably because *ME* trees are better adapted to the cultivation area and to water stress than the *Wonderful* ones.<sup>9</sup>

The content of citric acid was higher in the *Wonderful* fruits than in the *ME* fruits, which agreed with a previous study stating that citric acid predominated in sour varieties, such as *Wonderful*.<sup>17</sup> On the other hand, the level of ascorbic acid was low in all treatments and in both cultivars as a result of the loss of this compound from metabolic activity during ripening, generating polymeric compounds.<sup>34</sup> Both identified organic acids were not affected by either the irrigation treatment (water stress) or the thinning treatment (crop load) in any of the two cultivars, probably because of the low level of water stress reached.

### Volatile compounds

A total of 12 and 14 different compounds were identified in the *Wonderful* and *ME* juices under study, respectively. Although the number of the volatile compounds found in the present study was lower than those previously found in other studies (18 compounds), it is a normal value for this fruit with a low odor/aroma intensity and complexity.<sup>35</sup> Table 3 shows the retention times and indices used for the identification of the compounds (together with the simultaneous use of standards) found in the pomegranate juices, as well as the main sensory descriptors of each one of the volatile compounds.<sup>36,37</sup>

For clarity, the pomegranate volatile compounds have been grouped into five chemical families:

- (i) *Aldehydes* (ALDs, total aldehydes): hexanal (V1), octanal (V2) and nonanal (V3);
- (ii) *Esters* (ESTs): benzyl acetate (V4), ethyl hexanoate (V5) and ethyl octanoate (V6);
- (iii) *Aliphatic alcohols* (ALCs): 2-ethyl-1-hexanol (V7), 3-hexen-1-ol (V8) and 1-hexanol (V9); and.
- (iv) *Monoterpenes* (MTEs):  $\beta$ -pinene (V10), *p*-cymene (V11) and  $\alpha$ -terpineol (V12); and (v) *Monoterpenoids* (MTOs): linalool (V13) and limonene (V14).

The relative abundance of each chemical family (sum of the percentages of all the members of the family) was significantly different between cultivars, and followed the order (Table 4):

- *Wonderful*: ALCs (mean of all treatments 67.0%) >> MTOs (11.5%) > MTEs (10.1%)  $\approx$  ALDs (9.9%) > ESTs (1.4%)
- *ME*: ALDs (mean of all treatments 29.6%) > MTOs (26.7%) > ALCs (23.6%) > MTEs (13.9%) > ESTs (6.2%).

In general, aliphatic alcohols (ALCs) were the predominant family [mainly, 1-hexanol (V9) and 3-hexen-1-ol (V8)] in *Wonderful* fruits (Table 4); these results agreed with those reported previously.<sup>38</sup> On the other hand, aldehydes, ALDs [mainly hexanal (V1) and nonanal (V3)] played an important role and were the most abundant chemical family in the *ME* juices, also in agreement with previous data.<sup>39</sup>

Changes on volatile composition as affected by irrigation or thinning treatments have been found in different fruits, such as jujube,<sup>40</sup> table olive<sup>13</sup> and pistachio,<sup>10</sup> amongst others. However, the present study is the first to evaluate the combined effects of these two factors on the volatile profile of pomegranate juices; different trends were found for each cultivar under study.

In the *Wonderful* cultivar, when water stress was applied, a reduction in ALD (only as a result of hexanal) and EST (as a result of benzyl

**Table 3.** Retention time (min) and indices and sensory descriptors (SAFC, 2012) of the volatile compounds of pomegranate juices, cultivars *Wonderful* and *Mollar de Elche*

| Code               | Compounds           | Retention time (min) | Retention indices |              | Descriptors                  |
|--------------------|---------------------|----------------------|-------------------|--------------|------------------------------|
|                    |                     |                      | Experimental      | UMH database |                              |
| Aldehydes          |                     |                      |                   |              |                              |
| V1                 | Hexanal             | 6.64                 | 826               | 835          | Green                        |
| V2                 | Octanal             | 14.30                | 1036              | 1029         | Herbaceous, citrus           |
| V3                 | Nonanal             | 19.16                | 1140              | 1154         | Citrus, vegetable            |
| Esters             |                     |                      |                   |              |                              |
| V4                 | Benzyl acetate      | 22.37                | 1206              | 1210         | Apple, floral, fruity, sweet |
| V5                 | Ethyl hexanoate     | 13.38                | 1016              | 1018         | Apple, banana, pineapple     |
| V6                 | Ethyl octanoate     | 22.61                | 1211              | 1212         | Floral, pear, pineapple      |
| Aliphatic alcohols |                     |                      |                   |              |                              |
| V7                 | 2-Ethyl-1-hexanol   | 15.72                | 1067              | 1070         | Rose, sweet                  |
| V8                 | 3-Hexen-1-ol        | 8.70                 | 898               | 902          | Banana, green, vegetable     |
| V9                 | 1- Hexanol          | 8.98                 | 906               | 912          | Green, herbaceous            |
| Monoterpenes       |                     |                      |                   |              |                              |
| V10                | $\beta$ -Pinene     | 12.33                | 992               | 998          | Woody                        |
| V11                | <i>p</i> -Cymene    | 14.63                | 1043              | 1051         | Citrus                       |
| V12                | $\alpha$ -Terpineol | 24.03                | 1240              | 1250         | Lilac                        |
| Monoterpenoids     |                     |                      |                   |              |                              |
| V13                | Linalool            | 18.94                | 1135              | 1142         | Lemon, orange, sweet         |
| V14                | Limonene            | 14.46                | 1039              | 1046         | Lemon, orange, sweet         |

The UMH research group (Universidad Miguel Hernández) has created their own library of standards to provide proper retention indices for the identification of the volatile compounds found in different food matrixes. All 14 compounds found in the pomegranate juices have been identified by using Sigma-Aldrich (Merk KGaA, Darmstadt, Germany) standards.

acetate) contents, as well as an increase of MTEs ( $\beta$ -pinene), was observed; however, in the *ME* cultivar, ALDs also decreased (hexanal and nonanal), although MTEs decreased as well ( $\beta$ -pinene and  $\alpha$ -terpineol) and MTOs increased (limonene). Probably, the reduction of ALDs was a result of the effect of water stress on the synthesis of the compounds from this chemical family. The synthesis starts with the C18 fatty acids, linolenic and linoleic acids.<sup>41</sup> Furthermore, the effects seen in the MTEs family could be explained by the effects of water stress on the activity of the enzyme linalool synthase or in the contents of its substrates, mainly carotenoids.<sup>42</sup> The reduction of hexanal was observed in other fruits such as grapes under water deficit during grape development and this was associated with fruit maturity.<sup>43</sup>

On the other hand, the only common effect of thinning on the volatile composition of both pomegranate cultivars was the decrease of the EST content (ethyl hexanoate), whereas the behaviors of ALCs and MTEs were different. The effects on thinning (crop load) on the volatile composition of *Wonderful* fruits was limited to the noted effects on esters; however, in *ME* fruits, a smaller crop load implied reductions of ESTs and ALCs but an increase of MTEs.

## PCA

For a better understanding of the relationships among all the studied variables for the different treatments (interaction between irrigation and thinning treatments), a PCA was run for the *Wonderful* and *ME* cultivars, respectively (Fig. 2). All sensory data have been included in the PCA and are discussed here to avoid duplication (Table 5).

The first principal component (F1) accounted for 55.19% and 40.77% of the total data variance in *Wonderful* (Fig. 2A) and *ME* (Fig. 2B), respectively, whereas the second principal component

(F2) accounted for 27.72% and 32.36% of the total variance, respectively.

It is important to note that the higher the distance between two parameters, the lower their correlation. Considering F1 as the dimension that explained the main differences among treatments, in *Wonderful* fruits (Fig. 2A), TOA0 was positively linked with TPC, MTOs (MTOs normally have citric flavor notes), ALDs (which have vegetable flavor notes), ESTs (with fruity flavor notes), production (yield) and key descriptive sensory parameters (sweetness, color, fruity, floral and pomegranate ID). In *Wonderful* fruits, all other three treatments (TOA1, T1A0 and T1A1) were positively correlated with the sugar content, ALCs (vegetable notes), fruit weight, diameter, polymeric color, and apple and pear flavor notes.

On the other hand, in *ME* fruits (Fig. 2B), treatment T1A1 (trees under water stress and subjected to thinning) was positively correlated with ALCs (vegetable notes), MTOs (citric notes) and key descriptive sensory parameters (color, sourness, fruity, floral and pomegranate ID). The other treatments (TOA0, TOA1 and T1A0) were positively correlated with ALDs (vegetable notes), MTEs (citric notes), ESTs (fruity notes), production and citric acid content.

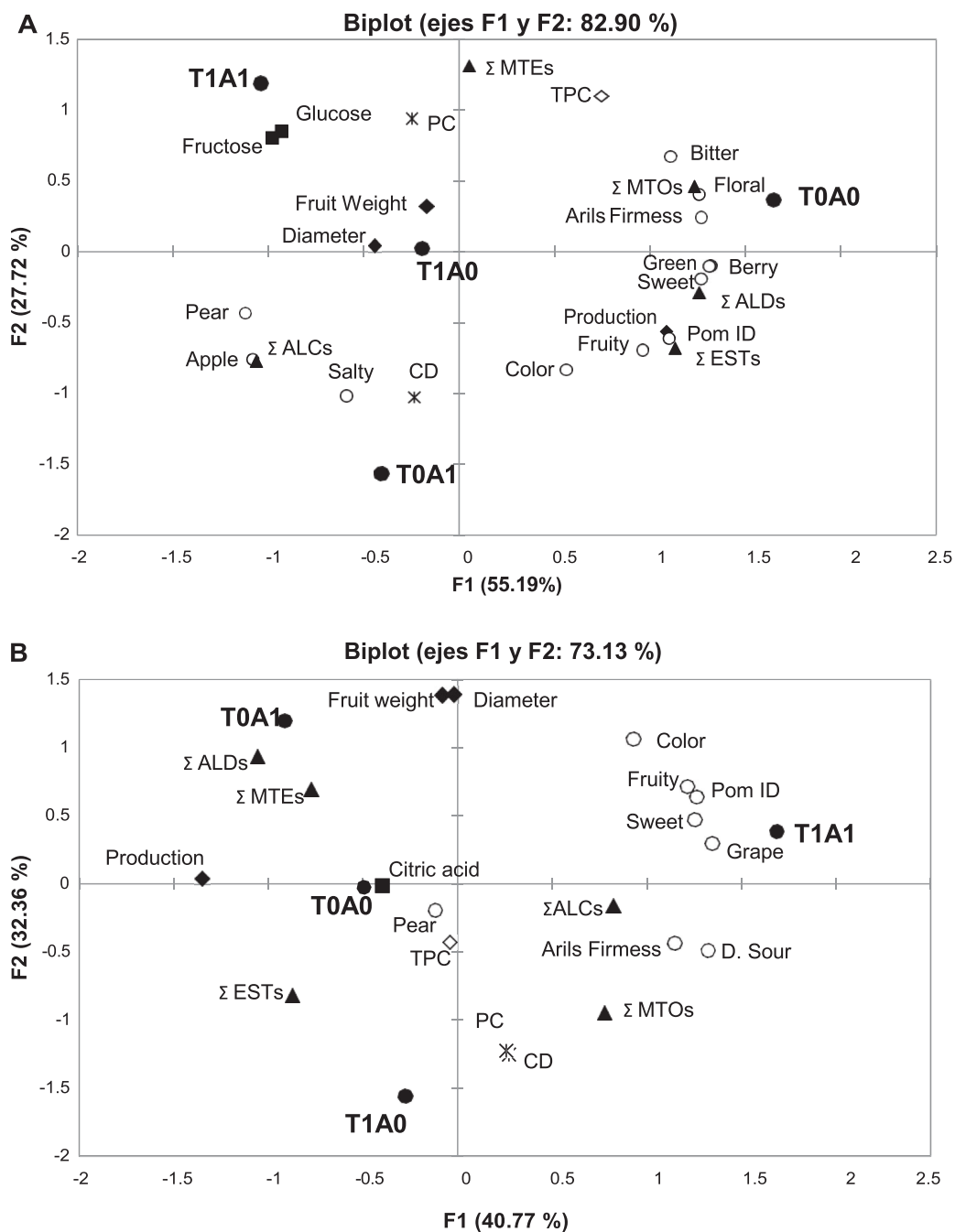
## CONCLUSIONS

Sustained deficit irrigation (T1) caused water stress in pomegranate trees. Although a decrease of marketable yield was observed after deficit irrigation (T1) and thinning (A1), the fruits remaining on the tree presented a higher weight and size, which is a positive fact and could be linked to the higher price of the commercial fruits. *Wonderful* fruits were more sensitive to changes in the sugar profile, with the values of glucose and fructose being increased by deficit irrigation strategies (T1),

**Table 4.** Volatile compounds (% of total area) of *Wonderful* and *Mollar de Eiche* pomegranates as affected by deficit irrigation and thinning treatments

| Compounds                   | Aldehydes       |        |         | Esters  |        |         | Aliphatic alcohols |         |         | Monoterpenes |         |         | Monoterpenoids |         |        |         |        |        |        |
|-----------------------------|-----------------|--------|---------|---------|--------|---------|--------------------|---------|---------|--------------|---------|---------|----------------|---------|--------|---------|--------|--------|--------|
|                             | V1 <sup>±</sup> | V2     | V3      | ∑ALD    | V4     | V5      | V6                 | ∑EST    | V7      | V8           | V9      | ∑ALC    | V10            | V11     | V12    | ∑MTE    | V13    | V14    | ∑MTO   |
| <i>WONDERFUL</i>            |                 |        |         |         |        |         |                    |         |         |              |         |         |                |         |        |         |        |        |        |
| ANOVA test                  |                 |        |         |         |        |         |                    |         |         |              |         |         |                |         |        |         |        |        |        |
| Irrigation                  | *               | NS     | NS      | *       | ***    | NS      | NS                 | **      | NS      | *            | *       | NS      | *              | NS      | NS     | *       | NS     | NS     | NS     |
| Thinning                    | NS              | NS     | NS      | NS      | NS     | *       | NS                 | *       | NS      | *            | *       | NS      | NS             | **      | *      | NS      | NS     | NS     | NS     |
| Irrigation x Thinning       | *               | NS     | *       | *       | *      | NS      | NS                 | *       | NS      | *            | **      | *       | *              | NS      | *      | *       | *      | *      | *      |
| Tukey's multiple range test |                 |        |         |         |        |         |                    |         |         |              |         |         |                |         |        |         |        |        |        |
| Irrigation                  | 6.15 a          | 0.00   | 4.66    | 10.8 a  | 1.27 a | 0.92    | 0.00               | 2.18 a  | 1.15    | 26.3 b       | 39.1 a  | 66.6    | 4.44 b         | 0.25    | 3.39   | 8.08 b  | 1.61   | 10.8   | 12.4   |
| T0                          | 3.08 b          | 0.00   | 5.19    | 8.26 b  | 0.00   | 0.70    | 0.00               | 0.70 b  | 1.08    | 34.5 a       | 31.6 b  | 67.2    | 7.65 a         | 0.31    | 4.14   | 12.1 a  | 1.14   | 9.77   | 10.9   |
| Thinning                    | 4.72            | 0.00   | 5.49    | 10.2    | 0.76   | 1.21 a  | 0.00               | 1.97 a  | 0.79 b  | 29.5         | 38.2 a  | 68.5    | 5.86           | 0.56 a  | 4.28 a | 10.7    | 1.87   | 11.3   | 13.2   |
| A0                          | 4.49            | 0.00   | 4.35    | 8.84    | 0.51   | 0.40 b  | 0.00               | 0.91 b  | 1.44 a  | 31.3         | 32.4 b  | 65.1    | 6.23           | 0.00 b  | 3.26 b | 9.49    | 0.88   | 9.22   | 10.1   |
| Irrigation x Thinning       | 6.25 a          | 0.00   | 5.66 a  | 11.9 a  | 1.51 a | 1.03 ab | 0.00               | 2.54 a  | 1.12 b  | 23.2 c       | 34.9 b  | 59.2 c  | 6.98 b         | 0.50    | 3.80 b | 11.3 b  | 2.34 a | 12.7 a | 15.1 a |
| TOA0                        | 6.04 a          | 0.00   | 3.66 b  | 9.70 ab | 1.02 a | 0.80 b  | 0.00               | 1.82 b  | 1.18 b  | 29.4 b       | 43.3 a  | 73.9 a  | 1.90 d         | 0.00    | 2.99 b | 4.88 d  | 0.87 c | 8.84 b | 9.71 b |
| T0A1                        | 3.16 b          | 0.00   | 5.28 a  | 8.44 b  | 0.00 b | 1.31 a  | 0.00               | 1.31 b  | 0.40 c  | 36.6 a       | 29.8 c  | 66.8 b  | 5.47 c         | 0.58    | 4.84 a | 10.9 b  | 1.43 b | 9.37 b | 10.8 b |
| T1A0                        | 2.93 b          | 0.00   | 5.05 ab | 7.98 b  | 0.00 b | 0.00 c  | 0.00               | 0.00 c  | 1.71 a  | 33.3 ab      | 33.2 bc | 68.2 ab | 9.83 a         | 0.00    | 3.53 b | 13.4 a  | 0.90 c | 9.60 b | 10.5 b |
| <i>MOLLAR DE EICHE</i>      |                 |        |         |         |        |         |                    |         |         |              |         |         |                |         |        |         |        |        |        |
| ANOVA test                  |                 |        |         |         |        |         |                    |         |         |              |         |         |                |         |        |         |        |        |        |
| Irrigation                  | *               | NS     | **      | ***     | NS     | *       | **                 | NS      | *       | *            | NS      | NS      | *              | NS      | **     | *       | **     | **     | **     |
| Thinning                    | NS              | NS     | NS      | NS      | NS     | *       | ***                | *       | NS      | NS           | *       | *       | **             | **      | **     | *       | *      | NS     | NS     |
| Irrigation x Thinning       | *               | **     | *       | *       | *      | **      | **                 | **      | **      | ***          | **      | ***     | **             | *       | *      | **      | *      | *      | *      |
| Tukey's multiple range test |                 |        |         |         |        |         |                    |         |         |              |         |         |                |         |        |         |        |        |        |
| Irrigation                  | 16.2 a          | 1.43   | 17.2 a  | 34.8 a  | 3.17   | 2.06 b  | 0.68 b             | 5.91    | 3.89 a  | 9.96 b       | 9.73    | 23.6    | 7.15 a         | 2.15    | 5.57 a | 14.9 a  | 3.63 a | 17.9 b | 21.5 b |
| T0                          | 13.5 b          | 1.41   | 9.51 b  | 24.4 b  | 2.67   | 3.02 a  | 0.86 a             | 6.55    | 2.97 b  | 11.8 a       | 9.49    | 24.3    | 6.57 b         | 2.10    | 4.06 b | 12.7 b  | 1.76 b | 30.3 a | 32.1 a |
| Thinning                    | 14.4            | 1.54   | 12.5    | 28.4    | 3.16   | 3.13 a  | 0.49 b             | 6.78 a  | 3.44    | 11.6         | 10.7 a  | 25.7 a  | 4.97 b         | 1.88 b  | 5.63 a | 12.5 b  | 2.94 a | 23.7   | 26.6   |
| A0                          | 15.2            | 1.30   | 14.3    | 30.8    | 2.68   | 1.95 b  | 1.05 a             | 5.68 b  | 3.42    | 10.2         | 8.52 b  | 22.1 b  | 8.76 a         | 2.37 a  | 4.00 b | 15.1 a  | 1.76 b | 24.5   | 26.3   |
| Irrigation x Thinning       | 17.4 a          | 0.86 b | 13.8 b  | 32.0 b  | 3.09 a | 1.98 b  | 0.47 c             | 5.54 bc | 2.07 c  | 14.5 a       | 13.3 a  | 29.8 a  | 3.62 c         | 1.76 ab | 6.22 a | 11.6 c  | 4.64 a | 16.4 c | 21.0 c |
| TOA0                        | 15.0 ab         | 1.95 a | 20.7 a  | 37.6 a  | 3.26 a | 2.15 ab | 1.24 a             | 6.64 b  | 3.87 b  | 5.44 c       | 6.20 c  | 15.5 c  | 10.7 a         | 2.55 a  | 4.91 b | 18.2 a  | 2.62 b | 19.4 c | 22.0 c |
| T0A1                        | 11.3 b          | 1.86 a | 11.6 b  | 24.8 c  | 3.17 a | 4.43 a  | 0.44 c             | 8.04 a  | 4.49 a  | 8.16 b       | 7.66 c  | 20.3 b  | 7.13 b         | 1.57 b  | 4.81 b | 13.5 b  | 1.05 c | 32.3 a | 33.3 a |
| T1A0                        | 15.4 ab         | 0.66 b | 7.90 c  | 24.0 c  | 2.11 b | 1.76 b  | 0.85 b             | 4.72 c  | 2.96 bc | 14.9 a       | 10.8 b  | 28.7 a  | 6.83 c         | 2.19 ab | 3.09 c | 12.1 bc | 0.90 c | 29.6 b | 30.5 b |

NS, not significant; ∑, nonanal;  $\overline{\text{ALD}}$ , total aldehydes; V4, benzyl acetate; V5, ethyl hexanoate; V6, ethyl octanoate;  $\overline{\text{EST}}$ , total esters; V7, 2-ethyl-1-hexanol; V8, 3-hexen-1-ol; V9, 1-hexanol;  $\overline{\text{ALC}}$ , total aliphatic alcohols; V10,  $\beta$ -pinene; V11, *p*-cymene; V12,  $\alpha$ -terpineol;  $\overline{\text{MTE}}$ , total monoterpenes; V13, linalool; V14, limonene; \* MTO, total monoterpenoids.



**Figure 2.** PCA scores plot showing the relationship among production, morphological parameters, total polyphenolic content, polymeric color, organic acids and sugars contents, volatile composition, and descriptive sensory analysis and treatments (TOA0, TOA1, T1A0 and T1A1) in cultivars *Wond* (A) and *ME* (B). ◊ Production and morphologic parameters: fruit weight and diameter; ■ total polyphenolic content (TPC); ▲ organic acids and sugars content; \*, color density (CD) and % polymeric color (PC); ○ Volatile composition: total aldehydes (Σ ALDs), total esters (Σ ESTs), total aliphatic alcohols (Σ ALCs), total monoterpenes (Σ MTEs) and total monoterpenoids (Σ MTOs); ○, descriptive sensory attributes.

which is important because of the strong sourness of these fruits. Furthermore, T1 caused a reduction of total aldehydes (mainly hexanal) and terpenoids in both cultivars, losing vegetables notes. In the *Wond* cultivar, fruits of the treatment T1A1 were positively linked with the highest fruit weight and fruit diameter and high contents of glucose and fructose, which is essential for a sour cultivar, whereas control *Wond* fruits, TOA0, were linked with total phenolic content, monoterpenes (citric notes), aldehydes (vegetable notes), esters (fruity notes), production and key

descriptive sensory parameters (sweetness, color, fruity, floral and pomegranate ID). In the *ME* cultivar, the interaction between water stress and commercial thinning (T1A1) was positively correlated with aliphatic alcohols (vegetable notes), monoterpenoids (citric notes) and key descriptive sensory parameters (color, sourness, fruity, floral and pomegranate ID), whereas control *ME* fruits, TOA0, were only linked to parameters such as total phenolic content, content of citric acid and the pear flavor. Thus, the final recommendation is that the best treatment for both

**Table 5.** Descriptive sensory analysis of *Wonderful* and *Mollar de Elche* pomegranates as affected by deficit irrigation and thinning treatments

|                             | Color | Fruity | Pom ID | Apple | Pear  | Grape Berry | Green | Earthy | Salty | Sweet | Sour   | Bitter | Arls Firmness |        |
|-----------------------------|-------|--------|--------|-------|-------|-------------|-------|--------|-------|-------|--------|--------|---------------|--------|
| <i>WONDERFUL</i>            |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| ANOVA test                  |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| Irrigation                  | ***   | NS     | NS     | NS    | NS    | NS          | *     | NS     | NS    | NS    | *      | NS     | NS            | NS     |
| Thinning                    | NS    | NS     | NS     | *     | NS    | NS          | *     | NS     | NS    | NS    | NS     | NS     | *             | NS     |
| Irrigation x Thinning       | ***   | **     | **     | **    | **    | NS          | **    | **     | NS    | ***   | **     | *      | **            | **     |
| Tukey's multiple range test |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| Irrigation                  |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| T0                          | 9.3 a | 4.7    | 5.6    | 1.8   | 0.7   | 1.9         | 2.4 a | 1.8    | 0.2   | 3.8   | 4.2 a  | 1.3    | 1.7           | 7.1    |
| T1                          | 7.3 b | 4.1    | 5.0    | 2.1   | 1.2   | 1.5         | 1.5 b | 1.2    | 0.4   | 3.9   | 3.3 b  | 1.4    | 1.4           | 6.7    |
| Thinning                    |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| A0                          | 8.0   | 4.8    | 5.6    | 1.4 b | 0.8   | 1.8         | 2.4 a | 1.9    | 0.2   | 3.7   | 4.0    | 1.3    | 1.8 a         | 7.1    |
| A1                          | 8.5   | 4.0    | 5.0    | 2.6 a | 1.2   | 1.6         | 1.5 b | 1.1    | 0.4   | 4.0   | 3.5    | 1.4    | 1.3 b         | 6.7    |
| Irrigation x Thinning       |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| T0A0                        | 9.0 a | 4.9 a  | 5.7 a  | 0.7 c | 0.2 b | 1.9         | 3.1 a | 2.4 a  | 0.1   | 2.9 b | 4.6 a  | 1.6 a  | 2.1 a         | 7.5 a  |
| T0A1                        | 9.5 a | 4.6 a  | 5.4 a  | 3.0 a | 1.3 a | 1.8         | 1.8 b | 1.3 b  | 0.3   | 4.8 a | 3.8 ab | 1.1 b  | 1.2 b         | 6.8 b  |
| T1A0                        | 7.0 b | 4.8 a  | 5.4 a  | 2.1 b | 1.4 a | 1.8         | 1.8 b | 1.5 b  | 0.2   | 4.6 a | 3.4 b  | 1.0 b  | 1.4 b         | 6.8 b  |
| T1A1                        | 7.5 b | 3.5 b  | 4.6 b  | 2.1 b | 1.1 a | 1.3         | 1.3 b | 0.8 c  | 0.6   | 3.2 b | 3.2 b  | 1.7 a  | 1.5 b         | 6.7 b  |
| <i>MOLLAR DEELCHE</i>       |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| ANOVA test                  |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| Irrigation                  | NS    | NS     | NS     | NS    | NS    | NS          | NS    | NS     | NS    | NS    | *      | NS     | NS            | NS     |
| Thinning                    | ***   | NS     | **     | NS    | **    | NS          | NS    | NS     | NS    | NS    | *      | NS     | NS            | NS     |
| Irrigation x Thinning       | ***   | ***    | **     | NS    | **    | *           | NS    | NS     | NS    | NS    | **     | NS     | NS            | **     |
| Tukey's multiple range test |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| Irrigation                  |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| T0                          | 6.4   | 3.3    | 3.1    | 1.6   | 2.7   | 0.9         | 0.3   | 0.4    | 1.6   | 1.4   | 4.9 b  | 0.9    | 0.8           | 6.8    |
| T1                          | 6.4   | 3.7    | 3.6    | 1.7   | 2.2   | 1.3         | 0.5   | 0.6    | 1.5   | 1.5   | 5.5 a  | 1.3    | 1.0           | 7.3    |
| Thinning                    |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| A0                          | 4.9 b | 3.0    | 2.8 b  | 1.4   | 2.9 a | 0.9         | 0.3   | 0.4    | 1.7   | 1.5   | 4.8 b  | 1.1    | 0.9           | 7.1    |
| A1                          | 7.8 a | 4.0    | 3.8 a  | 1.8   | 2.0 b | 1.3         | 0.5   | 0.6    | 1.5   | 1.5   | 5.6 a  | 1.1    | 0.8           | 7.0    |
| Irrigation x Thinning       |       |        |        |       |       |             |       |        |       |       |        |        |               |        |
| T0A0                        | 6.0 b | 3.1 b  | 3.0 b  | 1.3   | 3.6 a | 0.9 b       | 0.4   | 0.4    | 1.9   | 1.2   | 4.6 b  | 1.0    | 0.8           | 7.3 ab |
| T0A1                        | 6.8 b | 3.4 b  | 3.1 b  | 1.9   | 1.8 b | 1.0 b       | 0.2   | 0.5    | 1.4   | 1.5   | 5.1 b  | 0.8    | 0.8           | 6.3 b  |
| T1A0                        | 3.9 c | 2.9 b  | 2.6 b  | 1.6   | 2.1 b | 1.0 b       | 0.3   | 0.4    | 1.5   | 1.6   | 4.9 b  | 1.1    | 1.1           | 7.0 ab |
| T1A1                        | 8.9 a | 4.5 a  | 4.5 a  | 1.8   | 2.3 b | 1.6 a       | 0.8   | 0.8    | 1.5   | 1.3   | 6.1 a  | 1.4    | 0.9           | 7.6 a  |

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

Values (mean of three replications) followed by the same lowercase letter, within the same column and factor, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test.

pomegranate cultivars under study (*Wond* and *ME*) was T1A1, which comprises the simultaneous application of soft deficit irrigation during fruit growth and ripening (T1) and the application of thinning (A1).

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## **7.2 Publication 2**

### **Publication 2 (Literal transcription):**

#### **A Comparative Study Between Labelling and Reality: The Case of Phytochemical Composition of Commercial Pomegranate-Based Products**

**Cano-Lamadrid, M.**, Lipan, L., Calín-Sánchez, Á., Hernández, F., and Carbonell-Barrachina, Á.A.

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# A Comparative Study Between Labeling and Reality: The Case of Phytochemical Composition of Commercial Pomegranate-Based Products

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**Abstract:** Manufacturers are deeply involved in the development of new pomegranate-based products, which have acquired great prestige due to many studies proving their potential health benefits. Commercial pomegranate products including capsules and supplements and juices and nectars were assayed. The contents of the key phytochemical compounds (punicalagin, ellagic acid, and total polyphenolic content) and the associated antioxidant capacity (DPPH<sup>•</sup>, ABTS<sup>•+</sup>, and FRAP) were analyzed. The experimental ranges of punicalagin and ellagic acid contents were 0.96 to 308 mg/g and 0.09 to 13.1 mg/g, respectively. Punicalagin content was positively correlated ( $P < 0.001$ ) with DPPH<sup>•</sup> and total polyphenolic content values. No significant ( $P > 0.05$ ) correlation was observed among ellagic acid with the antioxidant capacity. The labeling standardization of these products is necessary due to the wide variability among “theoretically” similar pomegranate-based products.

**Keywords:** antioxidant capacity, dietary supplements, polyphenol intake, *Punica granatum* L., punicalagin

**Practical Application:** There is a need for labeling “standardization” of these products due to the wide variability of the key compounds (punicalagins, ellagic acid, and the total polyphenolic compounds) among “theoretically” similar pomegranate-based products. The contents of the key compounds should be declared on product labels, as indicators of the potential health benefits, and should replace subjective functional descriptions.

## Introduction

Nowadays, foods with health benefits have become very popular. Functional foods are regularly introduced into human's diet especially those having elevate polyphenols levels (Viuda-Martos and others 2011). Considering that, the antioxidant capacity (AOC) is positively correlated with indicators such as the total contents of polyphenols and flavonoids, pomegranate (Pom; *Punica granatum* L.) can be considered as a functional food (Bchir and others 2012). Some of the most relevant substances in Pom are hydrolysable tannins (for example, PC and punicalin) and phenolic acids (gallic and ellagic acids [EAs]; Turrini and others 2015). Punicalagin (PC) isomers ( $\alpha$ -PC and  $\beta$ -PC) together with EA are the most important substances in Pom and represent about the 85% of its total AOC (Calín-Sánchez and others 2013); however, EA content is significantly lower than that of PC (Nuncio-Jaúregui and others 2015). These compounds also are well-known free-radicals hunters, possessing maximum inhibition of mutagenicity up to 90%, and are associated with prevention of different diseases (Turrini and others 2015).

Manufacturers are deeply involved in the development of new Pom-based products (PomP), because their benefits should led to improved human health (Alaei and Amiri Chayjan 2015). This is

the reason that explains the expanded promotion of Pom products and the increment of the sales from \$84500 in 2001 up to \$66 million in 2005 in United States (Vázquez-Araújo and others 2015). Meanwhile in European Union, where the Eastern part of Spain is one of the main producers, there is no data on total consumption of PomP (MFA 2015; Szychowski and others 2015).

There is no exact information about recommended daily intake of polyphenols, but is estimated to be around 1 g/d (Scalbert and Williamson 2000). Regardless of the amount of studies regarding the possible role of polyphenols on disease prevention, data concerning their consumption at the population levels is still a huge ambiguity due to their wide structural diversity (Scalbert and Williamson 2000; Grosso and others 2014), and the different conditions of application. For instance, Grosso and others (2014) provided valuable information on dietary reference intake (DRI), and estimated the mean consumption of polyphenols at 1757 mg/d for the Polish population. The estimated flavonoid daily intake in adults from the U.S. was 189.7 mg/d, and was mainly due to flavan-3-ols (83.5%; Chun and others 2007). Other studies involving miscellaneous countries from Europe reported that the country with the highest polyphenols intake was Denmark, with 1786 mg/d, and the one with the lowest was Greece, with 744 mg/d (Zamora-Ros and others 2016). In France, the total polyphenol content (TPC) recorded was about 1031 mg/day (Perez-Jimenez and others 2011), whereas this content reached a value of 820 mg/d in Spanish adults (Tresserra-Rimbau and others 2013), and 863 mg/d in the Finnish population (Ovaskainen and others 2008). Besides, the PomP are rather safe without any negative effect registered in humans (Al-Muammar and Khan 2012).

The fact that the demand for fresh Poms and their based products is higher than the production is leading to high prices and also to

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frauds, in some cases. Thus, there is a need to analyze and control commercial PomP and to develop new methods to detect potential fraud and adulteration (Nuncio-Ja'uregui and others 2014).

Considering the positive reputation and high demand of PomP is essential to quantify the total polyphenols content, not only for health benefits and product labeling but also for key facts such as traceability and adulteration (Qu and others 2012). Previous studies concluded that a therapeutic index must be established in PomP (pills, capsules, and juices) to have a good understanding of their potential health benefits.

Therefore, the aim of this study was to compare the phytochemical content (TPC, PC isomers [ $\alpha$  and  $\beta$ ], and EA) and AOC (DPPH<sup>•</sup>, FRAP, and ABTS<sup>•+</sup>) of different commercial PomP (CPomP) available on the international market.

## Materials and Methods

### Commercial CPomP

Samples ( $n=22$ ) from 2 Pom (*Punica granatum* L.) CPomP groups were analyzed: (i) capsules and supplements ( $n=11$ ) and (ii) pure and concentrated juices ( $n=11$ ). Products were purchased from pharmacy chains, through internet (on-line shops), or by mail order. The main labeling information of all tested samples is listed in Table 1 (product name, main ingredients list, commercial brand, country, weight, and recommended intake dose). The daily intake estimation of PC, EA, and total polyphenolic content was obtained considering the recommended dose from the labeling of each CPomP.

### Identification and quantification of phenolic compounds

PC ( $\alpha$  and  $\beta$  isomers) and EA contents were determined in all studied samples. A known amount of CPomP was diluted with 5 mL of acidified MeOH/water (80:20 v/v, 1% HCl), and centrifuged. Supernatants were filtered through a 0.45  $\mu$ m Millipore filter, and injected into a Hewlett-Packard-series 1200 high-performance liquid chromatograph, HPLC (Woldbronn, Germany), equipped with a LiChroCART 100 RP-18 reversed-phased column (250  $\times$  4 mm, particle size, 5  $\mu$ m; Merck, Darmstadt, Germany), and a precolumn C18 (LiChrospher 100 RP-18, 5  $\mu$ m; Merck, Darmstadt, Germany). Eluents were analyzed using a UV-Vis Diode Array detector. The mobile phase consisted of: (i) solvent A (1% acetic acid in Milli-Q water); and (ii) solvent B (1% acetic acid in MeOH). Flowrate was 1 mL/min using a gradient starting with 1% B for 5 min, and increasing it to 60% B at 40 min. The compounds were quantified using calibration curves of the standard compounds ( $\alpha$ -PC,  $\beta$ -PC, and EA); all analyses were run in triplicate.

### AOC and TPC

A methanol extract was obtained from each product, and analyzed for AOC using the DPPH<sup>•</sup>, ABTS<sup>•+</sup>, and FRAP methods. A known amount of product was mixed with 10 mL of acidified MeOH/water (80:20 v/v, 1% HCl), sonicated at 25 °C for 15 min, and left for 24 h at 4 °C. The extract was again sonicated for 15 min and centrifuged until the separation of the supernatant.

The radical scavenging activity was evaluated using the DPPH<sup>•</sup> radical method, as described by previous research (Brand-Williams and others 1995), allowing a reaction time of 15 min. The ABTS<sup>•+</sup> and FRAP methods were also used to evaluate the AOC, according to previous methods (Benzie and Szeto 1999; Re and others 1999). Calibration curves within the range 0.50 to 5.00 mmol Trolox/kg were used for the quantification of the AOC in the 3

methods; these calibration curves showed good linearity ( $R^2=0.998$ ). Results were expressed in mmol Trolox/kg.

Total polyphenols content (TPC) was quantified using Folin-Ciocalteu colorimetric method as previously described by Cano-Lamadrid and others 2016. The absorbance was measured at 765 nm using an UV-Visible spectrophotometer (Helios Gamma model, UVG 1002E, Merckers Row, Cambridge, U.K.). Quantification was done with respect to a standard curve of gallic acid. The results were expressed as mg gallic acid equivalents (GAE)/g.

### Statistical analysis

Data were subjected to analysis of variance (ANOVA) and later to Tukey's multiple-range test to compare the means. Differences were considered statistically significant at  $P < 0.05$ . All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, Md., U.S.A.). In addition, correlations among variables were determined by correlation analyses using Pearson correlation coefficient.

## Results and Discussion

### PC and EA contents

The contents of PC ( $\alpha$  and  $\beta$  isomers) and EA in all the CPomP were expressed in mg/g and their values are listed in Table 2.

According to the phytochemicals content, there was a very high variability among samples ( $P < 0.05$ ). Regarding total PC of the 1st group (capsules and supplements), the range of detected values was 4.23 to 308 mg/g ( $P < 0.001$ ). Sample 1A had the highest content (308 mg/g), followed by 1G and 1I with 123 and 85.3 mg/g, respectively. Sample 1C registered the lowest detected value 4.23 mg/g; meanwhile samples 1B, 1E, and 1K had no measurable contents of PC. It is important to highlight that the 1E sample with no detected PC was "theoretically" powdered Pom peel extract. In the 2nd group (pure and concentrated juices), it was observed that values ranged from 0.96 mg/g (sample 2F) up to 10.4 mg/g (sample 2E;  $P < 0.01$ ). However, samples 2G, 2J, and 2K showed no measurable contents of the 2 analyzed compounds (PC and EA). Two of these samples (2G and 2K, respectively) were "theoretically" concentrated Pom juices.

As it can be observed in Table 2, there was very big difference between the 2 groups of CPomP. In the 1st group (capsules and supplements), it can be noticed that the highest value was about to 308 mg/g, whereas in the 2nd group (juices), the most elevated registered value was around 10 mg/g. This might be caused not only by the conditions during manufacturing, storage, or agricultural practices (Fisher and others 2011), but also due to the differences between the nature of these products. As can be noticed, samples from 1st group were capsules obtained from Pom extract of peel and seeds, meanwhile, samples from 2nd were juices mainly from the Pom arils. Several authors have reported that the phenolic compounds are higher in the nonedible parts of the Poms, such as rind and carpelar membranes, than in the edible arils, and even higher when the fruits are at the beginning of growing season (Seeram and others 2005; Rummun and other 2013).

Concerning the PC isomers of the 1st group of samples,  $\alpha$ -PC ranged from nonmeasurable contents (1B, 1E, 1I, and 1K) up to 176 mg/g (1A), whereas  $\beta$ -PC registered also from nonmeasurable values (1B, 1E, and 1K) to 132 mg/g (1A). In the 2nd group, the values of  $\alpha$ -PC and  $\beta$ -PC varied from nonmeasurable values (2G, 2J, and 2K) to 5.90 and 4.52 mg/g, respectively (2E). It can be observed that sample 1I, which is Pom kernel flour, presented

Table 1–Main labeling information in the studied pomegranate samples.

| Sample | Main ingredients   |                         |              | Country            | Recommended dose |     | Amount (capsules) |
|--------|--|-------------------------|--------------|--------------------|------------------|-----|-------------------|
|        | Group 1: CAPSULES AND SUPPLEMENTS  | Commercial brand        | Capsules     |                    | (mL)             | (g) |                   |
| 1A     | Pomegranate extract 833 mg, Bulking agent: silicone dioxide 10 mg, magnesium stearate 25 mg  | Granatum Plus           | 1            | Spain              | -                | -   | 30                |
| 1B     | Pomegranate extract 250 mg, Rice flour, gelatin. Contains <2% of: silica, vegetable cellulose, vegetable magnesium stearate  | Puritan's Pride Premium | 1-2          | USA                | -                | -   | 120               |
| 1C     | Pomegranate extract 200 mg. Capsule: gelatin, rice flour, bulking agent: magnesium stearate and silica   | Solarray                | 1            | USA                | -                | -   | 60                |
| 1D     | Pomegranate extract (77%). Gelatin, ascorbate, bulking agent (corn starch), anti-adherent agent (magnesium stearate)   | Snact Bernhard          | 2            | Germany            | -                | -   | 90                |
| 1E     | Powdered pomegranate extract from peel   | ClubVitis               | 2            | UK                 | -                | -   | 30                |
| 1F     | 70% concentrate powder of pomegranate juice (pomegranate juice, cornstarch), 10% pomegranate extract, granulated sugar and palm oil  | RAAB Vitalfood          | 9            | Germany            | -                | -   | 250               |
| 1G     | Pomegranate 83% (of which 84% pomegranate juice and 16% pomegranate juice extract) vegetable capsule (hydroxypropylmethylcellulose), bulking agent (maltodextrin), grape extract, anti-adherent agent (silica) | Dr. Jacob's             | 2-4          | Germany            | -                | -   | 100               |
| 1H     | 350 mg pomegranate dry extract. Bulking agent (microcrystalline cellulose), anti-adherent agent (magnesium stearate and silicone dioxide)  | Naturliider             | 2            | European Union, EU | -                | -   | 60                |
| 1I     | 100% pomegranate kernel flour  | RAAB Vitalfood          | -            | USA                | -                | 240 | -                 |
| 1J     | Pomegranate juice (30 mL) from concentrated  | SuperDiet               | 1-2 ampoules | France             | 300              | -   | -                 |
| 1K     | Pomegranate powder made from the fleshy seeds of ripe pomegranate  | Biotona                 | 10 g         | Belgium            | -                | 200 | -                 |

| Sample | Main ingredients   |                  |               | Country | Recommended Dose |     | Amount (capsules) |
|--------|--|------------------|---------------|---------|------------------|-----|-------------------|
|        | Group 2: PURE AND CONCENTRATED JUICES  | Commercial Brand | (mL)          |         | (mL)             | (g) |                   |
| 2A     | Pomegranate concentrate, water. Preservatives: potassium sorbate E-211 and sodium benzoate E-202   | Granatum Plus    | 30            | Spain   | 500              | -   | -                 |
| 2B     | Pomegranate juice made from pomegranate concentrate  | Granatum Plus    | 200           | Spain   | 200              | -   | -                 |
| 2C     | Pomegranate juice squeezed   | Granatum Plus    | 200           | Spain   | 200              | -   | -                 |
| 2D     | Dehydrated pomegranate juice and maltodextrins   | Salengei         | 1-2 teaspoons | EU      | -                | 200 | -                 |
| 2E     | Direct pomegranate juice   | Salus            | 10-15         | Germany | 200              | -   | -                 |
| 2F     | Pomegranate juice based on concentrate (93.7%, agave syrup (6.1%), preservatives (potassium sorbate and sodium benzoate)   | Marnys           | 40            | Spain   | 500              | -   | -                 |
| 2G     | Pomegranate, 100% pure concentrated juice. Preservatives: potassium sorbate and sodium benzoate  | Specciasol       | 20            | Italy   | 500              | -   | -                 |
| 2H     | 100% pomegranate juice   | Plantis          | 30            | Spain   | 1000             | -   | -                 |
| 2I     | Pomegranate (95% juice and pulp concentrate partly live fermented), elderberry juice concentrate, potassium lactate, natural flavor (vanilla extract), setting agent: pectin and spices (0.1%) | Dr. Jacob's      | 10-20         | Germany | 500              | -   | -                 |
| 2J     | Pomegranate juice. 100% fruit content without sugar or preservatives   | Snact Bernhard   | 50            | Germany | 1000             | -   | -                 |
| 2K     | Concentrated double strength pomegranate fruit juice, ascorbic acid (vitamin C), potassium sorbate and sodium benzoate (preservative)  | Optima           | 20            | Spain   | 500              | -   | -                 |

**Table 2—Contents of punicalagin ( $\alpha$ -PC and  $\beta$ -PC) and ellagic acid (EA) in the studied pomegranate products, expressed in mg/g.**

| Sample             | $\alpha$ -PC        | $\beta$ -PC | $\Sigma$ PC | EA     |
|--------------------|---------------------|-------------|-------------|--------|
|                    | (mg/g)              |             |             |        |
| ANOVA <sup>a</sup> |                     |             |             |        |
| Group 1            | ***                 | ***         | ***         | ***    |
| 1A                 | 176 a <sup>b</sup>  | 132 a       | 308 a       | 13.1 a |
| 1B                 | nd <sup>c</sup>     | nd          | nd          | 0.84 d |
| 1C                 | 2.04 d              | 2.19 d      | 4.23 e      | 3.89 c |
| 1D                 | 6.53 c              | 8.14 d      | 14.7 d      | 6.53 c |
| 1E                 | nd                  | nd          | nd          | 0.84 d |
| 1F                 | 2.78 d              | 2.10 e      | 4.88 e      | 0.79 d |
| 1G                 | 56.1 b              | 66.9 c      | 123 b       | 9.03 b |
| 1H                 | 2.00 d              | 10.0 d      | 12.0 d      | 4.05 c |
| 1I                 | nd                  | 85.3 b      | 85.3 c      | 0.19 d |
| 1J                 | 7.54 c              | 0.09 e      | 7.63 de     | 0.25 d |
| 1K                 | nd                  | nd          | nd          | 0.11 d |
| ANOVA <sup>a</sup> |                     |             |             |        |
| Group 2            | **                  | **          | **          | *      |
| 2A                 | 0.89 c <sup>b</sup> | 1.00 cd     | 1.89 d      | 0.49 a |
| 2B                 | 0.56 c              | 0.57 d      | 1.13 d      | 0.09 b |
| 2C                 | 1.23 bc             | 0.50 d      | 1.73 d      | 0.09 b |
| 2D                 | 2.02 b              | 2.20 c      | 4.22 c      | 0.40 a |
| 2E                 | 5.90 a              | 4.52 b      | 10.4 a      | 0.39 a |
| 2F                 | 0.45 c              | 0.51 d      | 0.96 d      | 0.09 b |
| 2G                 | nd <sup>c</sup>     | nd          | nd          | 0.05 b |
| 2H                 | 0.63 c              | 0.71 d      | 1.34 d      | 0.11 b |
| 2I                 | 0.47 c              | 8.00 a      | 8.47 b      | 0.35 a |
| 2J                 | nd                  | nd          | nd          | 0.11 b |
| 2K                 | nd                  | nd          | nd          | 0.10 b |

<sup>a</sup> \*, \*\*, and \*\*\*, significant at  $P < 0.05$ ,  $0.01$ , and  $0.001$ , respectively;

<sup>b</sup> Values (mean of 3 replications) followed by the same letter, within the same column and group, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test;

<sup>c</sup>nd = below limit of quantification (LOQ).

**Table 3—Antioxidant capacity (mmol Trolox/kg) and total polyphenols content (mg GAE/g) in the studied pomegranate products.**

| Sample             | TPC                 | DPPH*            | ABTS <sup>+</sup> | FRAP    |
|--------------------|---------------------|------------------|-------------------|---------|
|                    | (mg GAE/g)          | (mmol Trolox/kg) |                   |         |
| ANOVA <sup>a</sup> |                     |                  |                   |         |
| Group 1            | ***                 | ***              | ***               | ***     |
| 1A                 | 353 a <sup>b</sup>  | 1129 a           | 71.0 a            | 9029 a  |
| 1B                 | 16.9 bc             | 47.6 d           | 31.4 c            | 31.7 ef |
| 1C                 | 18.7 b              | 74.8 c           | 47.6 b            | 199 c   |
| 1D                 | 22.6 b              | 75.6 c           | 47.3 b            | 5696 b  |
| 1E                 | 15.5 c              | 32.3 e           | 45.9 b            | 67.5 de |
| 1F                 | 16.6 bc             | 75.4 c           | 47.8 b            | 238 c   |
| 1G                 | 0.10 e              | 733 b            | 46.9 b            | 55.6 e  |
| 1H                 | 19.9 b              | 74.8 c           | 47.0 b            | 6185 b  |
| 1I                 | 10.7 cd             | 16.5 f           | 23.6 c            | 108 d   |
| 1J                 | 7.93 d              | 23.0 f           | 39.8 bc           | 24.8 f  |
| 1K                 | 15.8 c              | 41.6 d           | 45.8 b            | 38.6 ef |
| ANOVA <sup>a</sup> |                     |                  |                   |         |
| Group 2            | **                  | **               | **                | **      |
| 2A                 | 17.5 a <sup>b</sup> | 42.3 a           | 46.4 a            | 51.9 c  |
| 2B                 | 6.13 c              | 14.3 c           | 16.3 d            | 11.9 d  |
| 2C                 | 4.32 cd             | 13.9 c           | 18.7 d            | 12.3 d  |
| 2D                 | 9.72 b              | 23.8 b           | 39.8 b            | 12.2 d  |
| 2E                 | 10.0 b              | 37.7 a           | 23.9 c            | 211 b   |
| 2F                 | 5.38 c              | 11.6 c           | 14.5 d            | 8.12 d  |
| 2G                 | 3.09 d              | 10.2 c           | 11.0 d            | 15.2 d  |
| 2H                 | 8.09 bc             | 25.9 b           | 23.9 c            | 12.4 d  |
| 2I                 | 10.8 b              | 37.4 a           | 20.8 c            | 2973 a  |
| 2J                 | 4.89 cd             | 13.4 c           | 23.7 c            | 7.90 d  |
| 2K                 | 7.71 bc             | 19.2 b           | 23.5 c            | 17.9 d  |

<sup>a</sup> \*\* and \*\*\*, significant at  $P < 0.01$  and  $0.001$ , respectively;

<sup>b</sup> Values (mean of 3 replications) followed by the same letter, within the same column and group, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test;

<sup>c</sup>nd = below limit of quantification (LOQ).

no detectable values of  $\alpha$ -PC but remarkable  $\beta$ -PC content (85 mg/g). This statement agreed quite well with previous studies, where a higher concentration of  $\beta$ -PC than  $\alpha$ -PC was reported in similar products, such as Pom juices (Qu and others 2012).

Regarding the EA content, samples of the 1st group recorded values ( $P < 0.001$ ) between 0.11 mg/g (1K, powder sample made from Pom seeds) up to 13 mg/g (sample 1A is a Pom extract, without indication from which part of the fruit it comes from), followed by sample 1G (9.03 mg/g), which underwent a controlled fermentation step during its preparation. The high content of sample (1G) respect the other products can be explained by previous results which indicated that the fermentation of PomP with *Lactobacillus plantarum* increases the concentration of EA (Filannino and others 2013). Sample 1A (Pom extract) presented much higher EA content than sample 1K (powder from arils), because of the different nature of the product, whole fruit and arils, respectively (Qu and others 2012). Peels and carpelar membranes are rich in polyphenols, flavonoids, and tannins apart from EA, meanwhile arils, including the woody portions (high amount of fatty acids) and juices are not characterized by high contents of these compounds (MFA; Viuda-Martos and others 2011; Rinaldi and others 2013). Concerning the 2nd group, the highest EA content was observed in a Pom concentrated juice sample, 2A (0.49 mg/g) followed by 2D (dehydrated Pom juice), 2E (direct Pom juice), and 2I (Pom concentrate mixed with other berries concentrate). As it can be seen in Table 1, generally all these products were Pom concentrated or pure juices. All these samples had a

higher content of EA as compared to juices prepared from just arils; this experimental situation agreed with results from other authors, who concluded that commercial manufacturers produce juice by squeezing whole fruit, including some nonedible parts of Pom (membrane or peel), and is during this process when the polyphenols are extracted (Qu and others 2012). On the other hand, the lowest EA content from 2nd group was registered in sample 2G (0.05 mg/g), which is a product commercialized as 100% Pom concentrate juice. Very similar values have registered in samples 2B (Pom juice from concentrate), 2C (squeezed juice from direct extraction), and 2F (Pom juice sweetened with agave syrup), with values around 0.09 mg/g. The variation among all these products can be explained by the use of different Pom varieties, and manufacturing conditions (Qu and others 2012).

#### AOC and TPC

The AOC in foods must be evaluated using a mixture of methods to cover all the aspects of the antioxidant efficacy (Viuda-Martos and others 2011). There are miscellaneous methods because none of them is precise and global when evaluating AOC (Cano-Lamadrid and others 2016). This happens due to the considerable differences in sample preparation techniques, extraction methods (solvents, temperature, and so on), choice of limits, and pronouncement of results (Viuda-Martos and others 2011). Electron transfer-based trials (ABTS<sup>+</sup>, FRAP, and DPPH\*) measure the ability of an antioxidant to reduce an oxidant, whereas color changes with the redox process. Nevertheless, the methods

**Table 4–Pearson’s correlation coefficients (R) among phytochemical parameters (TPC, punicalagin [PC], and ellagic acid [EA]) and antioxidant capacity (DPPH’, ABTS<sup>++</sup>, and FRAP).**

|                                   | TPC      | PC      | EA    | DPPH’ | ABTS <sup>++</sup> | FRAP |
|-----------------------------------|----------|---------|-------|-------|--------------------|------|
| Group 1: Capsules and supplements |          |         |       |       |                    |      |
| TPC                               | 1        |         |       |       |                    |      |
| PC                                | 0.88***a | 1       |       |       |                    |      |
| EA                                | 0.23     | 0.27    | 1     |       |                    |      |
| DPPH’                             | 0.80***  | 0.88*** | 0.07  | 1     |                    |      |
| ABTS <sup>++</sup>                | 0.68*    | 0.55    | 0.00  | 0.68* | 1                  |      |
| FRAP                              | 0.73**   | 0.59    | 0.46  | 0.54  | 0.65*              | 1    |
| Group 2: Juices and concentrated  |          |         |       |       |                    |      |
| TPC                               | 1        |         |       |       |                    |      |
| PC                                | 0.43     | 1       |       |       |                    |      |
| EA                                | 0.62*    | -0.15   | 1     |       |                    |      |
| DPPH’                             | 0.93***  | 0.66 *  | 0.49  | 1     |                    |      |
| ABTS <sup>++</sup>                | 0.85**   | 0.15    | 0.64* | 0.68* | 1                  |      |
| FRAP                              | 0.30     | 0.59    | -0.11 | 0.46  | 0.00               | 1    |
| All samples                       |          |         |       |       |                    |      |
| TPC                               | 1        |         |       |       |                    |      |
| PC                                | 0.88***  | 1       |       |       |                    |      |
| EA                                | 0.18     | 0.17    | 1     |       |                    |      |
| DPPH’                             | 0.80***  | 0.94*** | 0.07  | 1     |                    |      |
| ABTS <sup>++</sup>                | 0.61*    | 0.54    | 0.27  | 0.62* | 1                  |      |
| FRAP                              | 0.74**   | 0.63*   | 0.25  | 0.58  | 0.58               | 1    |

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

are distinct: the ABTS<sup>++</sup> method measures both hydrophilic and lipophilic capacity, while DPPH’ only considers lipophilic compounds. The FRAP assay is a ferric to ferrous ion reduction that in an acid pH produces a colored ferrous-tripyridyltriazine complex (Benzie and Strain 1996). For all these reasons, the AOC of PomP was evaluated using 3 different analytical methods as previously done by other researchers in similar products (Nuncio-Ja’uregui and others 2015). The AOC in Pom can be associated to the phenolic compounds, basically PC, EA, and anthocyanins. All these compounds are important due to its properties to hunt free radicals and to prevent lipid oxidation (Viuda-Martos and others 2011).

Table 3 shows the AOC results of all 22 studied CPomP, expressed in mmol Trolox/kg. Among the samples in the 1st group, the values of the DPPH’ assay ranged between 16.5 and 1129 mmol Trolox/kg ( $P < 0.001$ ). The highest DPPH’ value was reported for sample 1A (1129 mmol Trolox/kg), followed by 1G (733 mmol Trolox/kg), whereas the lowest value was found in sample 1J (16.5 mmol Trolox/kg). For ABTS<sup>++</sup>, the values ranged from 23.6 (1I) to 71.0 mmol Trolox/kg (1A;  $P < 0.001$ ). Finally, the samples 1A, 1H, and 1D showed highest FRAP values (9029, 6185, and 5696 mmol Trolox/kg, respectively;  $P < 0.001$ ), whereas the lowest value was found in sample 1J, with 24.8 mmol Trolox/kg. It can be noticed that sample 1A showed the highest ability to inhibit both DPPH’ and ABTS<sup>++</sup> radicals (1129 and 71 mmol Trolox/kg), and also the highest ferric reducing activity (9029 mmol Trolox/kg). It is important to remember that sample 1A were capsules of Pom extract.

Regarding the samples of the 2nd group (juices), sample 2A showed the highest values (42.3 mmol Trolox/kg) followed by 2E and 2I (37.7 and 37.4 mmol Trolox/kg, respectively), using DPPH’ method ( $P < 0.01$ ). However, sample 2G showed the lowest value, 10.2 mmol Trolox/kg. The ABTS<sup>++</sup> method also classified the sample 2A as having the highest AOC value (46.4 mmol Trolox/kg), whereas the sample 2G had the lowest one (11.0 mmol Trolox/kg). The range of values when using the FRAP assay was 7.90 to 2973 mmol Trolox/kg ( $P < 0.001$ ), with

the highest value being found in the sample 2I, followed by 2E and 2A (211 and 51.9 mmol Trolox/kg, respectively). The samples with the lowest values were 2J and 2F (7.90 and 8.11 mmol Trolox/kg, respectively).

In general, samples of 2nd group were described as samples with high AOC assayed by 3 methods of AOC determination. This may be explained because these samples present in their composition a greater concentration of total polyphenols content as can be observed in Table 3. It might be distinguished that the samples with the highest values of AOC are Pom concentrates and direct Pom juices whereas the samples with the lowest AOC were Pom juices based on concentrate, 100% Pom juice and Pom 100% pure concentrated juice.

The highest TPC value in the 1st group of samples was represented by sample 1A (353 mg GAE/g) followed by 1D and 1H (22.6 and 19.9 mg GAE/g, respectively), and the lowest one was 1G (0.10 mg GAE/g;  $P < 0.001$ ; Table 3). However, group 2 (juices) showed a range of values between 3.09 to 17.5 mg GAE/g ( $P < 0.01$ ), with the sample 2A having the highest value (17.5 mg GAE/g), followed by samples 2E and 2I (10.8 and 10.0 mg GAE/g, respectively), whereas the lowest value was that of 2G (3.09 mg GAE/g).

#### Pearson’s correlation coefficients

Table 4 shows the Pearson’s correlation coefficients between variables. The TPC was highly correlated ( $R = 0.88$ ;  $P < 0.001$ ) with the PC content in the 1st group but it was not correlated ( $R = 0.23$ ;  $P > 0.05$ ) with the EA content. Contrary, TPC among samples belonging 2nd group was not correlated with PC ( $R = 0.43$ ;  $P > 0.05$ ) but was correlated with EA ( $R = 0.62$ ;  $P < 0.05$ ). The lack of a positive correlation among TPC and the contents of PC and EA confirmed that polyphenols are not the only substances implied in the antioxidant activity (Rinaldi and others 2013) of PomP. Among samples belonging to both 1st and 2nd groups, DPPH’ was positively correlated with PC and TPC (for example, for the 1st group samples,  $R = 0.88$  and  $0.80$ ;

**Table 5-Daily intake according to recommended doses of each commercial pomegranate based products.**

| Sample         | Daily PC (mg)       | Daily EA (mg) ANOVA <sup>a</sup> | Daily TPC (mg GAE) |
|----------------|---------------------|----------------------------------|--------------------|
| <b>Group 1</b> | ***                 | ***                              | ***                |
| 1A             | 256 ab              | 10.9 a                           | 294 a              |
| 1B             | nd <sup>c</sup>     | 0.2-0.4 e-f                      | 4-8 e-f            |
| 1C             | 1.05 e              | 1.4 d                            | 7.0 e              |
| 1D             | 14.7 c              | 6.5 b                            | 23.0 d             |
| 1E             | nd                  | 1.2 d                            | 22.0 d             |
| 1F             | 15.4 c              | 2.5 c                            | 52.0 c             |
| 1G             | 150-310 b-a         | 11-22 a-a                        | Traces             |
| 1H             | 8.4 d               | 2.8 c                            | 140 b              |
| 1I             | —                   | —                                | Traces             |
| 1J             | 229-458 b-a         | 7.5-15 b-b                       | 238-476 a-a        |
| 1K             | nd                  | 1.1 d                            | 158 b              |
|                |                     | <b>ANOVA<sup>a</sup></b>         |                    |
| <b>Group 2</b> | ***                 | ***                              | ***                |
| 2A             | 56.7 d <sup>b</sup> | 14.7 a                           | 3524 a             |
| 2B             | 226 b               | 18.0 a                           | 1226 b             |
| 2C             | 346 a               | 18.0 a                           | 864 c              |
| 2D             | 67.5-135 d-b        | 6.4-12.8 c-b                     | Traces             |
| 2E             | 104-156 c-b         | 4.0-6.0 c-c                      | Traces             |
| 2F             | 38.4 d              | 3.6 c                            | 215 d              |
| 2G             | nd                  | 1.0 d                            | 62.0 f             |
| 2H             | 40.2 d              | 3.3 c                            | 243 d              |
| 2I             | 84.7-169 c-b        | 5.0-10 c-b                       | 108-215 e-d        |
| 2J             | nd                  | 5.5 c                            | 245 d              |
| 2K             | nd                  | 2.0 d                            | 154 e              |

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

<sup>b</sup>  $V$  values (mean of 3 replications) followed by the same letter, within the same column, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test.

<sup>c</sup> nd, the estimate cannot be calculated because the contents were below the limit of quantification (LOQ).

$P < 0.001$ ), whereas no correlation with EA (for example, for the 1st group samples,  $R = 0.07$ ;  $P > 0.05$ ) was found. The results were in agreement with a previous study (Aloqbi and others 2016), where Pom juice and PC showed no significant differences in DPPH radical inhibition; thus implying that the Pom AOC was mainly due to the PC content.

If the set of all the studied samples is considered, Pearson's correlation coefficient showed that PC content was positively correlated ( $R = 0.94$  and  $0.88$ ;  $P < 0.001$ ) with DPPH and TPC values. TPC was highly correlated ( $R = 0.80$ ;  $P < 0.001$ ) with DPPH assay. FRAP and ABTS<sup>+</sup> values were correlated ( $P < 0.05$ ) with TPC, PC, and DPPH. No significant ( $P > 0.05$ ) correlation was observed among EA and any of the 3 AOC assays used in this study; this lack of correlation can be due to the degradation of EA and generation of others compounds with AOC during processing and storage conditions, and/or adulteration (Talcott and Lee 2002; Zhang and others 2009).

Daily intake of PC, EA, and total polyphenols according to recommended dose of each commercial PomP

Table 5 shows the estimation of the daily intakes of PC, EA, and TPC, calculated using the recommended dose labeled by each manufacturer of the different CPomP. In some cases, the recommended dose was not a single value but a range.

Regarding the daily PC intake, the values registered in 1st group ranged between not measurable values (1B, 1E, 1I, and 1K) and 458 mg/d in sample 1J. Among all samples belonging to the capsules and supplements group, the daily intake of 70%

of them was below 15 mg; the estimates for the rest of products were higher, and took the values 256, 150 to 310, and 229 to 458 mg/d in samples 1A, 1G, and 1J, respectively. However, the daily intake of 80% of the CPomP of 2nd group was  $> 15$  mg/d (range between 38.4 and 346 mg/d); the PC intake in the rest of samples of samples could not be estimated because their PC contents were below the limit of quantification.

The daily EA intake values of 1st group ranged from 0.2 to 0.4 up to 11 to 22 mg. Sample 1G had the highest value (11 to 22 mg/d depending on the recommended dose), followed by sample 1A (10.9 mg) and sample 1J (7.5–15 mg depending on the recommended dose). Finally, the EA values for the juices group ranged from 1.0 (1G) up to 18 mg/d (2B and 2C).

Regarding the intake of TP (total polyphenols) of capsules and supplements, the range was 7.0 to 476 mg GAE, with the highest value being found in the sample 1J (238 to 476 mg GAE, depending on the dose), followed by samples 1A and 1K (196 and 158 mg GAE, respectively). Nevertheless, the daily intake estimates of the samples 1G and 1I only reached trace levels. In all samples, especially in the case of sample 1K (product from Pom seeds), it was observed that the intake of TP was higher than those of PC and EA. Considering the intake of the 2nd group of TP, a very high difference among samples was observed (ranging between 62 and 3524 mg GAE; only the intake of trace levels were estimated for samples 2D and 2E). Similarly to what was observed in the 1st group of samples, the estimated daily intake of TPC was higher those of PC and EA.

## Conclusion

The quality of PomP can be affected by many factors during their formulation and industrial processing. The experimental data showed high variability. The main conclusion was that there is a need for labeling "standardization" of these products. The contents of the key compounds, PCs, EA, and the total polyphenolic compounds should be declared on product labels, as indicators of the potential health benefits, and should replace subjective functional descriptions (no health statements are still allowed in PomP in the EU), such as "SuperFoods" or "SuperAntioxidant." In most cases, the reality did not match the label declarations, which indicated indirectly (through images) and/or directly with declarations such as "source of antioxidants," "healthy product," or "high content of EA." This lack of correlation is certainly misinforming the potential consumers of these products.

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### **7.3 Publication 3**

#### **Publication 3 (Literal transcription):**

### **A Critical Overview of Labeling Information of Pomegranate Juice-Based Drinks: Phytochemicals Content and Health Claims**

**Cano-Lamadrid, M.,** Turkiewicz, I.P., Tkacz, K., Sánchez-Rodríguez, L., López-Lluch, D., Wojdyło, A., Sendra, E., and Carbonell-Barrachina, A.A.

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# A Critical Overview of Labeling Information of Pomegranate Juice-Based Drinks: Phytochemicals Content and Health Claims

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**Abstract:** Punicalagin is responsible for over 50% of the antioxidant activity of pomegranate, but ellagic acid (EA) and total polyphenol content (TPC) are also key parameters regarding pomegranate bioactivity. Many juices and other drinks based on pomegranate take advantage from the widespread healthy image of this fruit, whereas their real content of bioactive phytochemicals is low. For that reason, the objective of this study was to compare the labelling information with the real phytochemical contents (TPC, punicalagin [Pn] [isomers  $\alpha$ ,  $\beta$ ], and EA). Titratable acidity (TA), color density (CD), polymeric color (PC), and sensory profiles (trained panel) were assessed. The experimental ranges of TPC and Pn contents were 2.75 to 70.9 mg/g and traces to 3.18 mg/g, respectively. The percentage of pomegranate juice was highly correlated with the intensity of the pomegranate ID attribute ( $R = 0.85$ ;  $p < 0.001$ ), Pn content ( $R = 0.71$ ;  $p < 0.001$ ), and EA content ( $R = 0.36$ ;  $p < 0.001$ ). The experimental results showed a high variability in the content of bioactive compounds and the need to urge food companies to optimize processes and storage conditions. Although no health claim for pomegranate has been authorized so far at the European Union, significant mismatches among labelling and bioactive compounds content are misleading consumers.

**Keywords:** antioxidant capacity, health claims, labelling, *Punica granatum* L., punicalagin

**Practical Application:** Pomegranate juice and nectar producers need to evaluate the real content of bioactive Pn, EA, and TPC in their products to optimize formulation, heat treatment, packaging and storage conditions to guarantee high levels of bioactive compounds during shelf life. Producers' organizations may benefit from harmonizing Pom products labelling, so they may fulfill consumer expectations and may be ready if health claims are finally authorized for these products. The development of a new sensory quality marker will be an interesting option.

## Introduction

Pomegranate (Pom) has been considered as a superfruit since ancient civilizations such as Greeks and Egyptians (Aboelsoud, 2010; Sidhu & Zafar, 2012). It has been used as an ingredient in traditional medicine, especially Ayurvedic, for the treatment of diseases: disorders of the digestive system, bleeding and parasitic illness (Bhandari, 2012). Currently, pomegranate extract is a component of many drugs, dietary supplements and cosmetics, while in the food industry pomegranate fruits are used to prepare juices, nectars, soft drinks, concentrates, syrups, and jams (Les, Prieto, Arbonés-Mainar, Valero, & López, 2015). Spain is the

largest pomegranate producer in Europe, and its cultivation is mainly located in the Valencian Community (Costa & Melgarejo, 2000; Kaur & Malik, 2016). Currently, the pomegranate cultivation area in Spain is 2,791 ha, and the annual production of these fruits is 36,000 t (Palou, Rosales, Montesinos-Herrero, & Taberner, 2016).

The edible part of the pomegranate fruits, arils, contain 80% juice and 20% seeds, hence the main use of fruit processing is the production of juices and nectars. Moreover, pomegranate juice contains different types of biologically active compounds, such as hydrolyzed tannins (ellagitannins), condensed tannins, anthocyanins, and phenolic acids (gallic and ellagic) (Fernandes et al., 2017). Ellagitannins can be hydrolyzed to ellagic acid (EA) under certain chemical or microbiological processes (Sun, Xin, Men, Xu, & Tian, 2017). The main ellagitannin, punicalagin, has been found in the pomegranate peel, making up about 65% of the total amount of polyphenols in the fruit (Li, Li, Zhao, & Yu, 2009). As a result of punicalagin hydrolysis, punicallin may be formed, while both compounds may be further hydrolyzed to EA. Punicalagin, punicallin, and EA have antioxidative, anti-inflammatory, hepatoprotective, antibacterial, and anticancer effects (Sun et al., 2017). Seeram et al. (2005) reported that punicalagin is responsible for more than 50% of the strong antioxidant capacity of the pomegranate juice. In fact, Seeram et al. (2008) stated that this juice has higher antioxidant activity than red wine, grape juice, açai juice, or blueberry juice.

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Consumers' awareness of the impact of food on health and well-being has been increasing in recent years. Therefore, they are looking for supplementing their poor diet with bioactive compounds. However, it should be emphasized that consumers are no longer interested in tablets or powders, but conventional food products that can enrich their daily diet with essential and healthy ingredients (Abountiolas & Nascimento Nunes, 2018). The health benefits of punicalagin have been not directly associated with their high levels of polyphenolic compounds due to the limited bioavailability of ellagitannins and EA. It has been suggested that potential health benefits rendered by these compounds *in vivo* are due to the activity of the intestinal microflora, which converts them into metabolites called urolithins (Saha et al., 2016).

Nowadays, among applicable regulation about fruit drinks in Spain (Asozumos, R1040/2014, D2001/112(CE), RD1518/2007, RD781/2013, D2012/12(UE), RD650/2011), the use of pomegranate ingredient is only regulated by D2012/12/UE (Codex General Standard for fruit juices and nectars CODEX-STAN 247-2005) in juices and nectars (minimum 25% pomegranate juice), while there is not specific regulation on pomegranate in the rest of juice drinks and/or soft drinks. Increasingly, consumers follow the labeling slogans on the packaging and/or advertisement, which have had to be approved previously by Spanish government (Nutriscore and AECOSAN) and European regulations (EFSA), and companies use words such as "antioxidant" or "polyphenols" to attract attention on their products. Very often those slogans do not correspond to reality because some beverages so labeled have either no or minimal levels of polyphenols (Abountiolas & Nascimento Nunes, 2018). As there is no established reference daily intake (RDI) for polyphenols, their presence in food and beverages cannot be used to claim antioxidant activity and reference values cannot be indicated in the label. In fact, only nutrients with proven healthy effects and a defined RDI are allowed to be highlighted in the label when a portion of the food provides at least 10% of the RDI (FDA, 2008).

Recently, González-Díaz, Gil-González, and Álvarez-Dardet (2018) clearly indicated the scientific requirements to include "antioxidants" as a health claim in the label: definite, identify, and validate functional biomarker of these compounds with a relationship with the mentioned claims, able to classify them as high level (EFSA) (BIOCLAIMS Project). Additionally, these concluded that the human studies must be intervention not only observation studies. Regarding European regulations, no health claim is yet authorized for "antioxidants" (68 opinions from EFSA), "punicallagins" (0 opinions), "EA" (3 EFSA opinions), "anthocyanins" (7 EFSA opinions), or "pomegranate" (12 EFSA opinions). Only for polyphenols, there is one authorized claim out of 18 opinions: the presence of polyphenols in olive oil for protection of LDL particles from oxidative damage. The reason for the negative opinion from EFSA is the noncompliance with the Regulation because on the basis of the scientific evidence assessed; this claimed effect for this food has not been substantiated. At present there is none authorized health claim related to pomegranate and its components within the European Union. However, any food in compliance with regulation 432/2012 may indicate health claims related to other components/nutrients present in food.

The aim of this study was to compare the phytochemical contents in pomegranate juice based drinks (Group 1: pure, 100 % pomegranate; Group 2: juices with different percentage of pomegranate juice) with their label information. Phytochemical content (total polyphenol content [TPC], punicalagin [Pn] content [isomers  $\alpha$ ,  $\beta$ , and total], and EA), titratable acidity (TA),

color density (CD), polymeric color (PC), and sensory profile (pomegranate-ID) were used as reference parameters.

## Materials and Methods

### Commercial pomegranate juice-based drinks

Samples were 100% pomegranate (Pom) juices and pomegranate juice based drinks ( $n=30$ ); in the first group pure (100%) Pom juices ( $n=12$ ) were included, while the second group consisted of blended juices containing pomegranate at different percentage ( $n=18$ ). Products were purchased at local stores and supermarkets from Alicante province. Three bottles for each product were bought and if available each bottle belonging to a different batch; thus, each value is the mean of three independent bottles/batches (Df = 2). Table 1 shows the list of samples, their codes, and the claims included on their labels. Samples were stored under refrigerated conditions at 4.2 °C for a maximum of 1 week after purchase.

### Physicochemical parameters

Total TA, TA (g citric acid/L) was determined by automatic titration (877 Titrino plus, Metrohm, Herisau, Switzerland) with 0.1 N NaOH up to pH 8.1, using 1 mL diluted juice in 25 mL distilled water. The CD and the percentage of PC were determined according to a previous literature (Giusti & Wrolstad, 2001). All analyses were run in triplicate.

### Phytochemical content and TPC

Methanol extract was prepared as follows: pomegranate juice based drinks (1 mL) were mixed with 10 mL of MeOH/water (80:20, v/v) + % HCl, sonicated at 20 °C for 15 min, and left for 24 hr at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged at 15,000  $g$  for 10 min. Punicalagin (Pn) (sum of  $\alpha$  and  $\beta$  isomers) and EA contents were determined using the method proposed by Cano-Lamadrid, Lipan, Cal in-Sa nchez, Hernández, and Carbonell-Barrachina (2017a).

TPC was quantified using Folin-Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventós, 1999). Absorption ( $\lambda = 765$  nm) was measured using a UV-Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France).

### Sensory analysis by trained panel

Eight trained panelists (30 to 55 years; four females and four males) with more than 500 hr of experience from the Agro-Food Technology Dept. (UMH) participated in this study. The panel was selected and trained following the ISO standard 8586-1 (1993), and it is specialized in descriptive sensory evaluation of pomegranate products (Cano-Lamadrid et al., 2018; Cano-Lamadrid et al., 2017b). The panel evaluated only one attribute, Pom ID flavor (a full-rounded pomegranate identity), which was used as a control parameter of organoleptic quality of the samples under study. Pom ID was defined as a part of the lexicon of pomegranate products: "The foundation of flavor notes that may be fruity, musty, earthy, sweet and/or sour, with an astringent mouthfeel." These aromatics under the definition "fruity" were a blend of fruits (grape, cranberries, blackberries) and vegetables (beets and carrots). As a reference for trained panel, fresh Pom juice cultivar Mollar de Elche: cultivar Wonderful (1:1) diluted with water in a ratio 1:2 (juice:water), fresh Pom juice diluted in a 1:1 juice:water ratio, and fresh Pom juice were used as reference materials for the intensities 3, 6, and 9, respectively. The sample serving and analysis procedure (using the suitable lexicon

**Table 1-List of pomegranate juices, codes, and main claims on the labels of pomegranate juices and pomegranate juice based drinks.**

| Sample   | % Pom | Ingredients   | Claims   | Organic |
|--|-------|---|--|---------|
| <b>Group 1: 100% pomegranate juice</b>         |       |   |  |         |
| 1A   | 100   | Squeezed pomegranate juice  | Source of potassium and with all polyphenols from pomegranate  | No      |
| 1B   | 100   | Pomegranate juice   | None   | Yes     |
| 1C   | 100   | 100% squeezed Elche Var. Mollar pomegranate juice   | None   | Yes     |
| 1D   | 100   | Pomegranate juice drink made from concentrated pomegranate juice and enriched with extract from the skin of the pomegranate in powder form, as well as pomegranate seed extract   | The result is a product with a high content of antioxidants, especially Punicalagins and OMEGA 5 fatty acids   | No      |
| 1E   | 100   | Organic pomegranate juice   | None   | Yes     |
| 1F   | 100   | Squeezed pomegranate juice  | None   | Yes     |
| 1G   | 100   | Pomegranate juice   | None   | Yes     |
| 1H   | 100   | 100% pomegranate Mollar juice   | None   | Yes     |
| 1I   | 100   | Pomegranate juice   | Contains natural antioxidants  | No      |
| 1J   | 100   | 100% pomegranate juice  | Natural antioxidant. A new way of consuming health   | No      |
| 1K   | 100   | 100% pomegranate juice  | None   | No      |
| 1L   | 100   | Pomegranate juice   | None   | No      |
| <b>Group 2: Pomegranate juice based drinks</b> |       |   |  |         |
| 2A   | 10    | Water, concentrated apple juice (12%), concentrated pomegranate juice (10%), sugar, apple purée (5%), concentrated lemon juice, concentrated elderberry juice, vitamin E  | With vitamin E antioxidant   | No      |
| 2B   | 50    | Pomegranate juice (50%) made partially from concentrate, water, sweeteners (sodium cyclamate, sodium saccharin)   | None   | No      |
| 2C   | 30    | Water, pomegranate juice squeezed (30.0%), concentrated lemon (0.2%), pomegranate aroma (0.2%)  | None   | No      |
| 2D   | 50    | Water, pomegranate (50%), panela, black currant, mangostan  | Drink with high antioxidant capacity providing 20,000 water-soluble ORAC units per intake (1 glass of 200 mL) to our diet, which is three times the units suggested by the Food and Drug Administration (FDA) to fight the effects of free radicals in our body through food | Yes     |
| 2E   | 15    | Water, pomegranate juice from concentrate (15%), red grape juice from concentrate (10%), white grape juice from concentrate (10%), acidifier: citric acid, sweetener: E-955, antioxidant: ascorbic acid and aromas  | None   | No      |
| 2F   | 27.5  | Infusion of white tea (0.1 g/L), pomegranate juice (27.5%), concentrated agave juice (7%), chokeberry juice (6%), pear puree (4%)   | None   | Yes     |
| 2G   | 30    | Pomegranate concentrate, water, sugar, citric acid: acidulant, ascorbic acid: antioxidant   | None   | No      |
| 2H   | 24    | Water, pomegranate juice (24%): from concentrate, goji juice (1%), acidity regulator: citric acid and sweetener: steviol glycosides   | None   | No      |
| 2I   | 70    | Pomegranate juice (70%) and red grape juice (30%)   | Contains natural antioxidants  | No      |
| 2J   | 50    | Pomegranate juice (50%), grape juice (50%)  | None   | No      |
| 2K   | 20    | Fruit juices partially from concentrates (60%) (apple and pomegranate 20%), water, sugar, acidity regulator: citric acid, colorant: anthocyanins, natural aroma   | None   | No      |
| 2L   | 2.5   | Squeezed red grape juice (92.5%), raspberry puree (2.5%), blackberry puree (2.5%), squeezed pomegranate juice (2.5%)  | None   | No      |
| 2M   | 22    | Water, multi fruit juice from concentrate (22% pomegranate, 4% apple, 3% chokeberry, 1% elderberry), sugar, citric acid; vitamin C, aroma   | One glass (1/4 L) covers your daily need of vitamin C  | No      |
| 2N   | 8     | Water, pomegranate juice (8%), raspberry puree, orange fibre, herbal extracts: rice, apple, cherry, radish, and hibiscus, citruline, hyaluronic acid, F&V MacroAntioxidants <sup>®</sup> (pomegranate peel extract), sea collagen (fish), pomegranate punicalagins extract, hydroxytyrosol (natural olive fruit extract), fruit pectin, selenium salt, vitamins C and E, sodium benzoate and potassium sorbate, citric acid, sucralose, flavors | Skin regenerating drink with MacroAntioxidants <sup>®</sup>  | No      |
| 2Ñ   | 55    | Juices of red grapes and Pom from concentrate (46%), water, glucose-fructose syrup, raspberry puree (4%), sugar, acidity regulator: citric acid, vitamin C  | Source of vitamin C  | No      |
| 2O   | 25    | Red grape juice (65%), pomegranate juice (25%), and red currant juice (10%)   | None   | No      |
| 2P   | 65    | Pomegranate juice (65%), apple, grape, and quince juice   | None   | No      |
| 2Q   | 30    | Pomegranate (30%) and apple (30%) juices, water, natural pomegranate extract of punicalagins, natural extract of rooibos, vitamin C, sweetener: sucralose   | None   | No      |

and reference products) were conducted according to (Vázquez-Araújo, Koppel, Chambers Iv, Adhikari, & Carbonell-Barrachina, 2011). A scale from 0 to 10, with 0.5 increments, was used, where 0 represented no intensity, and 10 represented extremely strong intensity.

### Statistical analysis

Data was subjected to analysis of variance (ANOVA) and later to Tukey's multiple-range test to compare the means. Differences were considered statistically significant at  $p < 0.05$ . All statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD, U.S.A.). In addition, the relationship among variables was determined using the Pearson correlation coefficient. Instrumental parameters correlated with sensory descriptors were used to conduct a Principal Component Analysis (PCA regression map) using XLSTAT Premium 2016 (Microsoft Corp., Redmond, WA, U.S.A.).

## Results and Discussion

### Chemical parameters

Regarding the TA of the pomegranate juices, it ranged from 1.02 (1A) to 6.76 g citric acid/L (1E), with significant statistical differences being found ( $p < 0.001$ ) (Table 2). Todaro et al. (2016) reported that Sicilian and Spanish pomegranates (sweet cultivars such as *Dente di cavallo*, *Violetto*, *Mollar de Elche*, *Valenciana*) presented TA from 1.3 to 2.5 g citric acid/L. Besides, there are high differences in TA between sweet and sour cultivars; for instance, *Mollar de Elche* juice has a mean value of 1.51 g citric acid/L while *Wonderful* juice had a TA value of 7.32 g citric acid/L (Cano-Lamadrid, Lipan, Calín-Sánchez, Hernández, & Carbonell-Barrachina, 2017a). Therefore, most of the studied samples were probably made from a blend of cultivars. In the second group, it was observed that TA ranged from 1.02 (sample 2C) up to 4.33 g citric acid/L (sample 2O). The addition of 10% of red currant juice may have caused an increase of TA in sample 2O.

Color is one of the most important attributes influencing consumer preference (Cano-Lamadrid et al., 2018), and its intensity depends on the copigmentation of anthocyanins (ACNs, responsible of Pom juice color) with copigments (for example, phenolic acids). CD values, which has a high correlation with catechin-phlorogucinol and monomeric ACNs ( $R = 0.866$ ) (Türkylmaz & Özkan, 2014), are shown in Table 1. When the polymerization degree is high, the CD value increases. The CD values for juices ranged from 1.74 (sample 1I) to 3.73 (sample 1H). Among them, juices from organic farming (1B, 1C, 1E, 1F, 1G, and 1H) presented higher CD values than other samples (1B and 1H), indicating that more brown compounds were found in the organic Pom juices available in the Spanish market. On the contrary, Crecente-Campo, Nunes-Damaceno, Romero-Rodríguez, and Vázquez-Odériz (2012) reported that strawberries from organic cultivation have a significantly higher content of individual anthocyanins than samples from conventional production. Thus, differences may be due not only to agricultural practices, but also to the Pom cultivar used (Cano-Lamadrid et al., 2017a), and processing and storage conditions. Alper, Bahçeci, and Acar (2005) studied the influence of processing and pasteurization on the color of Pom juices. They reported that both filtration and pasteurization increased the CD values; for instance, pasteurization increased values from 3.36 to 5.87. In a similar study, Ferrari, Maresca, and Ciccarone (2010) indicated that CD of Pom juices increased with increasing temperature, pressure, and pressing time during

**Table 2—Titratable acidity (TA), color density (CD) and polymeric color (PC) of pomegranate juice and pomegranate juice based drinks.**

| Sample                   | Titratable acidity, TA<br>(g citric acid L <sup>-1</sup> ) | Color density,<br>CD | Polymeric<br>color, PC (%) |
|--------------------------|--|----------------------|----------------------------|
| ANOVA (LSD) <sup>a</sup> |  |                      |                            |
| Group 1                  | ***<br>(0.10)  | ***<br>(0.08)        | ***<br>(2.1)               |
| 1A                       | 1.02 d <sup>b</sup>  | 3.06 ab              | 83.3 b                     |
| 1B                       | 2.16 c   | 2.36 b               | 94.4 a                     |
| 1C                       | 1.65 cd  | 2.84 ab              | 83.5 b                     |
| 1D                       | 2.29 c   | 2.23 b               | 96.1 a                     |
| 1E                       | 6.76 a   | 3.32 a               | 79.7 c                     |
| 1F                       | 2.93 bc  | 2.83 ab              | 81.1 bc                    |
| 1G                       | 3.56 b   | 2.57 b               | 64.4 d                     |
| 1H                       | 2.93 bc  | 3.73 a               | 76.7 c                     |
| 1I                       | 3.56 b   | 1.74 c               | 61.8 d                     |
| 1J                       | 6.24 a   | 2.33 b               | 46.5 f                     |
| 1K                       | 2.35 c   | 3.18 a               | 63.6 d                     |
| 1L                       | 3.82 b   | 3.33 a               | 54.3 e                     |
| ANOVA (LSD) <sup>a</sup> |  |                      |                            |
| Group 2                  | ***<br>(0.11)  | ***<br>(0.09)        | ***<br>(2.1)               |
| 2A                       | 1.27 c <sup>b</sup>  | 1.95 d               | 60.4 e                     |
| 2B                       | 1.40 c   | 1.74 d               | 90.3 a                     |
| 2C                       | 1.02 c   | 0.76 e               | 54.9 f                     |
| 2D                       | 3.56 b   | 3.58 b               | 82.1 b                     |
| 2E                       | 4.07 a   | 1.26 e               | 41.2 g                     |
| 2F                       | 1.86 c   | 2.25 cd              | 72.0 c                     |
| 2G                       | 3.31 b   | 2.10 d               | 83.1 b                     |
| 2H                       | 3.56 b   | 2.03 d               | 70.6 c                     |
| 2I                       | 4.08 a   | 1.63 d               | 66.8 d                     |
| 2J                       | 2.91 bc  | 2.86 c               | 71.3 c                     |
| 2K                       | 3.56 b   | 2.70 c               | 68.4 d                     |
| 2L                       | 3.44 b   | 3.81 b               | 63.8 de                    |
| 2M                       | 3.56 b   | 3.08 bc              | 65.0 d                     |
| 2N                       | 1.65 c   | 1.42 de              | 68.7 d                     |
| 2Ñ                       | 3.69 b   | 1.15 e               | 86.7 b                     |
| 2O                       | 4.33 a   | 2.64 c               | 72.2 c                     |
| 2P                       | 3.06 b   | 5.27 a               | 13.0 h                     |
| 2Q                       | 3.56 b   | 2.91 c               | 70.0 c                     |

<sup>a</sup>\*, \*\*, and \*\*\* significant at  $p < 0.05$ , 0.01, and 0.001, respectively, and LSD means lower significant difference.

<sup>b</sup>Values (mean of three replications) followed by the same letter, within the same column and group, were not significantly different ( $p > 0.05$ ), according to Tukey's least significant difference test.

processing. Regarding pomegranate juice based drinks, CD ranged from 0.76 (sample 2C) to 5.27 (sample 2P). It has been noticed that pomegranate juice based drinks including juices of grape, blackberry, raspberry, redcurrant, blackcurrant, chokeberry, and elderberry presented high values of CD, for instance 2P (5.27), 2L (3.81), 2D (3.38), and 2M (3.08). Abountiolas and Nascimento Nunes (2018) reported that anthocyanins constitute a significant part of the pool of polyphenolic compounds of berries; hence, there was a direct influence of the addition of anthocyanin-rich fruits on the CD values.

Regarding the percentage of PC (high values mean high degree of ACN polymerization, leading to less intense red color), the control value for fresh pomegranate juices should be less than 10%. In this study, as expected, the values of samples from Group 1 and Group 2 were significantly higher than that control value (ranges of 46.5 to 94.4% and 41.2 to 90.3%, respectively, except sample 2P, 13.0%). The variability among samples from the first group may be due to different factors, such as heat treatment

**Table 3—Contents of punicalagin ( $\alpha$ -,  $\beta$ -Pn, and sum), ellagic acid (EA), and total polyphenolic content (TPC) in the studied pomegranate juices and pomegranate juice based drinks.**

| Sample                   | $\alpha$ -Pn         | $\beta$ -Pn     | $\Sigma$ Pn   | EA          | TPC                  |
|--------------------------|----------------------|-----------------|---------------|-------------|----------------------|
|                          | (mg/mL)              |                 |               |             | (mmol gallic acid/L) |
| ANOVA (LSD) <sup>a</sup> |                      |                 |               |             |                      |
| Group 1                  | ***<br>(0.04)        | **<br>(0.01)    | ***<br>(0.05) | *<br>(0.01) | ***<br>(0.80)        |
| 1A                       | nd <sup>c</sup>      | nd              | nd            | nd          | 12.5cd               |
| 1B                       | 3.08 a <sup>b</sup>  | 0.68 a          | 3.76 a        | nd          | 15.4 c               |
| 1C                       | 1.58 bc              | 0.71 a          | 2.29 b        | nd          | 16.5 c               |
| 1D                       | 2.03 b               | 0.73 a          | 2.76 ab       | nd          | 17.4 c               |
| 1E                       | 2.08 b               | 0.69 a          | 2.77 ab       | nd          | 14.0 cd              |
| 1F                       | 1.19 c               | 0.77 a          | 1.96 bc       | 0.09 a      | 13.6 cd              |
| 1G                       | 1.21 c               | nd              | 1.21 c        | 0.02 a      | 9.0 e                |
| 1H                       | 1.61 bc              | 0.79 a          | 2.41 b        | nd          | 15.5 c               |
| 1I                       | 1.52 bc              | 0.67 a          | 2.18 b        | nd          | 11.4 d               |
| 1J                       | 0.97 c               | nd              | 0.97 c        | nd          | 66.7 a               |
| 1K                       | 1.76 bc              | 0.66 a          | 2.42 b        | 0.06 a      | 70.9 a               |
| 1L                       | 2.52 ab              | 0.66 a          | 3.18 a        | 0.05 a      | 60.8 b               |
| ANOVA (LSD) <sup>a</sup> |                      |                 |               |             |                      |
| Group 2                  | ***<br>(0.05)        | *<br>(0.02)     | ***<br>(0.07) | *<br>(0.01) | ***<br>(0.80)        |
| 2A                       | 0.66 cd <sup>b</sup> | nd <sup>c</sup> | 0.66 cd       | nd          | 3.04 f               |
| 2B                       | 1.39 b               | 0.63 a          | 2.02 a        | nd          | 46.4 b               |
| 2C                       | 0.90 c               | nd              | 0.90 c        | nd          | 17.1 d               |
| 2D                       | 2.09 a               | nd              | 2.09 a        | nd          | 10.4 rf              |
| 2E                       | 0.53 d               | nd              | 0.53 d        | nd          | 28.2 c               |
| 2F                       | 0.95 c               | nd              | 0.95 c        | nd          | 26.1 c               |
| 2G                       | 0.95 c               | nd              | 0.95 c        | nd          | 5.57 f               |
| 2H                       | 0.83 c               | nd              | 0.83 c        | nd          | 2.75 f               |
| 2I                       | 1.21 b               | 0.49 a          | 1.71 ab       | nd          | 11.0 e               |
| 2J                       | 1.65 b               | nd              | 1.65 b        | nd          | 7.65 ef              |
| 2K                       | 1.27 b               | nd              | 1.27 b        | nd          | 6.30 ef              |
| 2L                       | nd                   | nd              | nd            | nd          | 7.52 ef              |
| 2M                       | nd                   | nd              | nd            | nd          | 20.8 cd              |
| 2N                       | nd                   | nd              | nd            | nd          | 10.7 e               |
| 2Ñ                       | 0.84 c               | nd              | 0.84 c        | nd          | 4.48 f               |
| 2O                       | 0.76 c               | nd              | 0.76 c        | nd          | 7.73 e               |
| 2P                       | 0.97 c               | nd              | 0.97 c        | 0.04 a      | 54.4 a               |
| 2Q                       | 1.00b c              | nd              | 1.00 c        | nd          | 43.7 b               |

<sup>a</sup>\*, \*\*, and \*\*\* significant at  $p < 0.05$ ,  $0.01$ , and  $0.001$ , respectively, and LSD means lower significant difference.

<sup>b</sup>Values (mean of three replications) followed by the same letter, within the same column and group, were not significantly different ( $p > 0.05$ ), according to Tukey's least significant difference test.

<sup>c</sup>nd = below limit of quantification (LOQ).

and storage conditions. According to Cao et al. (2011), high PC values were observed in nonheat treated juices due to enzymatic browning catalyzed by oxidase and peroxidase. Also, Zou et al. (2016) proved significant increase in the value of PC of mulberry juices subjected to HTST (high temperature short time) treatment, which may be due to the condensation of ACNs with other ACNs and/or other condensed with tannins during thermal processing. Moreover, condensation reactions of ACNs with protein, phenolic acids or other phenolic compounds during refrigerated storage can also lead to increased PC values (Rein, Ollilainen, Vahermo, Yli-Kauhaluoma, & Heinonen, 2005).

**Phytochemical parameters and total polyphenolic content.** The content of punicalagin, Pn [ $\alpha$ ,  $\beta$  isomers, and total ( $\alpha$ - $\beta$ )] and EA, in all samples were expressed in mg/mL and their values are listed in Table 3. Regarding the juice Pn, values ranged from nondetectable (below limit of quantification) to

3.76 mg/mL ( $p < 0.001$ ). The highest Pn content was found in sample 1B, followed by 1L with 3.18 mg/mL. On the other hand, samples 1J and 1G presented the lowest detected value (0.97 and 1.21 mg/mL, respectively). It is worth mentioning that among 100% pomegranate juices, an unexpected result was found in sample 1A, presenting nondetectable contents of Pn, which may have been due to processing and/or storage conditions (Mena, Martí, Saura, Valero, & García-Viguera, 2013). Considering the content of  $\alpha$ - and  $\beta$ -Pn, the  $\alpha$ -Pn isomer was the predominant one in the juices, representing  $>60\%$  of the total Pn content. Contrary, Qu, Breksa, Pan, and Ma (2012) reported higher values of  $\beta$ -Pn in commercial 100% Pom juices, with values being 0.1 to 0.24 mg  $\alpha$ -Pn/mL and 0.12 to 0.28  $\beta$ -Pn mg/mL. Besides, Akhavan, Barzegar, Weidlich, and Zimmermann (2015) reported also the same trend, from 0.02 to 0.29 mg/mL and 0.13 to 0.88 mg/mL, for  $\alpha$ -Pn and  $\beta$ -Pn, respectively.

Several factors affect Pn content and variability, and have been identified in previous studies, such as the effect of cultivar (Sánchez-Salcedo, Mena, García-Viguera, Martínez, & Hernández, 2015), the part of Pom fruit and tree (Tzulker et al., 2007), juice processing conditions (Akhavan et al., 2015) and farming practices (Cano-Lamadrid et al., 2016). For instance, Pn content was maximum in Pom peel (Tzulker et al., 2007), being the reason why commercial Pom juices obtained by pressing the whole fruit have higher Pn content than commercial Pom juices from only arils (Akhavan et al., 2015; Tezcan, Gültekin-Özgüven, Diken, Özçelik, & Erim, 2009). Fifty percent of the juices were organic (1B, 1C, 1E, 1F, 1G, and 1H), with sample 1B having the highest total Pn content (3.76 mg/mL). On the other hand, the samples 1A and 1J came from conventional agricultural practices, and were those having the lowest total Pn content (no detected and 0.97 mg/mL), respectively. To summarize results, the total content of Pn in commercial juices was slightly higher in organic Pom juices than in the conventional ones. Also, Borges, Mullen, and Crozier (2010) reported higher Pn content in Pom juices from organic farming (mean value 2.11 mg/mL) than in conventional farming (from 0.36 to 1.11 mg/mL). However in a recent study, Cano-Lamadrid et al. (2016) observed that the Pn content in organic Pom Spanish juices was approximately half the content of the conventional juices, presenting 0.10 and 0.20 mg/mL, respectively. Additionally, Nuncio-Jauregui, Cano-Lamadrid, Hernández, Carbonell-Barrachina, and Calín-Sánchez (2015) also reported conventional juices having higher Pn content than organic ones. Although differences in the content of biologically active compounds have been reported among organic and conventional juices, no relationship can be established with farming practices given that ellagitannin content depends on the combination of cultivar and climate conditions, variety, processing methods, and research techniques (Bunea et al., 2012).

In  $<100\%$  pomegranate juice based drinks, it was observed that Pn content ranged from nondetectable to 2.09 mg/mL (sample 2D), followed by 2B (2.02 mg/mL). The highest total Pn content was found with 50% Pom juice in its composition (2D, 2B, and 2J), 2.09, 2.02, and 1.62 mg/mL, respectively. Unexpectedly, pomegranate juice based drinks with Pom content over 50% (70%, 65%, and 55%) (2I, 2P, and 2Ñ) presented lower total Pn content than with 50% of Pom juice (1.71, 0.97 and 0.84 mg/mL, respectively). This agreed with another study (Borges et al., 2010) which indicated that in commercial juices the content of Pom juice was not directly correlated with high Pn content. Such inconsistency can be explained by the use of different qualities of Pom juices (for example, type of extraction) but also a wide

**Table 4–Pearson’s correlation coefficients (R) among the contents of different phytochemicals (TPC, punicalagin [Pn], and ellagic acid [EA]) and percentage of pomegranate juice (% Pom), sensory quality (Pom ID), titratable acidity (TA), color density (CD), and polymeric color (PC).**

|        | %Pom         | Pom-ID       | TA           | CD           | PC           | TPC          | Pn           | EA           |
|--------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| % Pom  | <b>1.000</b> |              |              |              |              |              |              |              |
| Pom-ID | 0.854***     | <b>1.000</b> |              |              |              |              |              |              |
| TA     | 0.075        | 0.082        | <b>1.000</b> |              |              |              |              |              |
| CD     | 0.208        | 0.256        | 0.187        | <b>1.000</b> |              |              |              |              |
| PC     | 0.241        | 0.321        | -0.220       | -0.246       | <b>1.000</b> |              |              |              |
| TPC    | 0.354        | 0.228        | 0.124        | 0.286        | 0.446**      | <b>1.000</b> |              |              |
| Pn     | 0.709***     | 0.706***     | 0.086        | 0.153        | 0.344        | 0.216        | <b>1.000</b> |              |
| EA     | 0.364**      | 0.425**      | -0.021       | 0.353        | -0.226       | 0.427**      | 0.270        | <b>1.000</b> |

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

range of complementary ingredients such as other fruit juices, sweeteners and preservatives. Concerning the Pn isomers in <100% pomegranate juice based drinks, the content of  $\beta$ -Pn isomer only reached measurable levels in two samples (2B and 2I), while the isomer  $\alpha$ -PC was the predominant one in all nectar samples. Recently, Borges et al. (2010) reported in that the  $\alpha$ -Pn and  $\beta$ -Pn contents ranged from nondetected to 0.03 mg/mL and to 0.06 mg/mL, respectively, in commercial Pom based drinks.

The content of EA in juices ( $p < 0.001$ ) ranged from nondetectable up to 0.09 mg/mL (sample 1F). In most samples, (1A, 1B, 1C, 1D, 1E, 1H, 1I, and 1J) the EA content was below 0.02 mg/mL. Concerning <100% pomegranate juice based drinks, EA was only detected in sample 2P (0.04 mg/mL). Several authors indicated that more than 50% of total phenolic compounds in Pom fruit are concentrated in the peel (Fawole, Makunga, & Opara, 2012). As mentioned above, the way to squeeze the fruits to obtain juice affects the polyphenolic content of juice. This is the reason why EA content can be below the limit of quantification in many of the studied products. Moreover, the storage conditions can also affect EA content as Pn can be hydrolyzed to EA under certain conditions, thus increasing its amount in certain Pom product (Sun et al., 2017); however, low levels of EA were found, indicating that the transformation of Pn into EA was not a dominant reaction.

High values of TPC were found in first group (100% juices), ranging from 9.0 mmol gallic acid/L (sample 1G) to 70.9 mmol gallic acid/L (sample 1K) (Table 3). Other authors reported values between 11.02 to 66.13 mmol gallic acid/L from arils and whole fruit juice (Tzulker et al., 2007). Also, Tezcan et al. (2009) found similar results (from 15.30 to 59.29 mmol gallic acid/L) in 100% commercial Pom juices from the Turkish market. Likewise, Borges et al. (2010) reported that TPC of European commercial Pom juice ranged between 10.3 and 20.7 mmol gallic acid/L, being lower by about 3.5-fold in comparison to the results of the current study.

The TPC of organic juices ranged from 9.0 to 16.5 mmol gallic acid/L, while the TPC levels in conventional juices were higher, from 11.4 to 70.9 mmol gallic acid/L (which was even fourfold more for sample 1K). On the contrary, Mena et al. (2011) reported lower values of TPC in conventional Pom juices (9.18 mmol gallic acid/L) than organic juices (13.8 mmol gallic acid/L). No significant differences of TPC values were found in organic and conventional commercial Pom juice when investigated by Nuncio-Jauregui et al. (2015).

TPC of <100% pomegranate juice based drinks ranged from 2.75 mmol gallic acid/L (sample 2H) to 54.4 mmol gallic acid/L (sample 2P), being the highest for the formulation containing 65% Pom, apple, grape, and quince. Previously, Abountiolas and Nascimento Nunes (2018) studied TPC in nectars based on Pom

and reported lower contents: from 5.88 to 18.81 mmol gallic acid/L. Borges et al. (2010) indicated that similar products with 25% of Pom juice and 5% of blueberry presented 13.3 mmol gallic acid/L. In the current study, TPC of pomegranate juice based drinks with 25% (2O) and 27.5% (2F) Pom juice presented 7.73 and 26.1 mmol gallic acid/L, respectively. Moreover, nectar containing 8% Pom juice had 3.8 mmol gallic acid/L, while in our study nectar with the same percentage of Pom juice corresponded to 10.7 mmol gallic acid/L (sample 2N). Among pomegranate juice based drinks, samples 2F (27.5%) and 2D (50%) from organic agricultural practices contained 10.4 and 26.1 mmol gallic acid/L, respectively. Whereas Abountiolas and Nascimento Nunes (2018) reported in similar studies lower quantities of TPC for organic nectars with Pom (from 8.82 to 10.87 mmol gallic acid/L). TPC content in commercial Pom products is highly variable and cannot be related to farming practices.

For a better understanding and summarizing the results it should be pointed that the presence of Pom in a juice or nectar does not assure the presence of the bioactive compounds typically found in pomegranate. Concisely, 8.3%, 25%, 50%, and 16.7% of the studied juices ( $n=12$ ) and 16.7%, 72%, 11.1%, and 0% of the studied pomegranate juice based drinks ( $n=18$ ) ranged from nd-0.5, >0.5-2, >2-3, and more than 3 mg Pn/mL, respectively. Regarding TPC, 8.3%, 66.7%, 0%, 0%, and 25% of the total of the juices and 44.4%, 22.2%, 16.6%, and 16.7% of the pomegranate juice based drinks ranged from nd-10, >10-20, >20-40, and more than 40 mmol gallic acid/L.

Table 1 includes the health claims indicated on the labels of the analyzed samples. As already mentioned there are no authorized health claims in the EU related to Pom, Pn, EA, anthocyanins, TPC, or antioxidants (EFSA, 2018). Overall, evaluated products do comply with European Regulations. Note that 20 out of the 32 evaluated labels do not mention nutrition nor health claims linked to pomegranate. 6 of them present mentions related to either pomegranate or antioxidant but not as a real health claim. The other six products contain claims substantiated under R432/2012 European Regulation (linked to potassium, vitamin C or E, among others). It needs to be taken into account that consumers are not aware of authorized nutrition and health claims and tend to take fruit properties for granted.

When labelling information is compared with the real content of bioactive substances in the Pom products results are sometimes inconsistent. For instance, the 1J juice which indicates “natural antioxidant. A new way of consuming health,” had the highest TPC (66.7 mmol gallic acid/L), but the lowest Pn content (0.97 mg/mL). Apart from that, “natural” is not appropriate due to nondefinition by EFSA. Similar claim “natural antioxidant” was present in the sample 1I, which had the lowest TPC

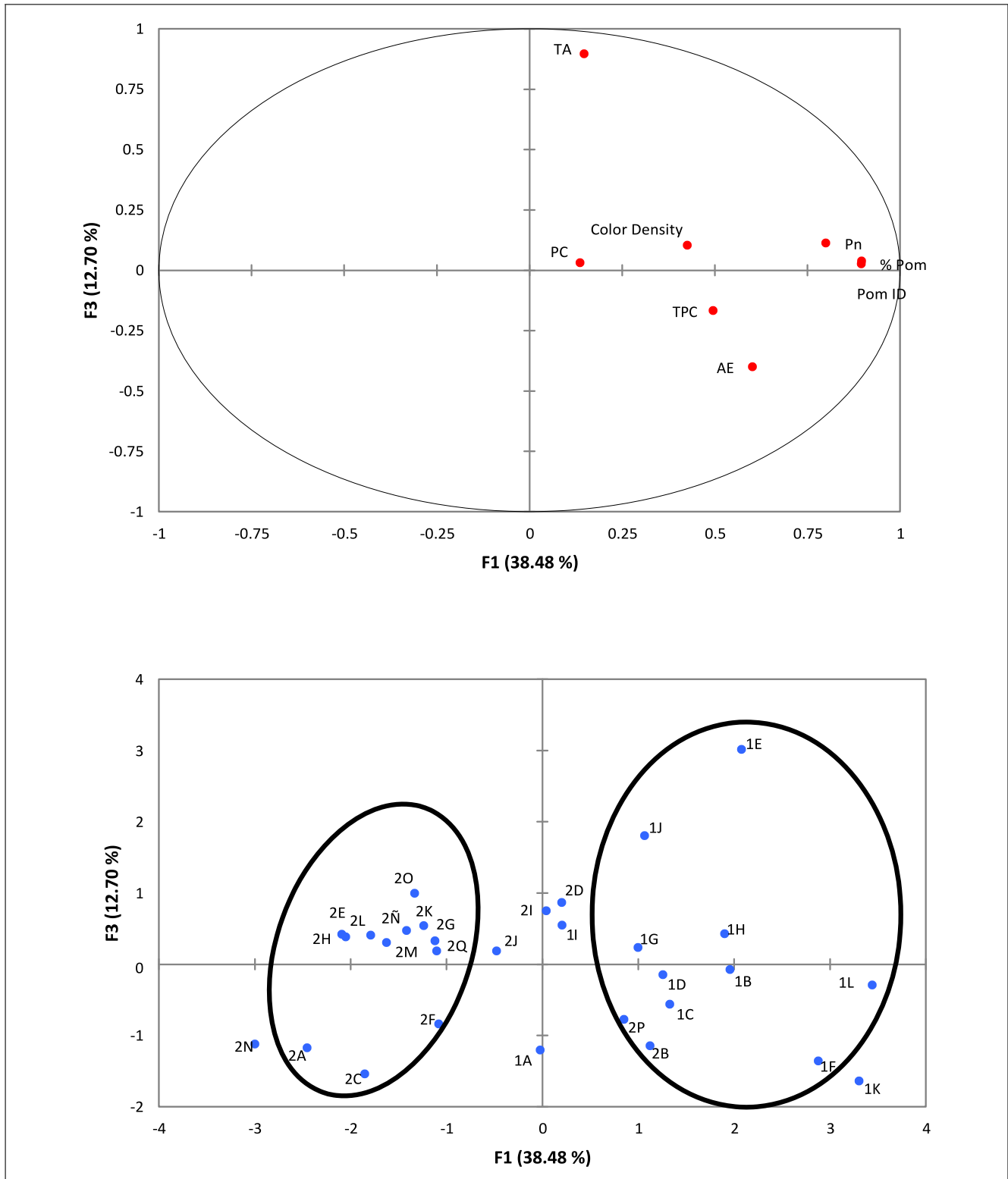


Figure 1—PCA map showing the relationships among chemical content (TPC, punicalagin [PC], and ellagic acid [EA]) and percentage of pomegranate juice (% Pom), sensory analysis (Pom ID), titratable acidity (TA), color density (CD), and polymeric color (PC) of pomegranate juice based drinks.

(11.4 mmol gallic acid/L). Moreover, juice 1D with suggested punicalagin content showed the highest Pn content (2.76 mg/mL); whereas sample 1A with Pn content below the limit of quantification claimed “with all polyphenols from Pom.” Among <100% pomegranate juice based drinks, 6 out of 18 mentioned properties

or nutritional and healthy claims; three of the samples (sample 2A, 2M, and 2N) contained nutritional claims related to vitamins C and E. The one indicating “high antioxidant capacity” had the highest Pn content, sample 2D (2.09 mg/mL). However, the nectar (sample 2I) with the largest percentage of Pom juice

(70%) presented low TPC (11.0 mg/mL) but a reasonable high Pn content (1.65 mg/mL). Finally, sample 2N with no detectable levels of Pn had a claim about presence of the Pom peel extract. This observation is strange given that the most valuable source of ellagitannins is Pom peel (Li et al., 2009). On the other hand, other authors (Nuncio-Ja'uregui et al., 2015) indicated that the effect of thermal treatment must take into account due to the fact that a temperature raise will increase Pn because of degradation of other ellagitannins. Aboutiolas and Nascimento Nunes (2018) tested 32 fruit juices from retail stores from United States. Seven juices contained Pom juice as their main ingredient, and four of them had health claims. Most of the statements concerned antioxidants as reported in seven of the present samples. It should be noted that current results were similar to that obtained by Aboutiolas and Nascimento Nunes (2018), Cano-Lamadrid et al. (2016), and Singh, Singh, Kaur, and Singh (2018), reporting that labelling information and formulation of some fruits drinks is not consistent with the levels of Pn and TPC.

**Pearson correlation and principal component analysis (PCA).** Table 4 shows the Pearson's correlation coefficients among variables. The Pom juice percentage was highly correlated with the Pom-ID sensory attribute ( $R=0.85$ ;  $p < 0.001$ ), Pn content ( $R=0.71$ ;  $p < 0.001$ ), and EA content ( $R=0.36$ ;  $p < 0.001$ ). Regarding the pomegranate-ID attribute, Laura Vázquez-Araújo, Chambers, Adhikari, and Carbonell-Barrachina (2010) also shown that consumers are more likely to choose drinks with the highest content of Pom juice. In agreement with previous data (Cano-Lamadrid et al., 2016), no correlation was noticed between TPC and Pn, confirming that Pn was not the only compound implied in the total polyphenolic content. On the other hand, TPC was positively correlated with PC as Türkylmaz, Tağı, Dereli, and Özkan (2013) reported; the total phenolic compounds of Pom juices significantly correlated with the PC ( $R=0.94$ ;  $p < 0.005$ ). Moreover, Pearson correlation showed that the correlation of TPC was affected by EA content, as mentioned in previous research (Borges et al., 2010; Cano-Lamadrid et al., 2017a). On the contrary, Todaro et al. (2016) stated that TPC did not correlate with EA content.

For an easy visualization of all the studied variables (Pn, % Pom, Pom-ID, PC, TPC, and CD) of the different pomegranate juice based drinks, a PCA was run for all samples ( $n=30$ ) (Figures 1A and 1B). The first two principal components explained 51.18% ( $F_1=38.48\%$  and  $F_2=12.70\%$ , respectively) of the total variation of the experimental data. Considering  $F_1$  as the dimension explaining the biggest percentage of differences among samples, here are some comments on the positioning of the samples as affected by the  $F_1$  axis. As previously mentioned, samples were positively correlated with the contents of EA, PC, CD and TPC, being specially associated with % Pom, Pom-ID, and Pn content in 100% Pom juices (first group), except sample 1A and 1I. Regarding <100% pomegranate juice based drinks (second group), samples were negatively linked with all analyzed variables, except sample 2B (50% Pom juice) and 2P (65% Pom juice) which presented a slight positive correlation with studied variables.

In conclusion, the pomegranate industry should focus on the selection of vegetal material, processing techniques and storage conditions to harmonize their products to guarantee the presence of bioactive compounds and so fulfill consumer expectations. It is also essential to better face the challenge of further studies to support new applications for health claims under European Regulations. The knowledge of phytochemical content is essential to

guarantee the quality of processed pomegranate products by the food industry, but small companies usually do not have access to expensive chromatographic equipments and techniques and so they should focus on good manufacturing practices to preserve bioactive compounds. The present study provides useful information to enhance the know-how on functional pomegranate juice based drinks and variability among commercial products. Also, the link among phytochemical content and sensory tools is clearly shown, suggesting that the development of a new sensory quality marker will be an interesting option.

## Conclusions

The present study showed high variability in bioactive compounds content among pomegranate juices based drinks available on the Spanish market and the fact that the presence of pomegranate in the ingredient list does not guarantee the presence of Pom-based bioactive compounds. Considering that consumers are not fully aware of EFSA opinions, the consumption of Pom juice is supported by the traditional healthy image of this fruit. However, pomegranate juice based drinks producers need to evaluate the real content of bioactive Pn, EA, and TPC in their products to optimize formulation, heat treatment, packaging and storage conditions to guarantee high levels of bioactive compounds during shelf life. In the present situation, studies aiming to evaluate the health properties and effects of pomegranate-based products are highly jeopardized by the high heterogeneity in the occurrence of their bioactive compounds. Producers' organizations (for example, DO Regulating Councils) may benefit from harmonizing Pom products labelling, so they may fulfill consumer expectations and may be ready if health claims are finally authorized for these products. Authors suggest a possible improvement of R(432/2012) reviewing the unregulated actions (antioxidants, antiaging) to improve labeling and avoid misleading or confusing the consumers (depending on labeling knowledge).

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## Author Contributions

Cano-Lamadrid, Turkiewicz, and Tkacz collected the physicochemical, sensory data, conducted part of the statistical analysis and manuscript writing; Sanchez-Rodríguez helped with phytochemical analysis and statistical analysis; Sendra and López-Lluch conducted part of the manuscript writing; Wodyło and Carbonell-Barrachina designed and coordinated the study.

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#### **7.4 Publication 4**

##### **Publication 4 (Literal transcription):**

**Influence of osmotic dehydration pre-treatment and combined drying method on physico-chemical and sensory properties of pomegranate arils, cultivar Mollar de Elche**

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# Influence of osmotic dehydration pre-treatment and combined drying method on physico-chemical and sensory properties of pomegranate arils, cultivar *Mollar de Elche*

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## abstract

### Keywords:

Convective drying

Vacuum-microwave drying

*Punica granatum* L.

Sensory analysis

Antioxidant capacity

Anthocyanins

“*Mollar de Elche*” is the most popular Spanish pomegranate cultivar (intense sweetness and easy-to-chew arils); however, arils have pale pink colour and flat sensory profile. “*Mollar the Elche*” arils first underwent an osmotic dehydration pre-treatment (OD) with concentrated juices: (i) chokeberry, (ii) apple, and/or (iii) pomegranate cultivar “*Wonderful*”, to improve their antioxidant capacity, colour, and sensory profile complexity, and later the arils were dried by a combined method (convective pre-drying + vacuum microwave finish drying). The use of OD provided dried arils with characteristic sweetness, and improved colour and aromatic complexity. The recommended OD methods were those using (i) pomegranate, and (ii) pomegranate with chokeberry juices; they improved the total anthocyanin content (mean of 368 mg kg<sup>-1</sup>), red colour ( $a^*$  coordinate 15.6), and antioxidant capacity (e.g. ABTS mean of 5.7 mmol Trolox 100 g<sup>-1</sup>). However, further research is still needed because freeze-dried arils had the highest anthocyanin content.

## 1. Introduction

Epidemiological studies concluded that high consumption of fruits and vegetables reduces the risk of chronic diseases (EUFIC, 2012). Among fruits, pomegranate (*Punica granatum* L.) and pomegranate-based products have been specifically associated with inhibition of prostate, breast, and lung cancer (Orgil, Spector, Holland, Mahajna, & Amir, 2016), reduction of dyslipidaemia, and cardiovascular issues (Haghighian et al., 2016), antioxidant stress effect (Orgil et al., 2016), and anti-diabetic properties.

Pomegranate owns its health-related properties to the unique composition of biologically active components, mainly polyphenols from the fruit peel (exterior rind) (Calín-Sánchez et al., 2015).

Pomegranates are usually available on the market as fresh fruits or as beverages, basically juices, concentrates or wine (Jaiswal, DerMarderosian, & Porter, 2010). In smaller amounts, they are available as an additive to jams, jellies, and are used for candy production (Tezcan, Gültekin-Özgülven, Diken, Özçelik, & Erim, 2009).

To prolong the arils shelf-life, different drying processes have been applied; however, they had a significant impact on the final products quality (Kingsly & Singh, 2007). Dried pomegranate arils are a great source of vitamins and minerals and are rich in biologically active components (Alaei & Amiri Chayjan, 2015). The main purpose for production of dried pomegranate arils is consumption of the arils as a nibbling snack.

Among Spanish pomegranates, the most popular cultivar is “*Mollar de Elche*”, which production is safeguarded by a Protected Designation of Origin (DOP) since 2016 [R (UE) 2016/83]. This cultivar is recognized worldwide, due to its high sweetness intensity and arils with soft woody portion. It has also disadvantages, such as pale pink colour that significantly decreases the quality of pomegranate-based products, especially the juice after the heat treatment. Besides, its sensory profile is too flat with a predominating sweetness and with very weak fruity notes (Vázquez-Araújo et al., 2014).

There are agronomic practices that can be used to improve the flavour of some fruits. Regulated deficit irrigation led to pomegranate fruits, cv. “*Mollar de Elche*” with a more complex sensory profile and enriched chemical composition (high punicalagin content); these fruits are called “*hydroSOStainable*” (Galindo et al., 2014).

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The appearance of commercial dried arils is mostly not acceptable for consumers, due to intense browning. A previous study (Calín-Sánchez et al., 2013) showed that high quality dried arils could be prepared using appropriate drying methods. It was indicated that the application of fruit or berry juices (e.g. chokeberry) during the osmotic dehydration (OD) step could improve the functionality, and colour of the dried products, by transferring bioactive and organoleptic-active compounds from the osmotic solution into the dehydrated pomegranate arils (Calín-Sánchez et al., 2013).

Considering all the above, the aim of this study was to evaluate the physico-chemical and sensory properties of the dried arils prepared using first osmotic dehydration (OD), with selected fruit juice concentrates, and later a combined drying technique [convective pre-drying (CPD) and vacuum-microwave finish drying (VMFD)] for dehydration of pomegranate arils cultivar “Mollar de Elche”. The drying kinetics, quality parameters (anthocyanin content, antioxidant capacity, colour, rehydration ratio), and descriptive sensory profile were studied.

## 2. Materials and methods

### 2.1. Material

Pomegranates [cultivars “Mollar de Elche” (used for preparation of dried arils) and “Wonderful” (used for preparation of osmotic solution)] were cultivated in a farm located in Murcia (Spain) under regulated deficit irrigation (RDI) (Galindo et al., 2014). Pomegranate fruits (~100 kg) were hand-harvested in mid-September 2015 at a commercial maturity stage (~15 °Brix), and immediately posted to Poland. The pomegranate fruits were submitted to short term storage (less than 2 weeks) at 5 °C at an approximate relative humidity (RH) of 90%, which are the optimal storage conditions for pomegranates (Elyatem & Kader, 1984).

Each fruit from *Punica granatum* L. “Mollar de Elche” was cut at the equatorial zone, and arils were manually separated. Arils were immediately used after its preparation. The main physico-chemical parameters of the fresh arils were: pH 7.8, titratable acidity (TA) 2.9 g L<sup>-1</sup>, total soluble solids (TSS) 15.2 °Brix, maturity index [TSS (°Brix)/TA (g per 100 mL)] 53.4, and moisture content 81.5%.

### 2.2. Drying processes

Freeze drying (FD) was carried out in freeze dryer OE-950 (Hungary) at a reduced pressure of 65 Pa for 24 h. The temperature within the drying chamber was 60 °C, while the heating plate was at -30 °C. The freeze-dried arils, without osmotic treatment, were considered as the control sample. Freeze-drying conditions for pomegranate arils were previously optimized by Calín-Sánchez et al. (2013).

The process of combined drying (CPD-VMFD) consisted of convective pre-drying (CPD) at temperature 60 °C for 2 h with an air velocity of 0.6 m s<sup>-1</sup> performed in the drier designed and built at the Institute of Agriculture Engineering (Wrocław, Poland), and followed by vacuum microwave finish drying (VMFD) with microwave power reduced from the initial 360 W–120 W to avoid overheating of the dried material. A similar combined procedure, CPD-VMFD, was also used during chokeberry drying (Calín-Sánchez et al., 2015).

The VMFD process of the CPD dried samples was performed in a SM 200 dryer (Plazmatronika, Wrocław, Poland) connected to a vacuum system consisting of a vacuum pump BL 30 P (Tepro, Koszalin, Poland), a vacuum gauge MP 211 (Elvac, Bobolice, Poland), and a compensation reservoir of 0.15 m<sup>3</sup>. A control sample of “Mollar de Elche” pomegranate arils dried by CPD-VMFD, with no

osmotic pre-treatment, was prepared using the above equipment for 240 min at 60 °C.

#### 2.2.1. Osmotic dehydration pre-treatment

Fresh “Mollar de Elche” arils were osmotically dehydrated at 45 °C for 90 min in different combinations and ratios of concentrated juices (40°Brix):

1. OD(POM): 100% pomegranate *Punica granatum* L., cv. “Wonderful”;
2. OD(POM + Ch): 50% pomegranate “Wonderful” and 50% chokeberry (*Aronia melanocarpa* L.);
3. OD(POM + A): 50% pomegranate “Wonderful” and 50% apple (*Pyrus malus* L.);
4. OD(A + Ch): 50% apple and 50% chokeberry; and
5. OD(AP + Ch): 75% apple and 25% chokeberry.

Chokeberry and apple concentrated juices (65 and 67 °Brix, and 22.2 and 5.0 g citric acid kg<sup>-1</sup>, respectively) were commercial products (Rauch Polska Sp z o.o., Płońsk, Poland). However, pomegranate concentrate was prepared under laboratory conditions by pressing pomegranate fruits, cv. “Wonderful” cut in halves, using a domestic citrus juicer (Braun, model CJ 3053, Barcelona, Spain) followed an evaporation step conducted in a vacuum evaporator (Rotavapor R 151, Donserv, Warsaw, Poland). The main quality parameters of this juice were: pH 3.5, total acidity 16.9 g L<sup>-1</sup>, total soluble solids 16.8 °Brix, and maturity index 10.1. Juices, after concentration to 40 °Brix, were immediately frozen until use. There were three main reasons for selecting these three fruit juices as osmotic solutions: (i) the three juices will improve the sensory quality of the dried pomegranate arils, and especially their appearance; (ii) chokeberry and apple juices are very abundant in Poland, and new applications must be sought; and, (iii) the pomegranate juice, cultivar “Wonderful” will lead to a final product which is 100%. The values of dynamic viscosity for the osmotic solutions 1–5, determined using viscometer SV-10 (A&D Co., Tokyo, Japan), were 5.13 ± 0.10, 4.13 ± 0.15, 4.76 ± 0.13, 4.20 ± 0.1 and 4.39 ± 0.12 mPa, respectively.

The ratio of osmotic solution to pomegranate arils was maintained at 200 mL to 100 g of fresh matter and the mixture was manually agitated every 5 min (Lech et al., 2015).

In previous studies using pomegranate arils, cultivar *Kandhari*, Mundada, Singh, and Maske (2010) used slightly different working conditions, 55 °Brix, 40 °C, 100 min, and fruit to solution ratio of 1:4 (w/w). However, the parameters of the osmotic dehydration step (time, temperature, and seed ratio) used in the current experiment were chosen on the basis of the results obtained in previous studies on the dehydration of similar matrices, such as chokeberries (Calín-Sánchez et al., 2015), beetroots (Lech et al., 2015), and sour cherries (Nowicka, Wojdyło, Lech, & Figiel, 2015b). These experimental conditions provided optimum mass exchange, with significant enrichment of the dried products but without significant changes in the chemical composition of the osmotic solutions which will limit their potential reuse in several osmotic steps.

After the OD treatment, the samples were removed from the osmotic solutions using a tea strainer, and were left in the strainer for ~20 min; then, the excess of osmotic solution was gently removed from their outer surface with absorbent paper.

### 2.3. Modelling of drying kinetics

The pomegranate arils drying kinetics was evaluated on the basis of the mass loss of the arils. During CPD, the samples were weighted every 5 min for the initial 20 min and the time intervals between the mass measurements were successively extended with the drying time. During VMFD samples were weighted at time 3, and every 8 min thereafter. The drying kinetics represents de

ing of moisture ratio  $MR$  in time of drying.  $MR$  is defined according to Eq. (1):

$$MR = \frac{M - M_0}{M_0} \quad (1)$$

Preliminary tests conducted in this study proved that the best fitting was obtained for the modified Page model (as given by Eq. (2)); consequently, only this model was used in this study: where  $A$ ,  $n$  and  $k$  are constants.

$$MR = A \cdot e^{-k \cdot t^n} \quad (2)$$

The good-fitting of a specific model to the experimental data was evaluated using: (i) coefficient of determination ( $R^2$ ) and (ii) Root Mean Square Error ( $RMSE$ ). The model fit is better, if the value of  $R^2$  is closer to 1.0 and the  $RMSE$  value is closer to 0.

#### 2.4. Extraction and LC-PDA/MS analysis of anthocyanins

The pomegranate extract of polyphenols was prepared as described previously (Wojdyło, Nowicka, Carbonell-Barrachina, & Hernández, 2016). Identification and quantification of polyphenols in all samples were carried out using an ACQUITY Ultra Performance LC™ system equipped with PDA (photodiode detector; UPLC™) with binary solvent manager (Waters Corporation, Milford, USA) series coupled with the mass detector G2 QToF Micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in positive mode. Separations of polyphenols were carried out using an Aquity BEH C18 column (1.7  $\mu$ m, 2.1  $\times$  100 mm, Waters Corporation, Milford, USA) at 30 °C using conditions previously reported by Wojdyło et al. (2016). The PDA spectra were measured over the wavelength range of 200–600 nm in steps of 2 nm. The runs were monitored at 520 nm for anthocyanins. Retention times and spectra were compared with those of pure standards. Calibration curves at concentrations ranging from 0.05 to 5.00 mg mL<sup>-1</sup> ( $R^2 \geq 0.9998$ ) were made using cyanidin-3-glucoside, cyanidin-3,5-diglucoside, pelargonidin-3-glucoside from Extrasynthese (Lyon, France). The results were expressed as mg per kg dry matter (dm).

#### 2.5. Antioxidant capacity (TEAC ABTS<sup>+</sup> and FRAP)

Approximately 1 g of dried pomegranate arils in 10 mL of 30% aqueous methanol (v/v) was sonicated for 15 min. After being kept for 24 h at 4 °C in the dark, the extracts were centrifuged (1500g, 10 min, 4 °C). The antioxidant capacity of the extracts was examined using the Trolox Equivalent Antioxidant Capacity test (TEAC ABTS<sup>+</sup>) according to Re et al. (1999). The ferric reducing ability was determined by FRAP assay (Benzie & Strain, 1999). Results were presented as mmol Trolox 100 g<sup>-1</sup> dry weight, dw ( $\pm$  standard deviation).

#### 2.6. Colour measurement

Colour coordinates  $L^*$ ,  $a^*$  and  $b^*$  were evaluated using a Minolta Chroma Meter CR-200 Reflectance System (Osaka, Japan), and colour difference (DE) was calculated by Eq. (3):

$$DE = \sqrt{\frac{1}{3} \left[ (L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2 \right]} \quad (3)$$

The DE indicates the degree of total colour change in comparison to the colour of fresh pomegranate arils, cv. "Mollar de Elche" ( $L^* = 33.13$ ,  $a^* = 18.40$ , and  $b^* = 13.12$ ). Low DE values represent high similarity to the ideal colour of fresh arils, and a good performance of the drying method (Dak, Sagar, & Jha, 2014). This analysis was conducted in 5 replications.

#### 2.7. Moisture content, water loss and solid gain and rehydration ratio

Moisture content (MC), water loss (WL) and solid gain (SG) were determined according to Nowicka, Wojdyło, Lech, and Figiel (2015a) and Bchir, Besbes, Karoui, Attia, et al. (2012). Rehydration of pre-osmotic dehydrated dried pomegranate arils was performed using 15 mL of distilled water during 1 h at room temperature. The rehydration ratio (RR) was calculated as using Eq. (4).

$$RR = \frac{m_1}{m_0} \quad (4)$$

where,  $m_1$  is the mass of the rehydrated arils (g), and  $m_0$  is the mass of the dried arils (g). These analyses were conducted in 5 replications.

#### 2.8. Conductivity

The conductivity of the rehydration liquid (distilled water conductivity = 6 mS cm<sup>-1</sup>) was measured with the EC-Meter GLP 31 (Crison Instruments S.A., Barcelona, Spain) to evaluate the loss of electrolytes at the end of rehydration step.

#### 2.9. Descriptive sensory evaluation

Eight highly trained panellists, (aged 25–50 years; 4 females and 4 males) from the department of Agro-Food Technology (UMH, Orihuela, Spain) participated in the study (Meilgaard, Civille, & Carr, 2007). The panel was selected and trained following the ISO standard 8586-1 (1993), and it is specialized in descriptive sensory evaluation of fruits and vegetables, including pomegranate products (e.g. Szychowski et al., 2015). For the current study, the panellists received two orientation sessions of 60 min, on fresh and dried pomegranate arils. The following attributes were chosen on the basis of the lexicon by Vázquez-Araújo et al. (2014): (appearance) colour and uniformity; (basic tastes and chemical feelings) sweetness, sourness, bitterness and astringency; (flavour) pomegranate ID, chokeberry ID, apple ID, fruity, caramel, citric, off-flavours, woody and burnt; and, (texture) crispiness, adhesiveness, and solubility in saliva. The panel used a numerical scale for quantifying the intensity of the pomegranate products attributes where 0 represents none and 10 extremely strong with 0.5 increments.

Samples (~4 g of dehydrated arils) were served monadically in a randomized order and coded using 3 digit numbers. Unsalted crackers and distillate water were provided to panellists to clean their palates between samples.

#### 2.10. Statistical analysis

All experiments and analyses were run, at least, in triplicate, and data reported are presented as the mean  $\pm$  standard deviation. All data were subjected to analysis of variance (ANOVA) test and later to Tukey's multiple range test to determine significant differences among treatments at  $p < 0.05$ . The statistical analyses were done using Statgraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD, USA). Table Curve 2D Windows v. 2.03 enabled mathematical modelling with the best determination coefficient.

### 3. Results and discussion

#### 3.1. Moisture content (MC), water loss (WL), solid gain (SG), and drying kinetics

The osmotic dehydration in different fruit solutions reduced MC of "Mollar de Elche" arils from an initial  $81.5 \pm 0.7\%$  down to an average value of  $72.1 \pm 0.4\%$  (Table 1). The WL was not affected

by the nature of the osmotic solution; it reached a mean of  $30.2 \pm 0.6\%$ , and ranged from 28.9% and 30.7% (Table 1). Similarly to previous studies (Lech et al., 2015), a mean increase of  $19.2 \pm 1.6\%$  in SG was observed after osmotic pre-treatment, with the OD treatment using pomegranate juice cultivar “Wonderful” leading to the highest SG increase (23.3%). The SG reached the lowest value when chokeberry was used in the osmotic solution; this observation was connected with the high particle size of this juice and the accumulation of solids near the surface, causing compaction of the surface layers and increased mass transfer resistance for both water and solids (Bchir, Besbes, Karoui, Paquot, et al., 2012; Kulling & Rawel, 2008). A significant ( $p < 0.05$ ) positive correlation ( $R^2 = 0.789$ ) was observed between dynamic viscosity and SG; the higher the viscosity, the higher the adhesive forces maintaining adsorbed solids on the surface and inner capillaries of the pomegranate arils (Juszczak, Witczak, & Galkowska, 2009).

The modified Page model has been already successfully used to describe the drying kinetics of pomegranate arils cultivar *Hicaz* (Horuz & Maskan, 2015; Kingsly & Singh, 2007) and cultivar *Kandhari* (Mundada, Hathan, & Maske, 2011). In the current study, the mean square error (MSE) ranged between  $4.2 \times 10^{-3}$  and  $7.0 \times 10^{-3}$  for CPD and  $5.0 \times 10^{-4}$  and  $1.9 \times 10^{-3}$  for VMFD, with the coefficient of determination ( $R^2$ ) being above 0.9822 (Table 2). These values (high  $R^2$  and low MSE) proved the good agreement between the thin layer modelling equation and the experimental data.

The MR at the very beginning of CPD was influenced by the composition of the juice used for the osmotic dehydration (Fig. 2a).

The constant  $A$  ranged from 0.556 and 1.00 (Table 2), with lowest  $A$  values being indicative of high WL. Similarly, the VMFD step was affected by the WL during CPD and, thus, the moisture content of the samples after CPD. The values of  $A$  indicated the MR of samples dehydrated by combination of OD-CPD at the initial time of the VMFD step (Fig. 2b). The constant  $A$  and the drying time followed a positive relationship, with lower  $A$  values leading to shorter drying times due to an increased WL. The OD and CPD steps had fixed times, 90 and 240 min, respectively (Table 2). Thus, the final drying time depended basically on the VMFD time. The longest VMFD time was found for arils that were not OD, as they contained higher moisture content to be removed as compared to samples that lost some water during OD. The constant  $A$  took the lowest value during VMFD (Table 2) when only pomegranate concentrated juice was used, influencing the final time of dehydration and making it the shortest one. In this case, i.e. POM, POM + Ch and POM + A, the final drying time was 2.4 times shorter than in the control CD-VMFD sample (Fig. 2b).

In this way, the composition of the osmotic solution affected the final drying time of samples, and the application of pomegranate juice in OD solutions reduced the final drying time of arils in contrast to apple juice which hindered the process of CD-VMFD. It is worth noting, that enriching pomegranate arils with solids originated from pomegranates increased the drying rate during CD-VMFD by increasing water diffusivity, which on other hand was decreased by gaining of solids from apple concentrate. These trends can be explained in terms of physicochemical mechanisms

Table 1  
Moisture content (MC, g/100 g fresh weight, fw), water lost (WL, g/100 g fw), solid gain (SG, g/100 g fw), rehydration ratio (RR), and rehydration solution conductivity ( $\sigma$ ) of pomegranate arils before and after osmotic dehydration (OD) in different fruit juices.

| Samples <sup>†</sup>  | Before OD        |      | After OD |                 |                  | Rehydration |                        |
|-----------------------|------------------|------|----------|-----------------|------------------|-------------|------------------------|
|                       | MC               |      | MC       | WL <sup>‡</sup> | SG <sup>‡‡</sup> | RR          | P                      |
|                       | (g per 100 g fw) |      |          |                 |                  |             | (mS cm <sup>-1</sup> ) |
| FD                    | –                | –    | –        | –               | –                | 1.90 c      | 263 b                  |
| CPD-VMFD              | –                | –    | –        | –               | –                | 3.14 a      | 200 e                  |
| OD(POM)-CPD-VMFD      | 81.5             | 71.4 | 30.2 a   | 23.3 a          | 2.95 ab          | 284 a       |                        |
| OD(POM + Ch)-CPD-VMFD | 81.5             | 73.1 | 28.9 a   | 15.3 c          | 3.00 a           | 250 c       |                        |
| OD(POM + A)-CPD-VMFD  | 81.5             | 71.0 | 32.1 a   | 22.2 a          | 1.07 d           | 229 de      |                        |
| OD(A + Ch)-CPD-VMFD   | 81.5             | 72.4 | 29.3 a   | 18.9 b          | 2.80 b           | 245 d       |                        |
| OD(AP + Ch)-CPD-VMFD  | 81.5             | 72.4 | 30.7 a   | 16.4 c          | 2.85 b           | 244 d       |                        |

<sup>†</sup>Mean values followed by the same letter, within the same column, were not significantly different ( $p < 0.05$ ), according to HSD Tukey’s least significant difference test.

Table 2  
Values of the parameters  $A$ ,  $k$ , and  $n$  of the functions describing drying kinetics of “Mollar de Elche” pomegranate arils dried by combined method consisted of convective pre-drying (CPD) and microwave finish vacuum drying (VMFD).

| Drying method | Osmotic treatment <sup>†</sup> | Drying kinetics $MR \frac{1}{A} A \cdot e^{-k \cdot t^n}$ |         |          |         |        | Partial time (min) | Total time (min) |
|---------------|--------------------------------|---|---------|----------|---------|--------|--------------------|------------------|
|               |                                | $A$   | $K$     | $n$      | MSE     | $R^2$  |                    |                  |
| CPD           | None                           | 1.000 a   | 0.012 c | 0.949 b  | 0.0092  | 0.9990 | 240 a              | –                |
|               | POM                            | 0.573 c   | 0.110 b | 1.060 a  | 0.0069  | 0.9990 | 240 a              | –                |
|               | POM + Ch                       | 0.617 b   | 0.009 c | 1.080 a  | 0.0042  | 0.9996 | 240 a              | –                |
|               | POM + A                        | 0.556 c   | 0.009 c | 1.090 a  | 0.0045  | 0.9994 | 240 a              | –                |
|               | A + Ch                         | 0.602 b   | 0.179 a | 0.850 c  | 0.0062  | 0.9985 | 240 a              | –                |
|               | AP + Ch                        | 0.604 b   | 0.019 c | 0.880 c  | 0.0070  | 0.9983 | 240 a              | –                |
|               | VMFD                           | Without OD  | 0.124 a | 0.107 c  | 0.640 a | 0.0019 | 0.9963             | 83 a             |
| OD-POM        |                                | 0.026 d   | 0.049 d | 0.681 a  | 0.0005  | 0.9822 | 35 c               | 365 c            |
| OD(POM + Ch)  |                                | 0.029 d   | 0.345 a | 0.198 d  | 0.0006  | 0.9822 | 35 c               | 365 c            |
| OD(POM + A)   |                                | 0.030 d   | 0.218 b | 0.338 c  | 0.0006  | 0.9834 | 35 c               | 365 c            |
| OD(A + Ch)    |                                | 0.096 b   | 0.238 b | 0.519 b  | 0.0016  | 0.9956 | 51 b               | 381 b            |
| OD(AP + Ch)   |                                | 0.069 c   | 0.129 c | 0.602 ab | 0.0012  | 0.9945 | 59 b               | 389 b            |

<sup>†</sup> POM, Ch, A, and AP stand for pomegranate, chokeberry, apple (at 50% in mixture with 50% chokeberry), and apple (at 75% in mixture with 25% chokeberry), respectively.

<sup>‡</sup> Mean values followed by the same letter, within the same column and drying method, were not significantly different ( $p < 0.05$ ), according to HSD Tukey’s least significant difference test.

<sup>‡‡</sup> The OD step lasted 90 min for all treatments.

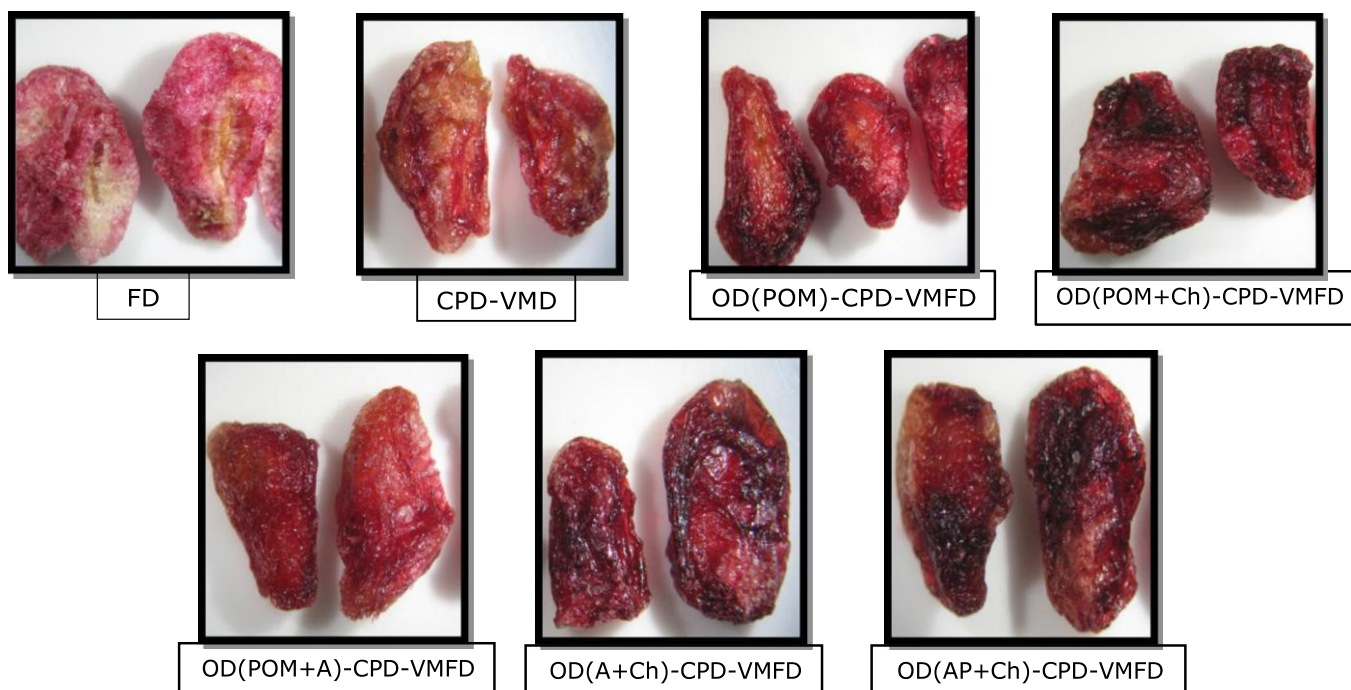


Fig. 1. “Mollar de Elche” pomegranate arils dried by freeze drying (FD), combined drying (CPD-VMFD), and osmotic dehydration (OD) before combined drying method (CPD-VMFD).

of water binding and changes in the cellular structure of pomegranate arils caused by solids gain. The sugars present in apple juice hinder water transport to a higher extent by clogging the pores of the dried material (Lech et al., 2015) and increasing mass transfer resistance for water (Bchir, Besbes, Karoui, Paquot, et al., 2012), which contributed to the rise in internal pressure under microwave heating associated with less intensive water evaporation confirmed by the highest maximal temperature during VMFD (Fig. 2c). The cooling effect resulting from intensive water evaporation (Figiel, 2010) decreased the maximal temperature of pomegranate arils (Fig. 2c) which were characterised by the highest moisture content at the very beginning of VMFD (Fig. 2b). The course of temperature of pomegranate arils pre-treated in different osmotic solutions shown in Fig. 2c resulted from the thermal balance between the energy generated by water dipoles inside the microwaved material and energy necessary for water evaporation to the ambient of lower temperature (Figiel, 2010).

Dried pomegranate arils might be consumed as ready-to-eat snacks (Kingsly, Singh, Manikantan, & Jain, 2006) or might be rehydrated before consumption. The *RR* (rehydration ratio) values ranged between 1.07 up to 3.14 (Table 1) and were similar to those previously found in other dried fruits (Megías-Pérez, Gamboa-Santos, Soria, Villamiel, & Montilla, 2014). The *RR* values were significantly affected by drying method and composition of the osmotic solution used for arils pre-treatment. The lowest *RR* (1.07) was found in OD(POM + A) arils, which was also characterised by the highest *WL*. Bchir, Besbes, Karoui, Paquot, et al. (2012) reported that the cells of OD pomegranate arils appeared shrunk and distorted due to solubilisation of polysaccharides that compose the cell walls, the intense *WL*, and the pre-concentration of sucrose on the surface of the tissue during the OD. Contrary to the previous research (Megías-Pérez et al., 2014), FD products had relatively low *RR* values (1.90) as it might result from the destruction of the outer layer structure of arils during freezing and freeze drying processes. As a result, the porous structure of outer layer allows the water for better incorporation into tissue during the rehydration process.

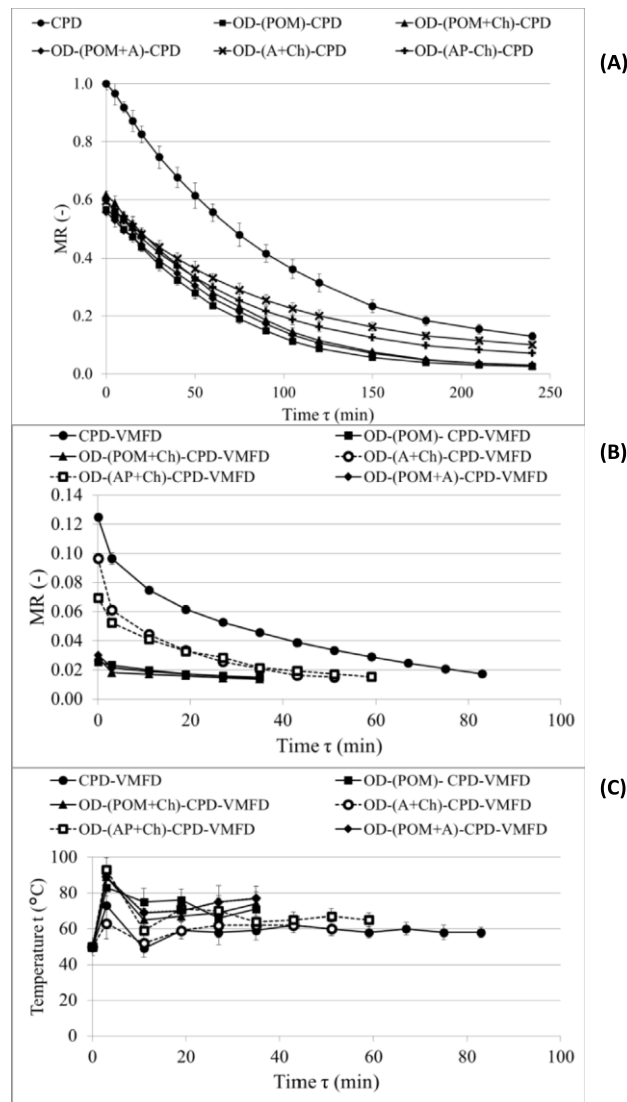
Finally, the electrical conductivity ( $\sigma$ ) is a measure of the soluble solids and electrolytes in the medium, which was indicative of the release of intercellular ions from sample tissue, due to an induced damage or creation of a porous structure in the material during drying. The values ranged between  $200 \pm 1$  and  $284 \pm 2$  mS  $\text{cm}^{-1}$  (FD sample, with porous structure), and were significantly higher than that of the water used for rehydration,  $6 \mu\text{S cm}^{-1}$  (Table 1). The OD pre-treatment increased the  $\sigma$  values as compared to the CPD-VMFD sample, because the solids retained during the OD were not irreversibly bonded to the aril matrix.

### 3.2. Anthocyanins

Initially, the anthocyanin profiles of the juices used for the osmotic dehydration consisted of the following compounds:

- Pomegranate juice: A1–A6 with contents being 79.2, 178, 72.2, 283, 75.3, and 5.9 mg  $100 \text{ mL}^{-1}$  of juice, respectively (total of 694 mg  $100 \text{ mL}^{-1}$ ).
- Chokeberry juice: A4 and A7 with contents being 28.2 and 1032 mg  $100 \text{ mL}^{-1}$ , respectively.
- Apple juice: A4 and A7 with 10.4 and 76.9 mg  $100 \text{ mL}^{-1}$ , respectively.

A total of 6 anthocyanins typical of pomegranate products were identified: delphinidin-3,5-diglucoside (A1), cyanidin-3,5-diglucoside (A2), delphinidin-3-glucoside (A3), cyanidin-3-glucoside (A4), pelargonidin-3-glucoside (A5), cyanidin-pentoside (A6), and another 4 anthocyanins not typical for pomegranate products, basically coming from the chokeberry juice used in the osmotic dehydration step were also found, and are quantified together as A7 (Table 3); the mass spectral characteristics and positive ions in LC-MS QToF of all anthocyanins are summarized in Table 1S (Supplementary material). These 4 chokeberry anthocyanins were identified as cyanidin-3-*O*-galactoside, -glucoside, -arabinoside, and -xyloside. All above mentioned compounds (A1–A5) were



**Legend:** POM, Ch, A, and AP stand for pomegranate, chokeberry, apple (at 50% in mixture with 50% chokeberry), and apple (at 75% in mixture with 25% chokeberry), respectively.

Fig. 2. Drying kinetics of “Mollar de Elche” pomegranate arils during the two steps of the combined drying method, CPD-VMFD: convective drying CPD (A); and, vacuum-microwave drying, VMFD (B); and, temperature profile during the VMFD step (C).

previously reported in various pomegranate products (Jaiswal et al., 2010; Mena, Martí, & García-Viguera, 2014; Trigueros, Wojdyło, & Sendra, 2014), except cyanidin-pentoside.

Among all samples analysed, FD led to the highest retention of total anthocyanins (Table 3), followed by CPD-VMFD. In comparison, a greater degradation of those constituents (even down to 61%) was noted when cabinet and sun drying was applied for dehydration of pomegranate arils (Jaiswal et al., 2010). In general, the application of an osmotic dehydration step led to a reduced anthocyanin content due to a migration of these compounds to the osmotic solutions, as previously reported by other researchers (Bchir, Besbes, Karoui, Attia, et al., 2012; Bchir, Besbes, Karoui, Paquot, et al., 2012). Among the OD treatments, the combination of “Wonderful” pomegranate and chokeberry juices (50%–50%; Pom + Ch) resulted in the highest content of total anthocyanins ( $441 \text{ mg kg}^{-1} \text{ dm}$ ) in dried arils and pomegranate juice ( $295 \text{ mg kg}^{-1}$ ). The high anthocyanin content of the first treatment could be due to high

contents of anthocyanins A7 from chokeberry. The use of apple juice with its high sugar content restricted the migration of A7 anthocyanins from the chokeberry juice to the dried arils.

The most abundant anthocyanin in all samples analysed was cyanidin-3,5-diglucoside (A2). On the other hand, chokeberry juice is a great source of polyphenolic compounds, with anthocyanins comprising between 25 and 50% of all polyphenol content (Oszmiański & Lachowicz, 2016). These constituents are natural food colouring agents that may significantly improve the colour of foods; these is the case of the samples OD(POM-Ch)-CPD-VMFD and OD(A-Ch)-CPD-VMFD. Previously, 7 anthocyanins (4 cyanidin glycosides: 3-galactoside, 3-glucoside, 3-arabinoside and 3-xyloside) were identified in chokeberry (Oszmiański & Lachowicz, 2016) among which six of them were detected in arils osmotically dehydrated in chokeberry juice, whereas none of them was identified in other pomegranate products. The OD-treatment including a “Wonderful” juice showed higher quantity of cyanidin-



**Table 3**  
Effect of drying conditions on anthocyanin content, antioxidant capacity and the colour of osmotic dehydrated pomegranate arils as affected by the drying method.

| Sample <sup>y</sup>   | Anthocyanins<br>(mg kg <sup>-1</sup> , dm) |         |         |         |          |        |        | Antioxidant capacity<br>(mmol Trolox 100 g <sup>-1</sup> ) |                   |        | Colour  |         |        |        |
|-----------------------|--|---------|---------|---------|----------|--------|--------|--|-------------------|--------|---------|---------|--------|--------|
|                       | A1   | A2      | A3      | A4      | A5       | A6     | A7     | Total A  | ABTS <sup>+</sup> | FRAP   | L*      | a*      | b*     | DE     |
| FD                    | 113 a                                      | 219 a   | 100 a   | 155 a   | 58.7 b   | 0.0 d  | 0.0 e  | 646 a  | 4.7 b             | 3.4 b  | 34.5 a  | 16.3 a  | 13.0 a | 2.5 c  |
| CPD-VMFD              | 41.8 bc                                    | 127.3 b | 49.8 b  | 62.3 cd | 77.3 a   | 0.0 d  | 0.0 e  | 358 c  | 3.6 d             | 2.7 d  | 24.3 b  | 15.2 ab | 11.2 a | 9.6 b  |
| OD(POM)-CPD-VMFD      | 48.8 b                                     | 102.2 c | 49.0 b  | 73.6 bc | 20.3 de  | 1.2 d  | 0.0 e  | 295 c  | 6.1 a             | 4.4 a  | 23.4 b  | 14.6 ab | 9.6 b  | 13.4 b |
| OD(POM + Ch)-CPD-VMFD | 19.5 de                                    | 94.3 cd | 37.7 bc | 92.0 b  | 36.3 cd  | 13.5 a | 147 a  | 441 b  | 5.3 b             | 4.0 a  | 21.5 c  | 16.6 a  | 6.8 bc | 21.8 a |
| OD(POM + A)-CPD-VMFD  | 31.1 cd                                    | 79.9 d  | 30.0 cd | 47.0 de | 16.0 e   | 0.0 d  | 8.5 d  | 213 d  | 4.4 bc            | 3.4 bc | 16.0 d  | 9.7 c   | 2.8 d  | 11.1 b |
| OD(A + Ch)-CPD-VMFD   | 10.4 e                                     | 51.0 e  | 33.3 bc | 31.1 e  | 38.7 c   | 6.5 b  | 63.2 b | 234 d  | 3.9 cd            | 3.1 c  | 13.9 e  | 7.8 d   | 2.8 d  | 24.3 a |
| OD(AP + Ch)-CPD-VMFD  | 11.6 e                                     | 56.2 e  | 14.0 d  | 27.9 e  | 23.3 cde | 4.5 bc | 39.4 c | 177 d  | 3.3 d             | 2.7 d  | 18.0 cd | 11.1 b  | 4.2 c  | 19.0 a |

<sup>y</sup> POM, Ch, A, and AP stand for pomegranate, chokeberry, apple (at 50% in mixture with 50% chokeberry), and apple (at 75% in mixture with 25% chokeberry), respectively.

<sup>z</sup> A1: delphinidin-3,5-diglucoside; A2: cyanidin-3,5-diglucoside; A3: delphinidin-3-glucoside; A4: cyanidin-3-glucoside; A5: pelargonidin-3-glucoside; A6: cyanidin pentoside; A7: non-pomegranate-typical anthocyanins.

<sup>†</sup> Mean values followed by the same letter, within the same column, were not significantly different ( $p < 0.05$ ), according to HSD Tukey's least significant difference test.

3-glucoside (A4) than CPD-VMFD (drying control without OD). These results agreed with previous studies showing that the amount of cyanidin-3-glucoside in “Wonderful” pomegranate variety juice was higher than in “Mollar de Elche” (Mena et al., 2014).

### 3.3. Antioxidant capacity (TEAC ABTS<sup>+</sup> and FRAP)

The ABTS<sup>+</sup> antioxidant capacity was affected by the osmotic dehydration pre-treatments as well as the drying methods (Table 3). The highest ABTS<sup>+</sup> values were found in the samples osmotically dehydrated in pomegranate juice and in pomegranate and chokeberry juice, and were 1.6- and 1.2-times higher than the values of FD and CPD-VMFD control samples, respectively. On the other hand, the lowest ABTS<sup>+</sup> value was found in samples osmotically treated with apple juice.

Similarly, the FRAP values of the same two treatments were 1.6- and 1.2-times higher than those of the control samples. Again, the lowest FRAP values were found in samples treated with apple juice.

The experimental results obtained demonstrated that an initial osmotic dehydration step with pomegranate and pomegranate/chokeberry concentrated juices can increase the antioxidant capacity of dried pomegranate arils. These results did not agree with results published in other fruits, such as dried sour cherry, in which the osmotic dehydration step led to lower antioxidant activities of dried products (Nowicka et al., 2015a). This decrease in the antioxidant capacity of dried sour cherries was justified by short drying time and polymerisation reactions (Yilmaz & Toledo, 2005), and could be also due leaching of natural solutes into the osmotic solutions (Bchir, Besbes, Karoui, Paquot, et al., 2012).

There were not significant correlations among the total anthocyanin content and the ABTS<sup>+</sup> or FRAP values; however, a positive and significant ( $p < 0.001$ ) correlation was found between ABTS<sup>+</sup> and FRAP, ( $R^2 = 0.977$ ). Thus, other compounds (not only anthocyanins) were responsible for the antioxidant capacity of dried pomegranate arils; it is well established that hydrolyzable tannins (punicalagins and punicalins) and phenolic acids (e.g. ellagic acid) are the key compounds in the antioxidant capacity of pomegranate fruits (Calín-Sánchez et al., 2013; Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000)

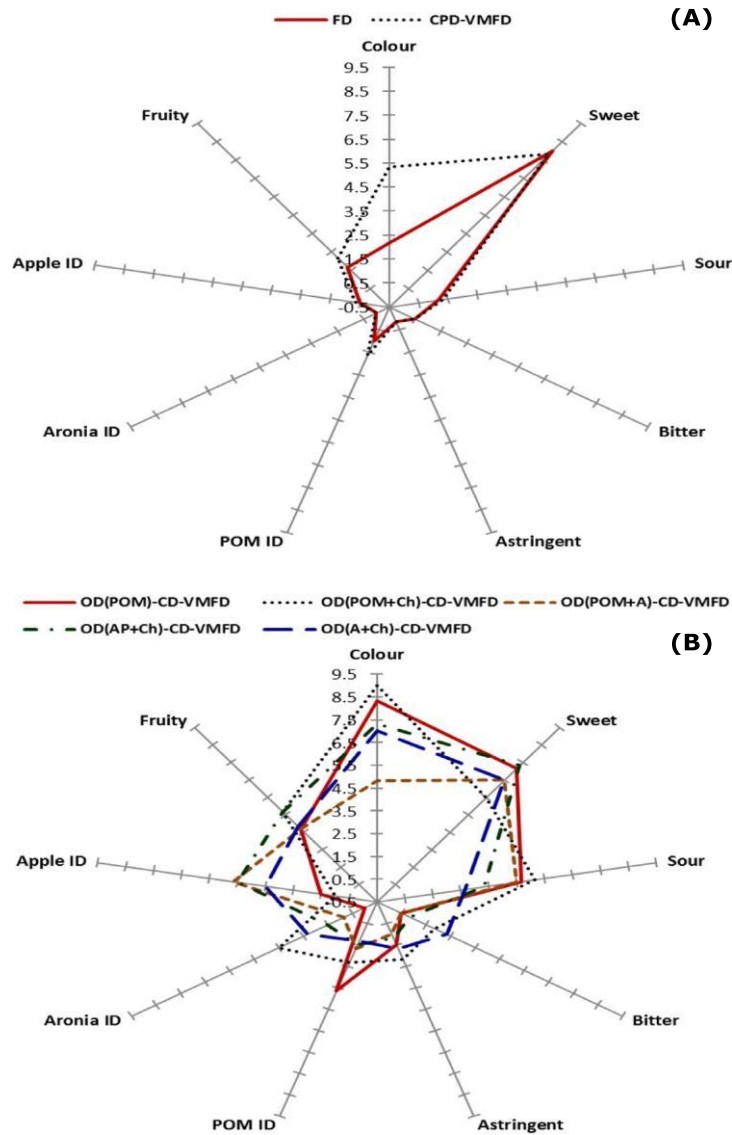
### 3.4. Colour

The dried pomegranate arils available in the market now are usually brownish and not attractive to consumers, due to degradation of arils anthocyanins (Maskan, Kaya, & Maskan, 2002).

The values of coordinate L\*, lightness, considerably decreased in all samples when compared to the freeze dried (FD) products (Table 3). Among the samples analysed, the darkest products (lowest L\* values) were found in the OD(POM + A), OD(A + Ch), and OD(AP + Ch) samples.

The level of red pigments, as described by the coordinate a\*, was affected by osmotic dehydration. The highest a\* values were found in the samples osmotically treated with pomegranate-chokeberry juices and pomegranate juice; their a\* values were statistically equivalent to those of the control samples (FD and CPD-VMFD). The high values of these two treatments (pomegranate-chokeberry and pomegranate juices) led to an equivalent red colour intensity to that previously reported in dried-pomegranate arils, cultivar “Hicaz” (<14.0) (Horuz & Maskan, 2015), which have a very intense red colour, which is lacking in the arils of the cultivar used in the current study “Mollar de Elche”.

The application of an osmotic dehydration step resulted in a significant decrease in the b\* values (Table 3), as compared to control samples. Osmotic dehydration in “Wonderful” pomegranate juice affected the b\* values to a lesser extent when compared to the rest of the analysed osmotic solutions.



**Legend:** POM, Ch, A, and AP stand for pomegranate, chokeberry, apple (at 50% in mixture with 50% chokeberry), and apple (at 75% in mixture with 25% chokeberry), respectively.

Fig. 3. Descriptive sensory analysis of dried “Mollar de Elche” pomegranate arils [control (A) and osmotic-dehydrated (B) samples].

The colour difference (DE) of samples ranged from 2.5 to 24.3 (Table 3). The lowest DE values were found in the control samples, followed by samples osmotically treated with pomegranate juice.

Pearson’s correlation coefficient showed that the total anthocyanin content was positively ( $p < 0.05$ ) correlated with  $L^*$ ,  $a^*$ , and  $b^*$  coordinates ( $R^2 = 0.81, 0.59, \text{ and } 0.62$ , respectively).

As a summary of this section, it can be stated that application of “Wonderful” pomegranate juice resulted in the best colour of the osmotically dried arils of “Mollar de Elche”, and that anthocyanins played an important role in the final colour of dried arils.

### 3.5. Descriptive analysis

The main objective of this study was to optimize the colour of the samples, because it is main attribute driving consumer acceptance.

The values of colour (the higher the value, the more intense reddish was the colour) obtained by the trained panel were: 2.2 (FD) < 4.8 [OD(POM-A)-CPD-VMFD] < 5.3 (CPD-VMFD) < 7.0 [OD(AP-Ch)-CPD-VMFD] < 7.3 [OD(A-Ch)-CPD-VMFD] < 8.3 [OD(POM)-CPD-VMFD] < 9.0 [OD(POM-Ch)-CPD-VMFD] (Fig. 3). The goodness of the appearance of the samples of the last two treatments can be seen in Fig. 1.

It is noteworthy that none of the samples had measurable off-flavours notes, supporting the high quality of the products.

Control samples (FD and CPD-VMFD) presented a flat (no complex) sensory profile, with their predominant attribute being sweetness (Fig. 3A). The profile changed considerably when “Wonderful” pomegranate juice was used during the osmotic dehydration [OD(POM)-CPD-VMFD]; this step enriched the sensory complexity of the “Mollar de Elche” dried arils by increasing the

intensities of sourness (4.7), fruity notes (3.7) and pomegranate flavour (3.7). The use of chokeberry juice significantly decreased sweetness, and increased astringency, bitterness, sourness, and chokeberry flavour. The use of apple juice led to samples with low colour intensity and low pomegranate flavour but high intensity of apple; the apple flavour masked that of pomegranate.

Besides, three texture attributes were evaluated: crispiness, adhesiveness, and solubility in the saliva. There were no significant differences among the dried samples. Samples were defined by low crispiness (<1 in scale 0–10) and low solubility (<1.5). Regarding the adhesiveness, the OD pre-treatment significantly increased them due to higher contents of soluble solids. Control samples (FD and CPD-VMFD) had values 1.6, while the OD samples had values ranging between 2.4 and 3.8. This experimental finding may be justified because samples with a pre-treatment had more quantity of sugar (OD with juice concentrated = 40 °Brix), and it seems reasonable that a positive relationship between high sugar content and high adhesiveness intensity may exist.

#### 4. Conclusions

Osmotic dehydration using “Wonderful” pomegranate and chokeberry concentrated juices improved the quality of dried “Mollar de Elche” pomegranate arils in terms of rehydration rate, antioxidant capacity, colour, and sensory profile; however still further research is needed to fully optimize this combined drying treatment because the freeze-dried sample still had higher anthocyanin content and better instrumental colour parameters. On the basis of the results obtained connected with colour, antioxidant capacity and anthocyanin contents, the combination of pomegranate and chokeberry juices resulted in the highest intensity of the key flavour notes; the second best results were obtained by using just “Wonderful” pomegranate juice. All treatments were acceptable in terms of sensory parameters (for example, had no off-flavours) but with different characteristic flavour notes. Improving the quality of dried pomegranate arils must increase the popularity of this product, even in groups with reduced fruit consumption, such as teenagers and children, leading to higher consumer acceptance, consequently higher product demand, and finally higher benefits for the farmers and industry. The energy consumption and cost of the drying treatments will be evaluated in future studies, as done by Calín-Sánchez et al. (2014).

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2017.04.033>.

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## **7.5 Publication 5**

### **Publication 5 (Literal transcription):**

#### **Consumers' Opinion on Dried Pomegranate Arils to Determine the Best Processing Conditions**

**Cano-Lamadrid, M.,** Vázquez-Araújo, L., Sánchez-Rodríguez, L., Wodyło, A., and Carbonell-Barrachina, Á.A.

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# Consumers' Opinion on Dried Pomegranate Arils to Determine the Best Processing Conditions

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**Abstract:** Consumers' preference is essential to improve processed food products quality, but small companies sometimes lack knowledge or tools to develop consumer studies. The aim of the present study was to investigate consumers' insight to recommend the best drying methodology for pomegranate arils. With the aim of providing information that industry can correlate to the drivers of liking, descriptive sensory characteristics, and volatile compounds of the samples were determined and related with consumers' responses. A total of 19 volatiles of dehydrated pomegranate arils were determined using solid-phase microextraction and gas chromatography-mass spectrometry. Partial least square regression (PLS) results indicated that consumers overall liking was positively correlated with "pom ID", "sweet", and "fruity" attributes, and also volatile compounds of the esters family. Overall liking was negatively correlated with the "off-flavor" and "burnt" attributes, related to the furan compounds family. Penalty analysis indicated that the sample corresponding with the current commercial product needed improvement on the "pom ID", "fruity", and "sweetness" parameters. All the samples processed using the proposed new drying techniques were more liked than the commercial sample, highlighting a sample dried using pre-osmotic dehydration in Wonderful concentrate pomegranate juice.

**Keywords:** *Punica granatum*, drivers of liking, drying, descriptive analysis, volatile compounds

**Practical Application:** Consumers' preference is essential to improve processed food products quality, but small companies sometimes lack knowledge or tools to conduct consumer studies. The present study provides useful information to understand consumers' preferences of a healthy product such as pomegranate dehydrated arils. Also, the link of physico-chemical and sensory tools is clearly described, providing information about possible sensory quality indicators.

## Introduction

Different and recent scientific publications have demonstrated the health benefits of pomegranate consumption. Some of the reported benefits are anti-mutagenic properties and prevention of oxidative inflammation and cardiovascular disease (Kalaycıoğlu & Erim, 2017; Karimi, Sadeghi, & Kokini, 2017). Because of these reported healthy properties, consumer interest in this fruit and its based-products has drastically increased. Nowadays, consumers are demanding novel and healthier ready-to-eat products such as dried pomegranate arils (Cano-Lamadrid et al., 2017; Dak, Sagar, & Jha, 2014), and the interest increases if the fruits are produced under friendly farming environmental conditions such as HydroSOStainable products (Cano-Lamadrid et al., 2018; Noguera-Artiaga et al., 2016).

The main problems of the scarce commercially available dried pomegranate arils are the undesirable appearance (browning) and

the off-flavors generation due to the drying process, sometimes uncontrolled. Also, some authors have reported specific textures in different pomegranate cultivars, for example Wonderful cultivar characterized by having a high seed-hardness (Szychowski et al., 2015). Therefore, the choice of the cultivar is a key step on developing successful products because the diverse dried arils might have important differences on their sensory properties, not only in flavor, but also in texture (Alcaraz-Mármol, Nuncio-Jáuregui, García-Sánchez, Martínez-Nicolás, & Hernández, 2017).

Nowadays, because of the increasing interest in healthy and ready to eat products, numerous pomegranate based-products are available in the market, providing consumers with different options: ready-to-eat fresh arils, pomegranate juice, pomegranate concentrate, dried arils, and so on. Some studies have been published during the last years providing useful information about the chemical characteristics and the technology useful to improve the quality of dehydrated fruits, but none of them included consumers' insights (for example, Vardin & Yilmaz, 2018; Wojdyło, Figiel, Lech, Nowicka, & Oszmiański, 2014). Consumers' studies are essential to determine the quality and to drive the possible improvement of foods, guaranteeing successful products and, in the present scenario, promote the consumption of healthy processed foods. To improve consumers' interest in pomegranate dried arils, quality of the product has been improved using novel techniques such as combined dried method (convective predrying with vacuum microwave finish drying, CPD-VMFD) and CPD-VMFD with a pre-osmotic dehydration (OD). Using these drying techniques led to an increment of antioxidant capacity, as well as a decrease of off-flavors and an improvement of the appearance

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of the product (Cano-Lamadrid et al., 2017). Although in this study an improvement on the sensory properties of the dried pomegranate arils was reported, no consumer research was conducted, and therefore the results might lead to the market launching of unsuccessful products. Also, none of the studies conducted on dehydrated pomegranate arils included information about the volatile composition, which might be important to link with sensory data for those small companies with limited access to sensory or consumer data.

The aim of the present study was to determine consumer insights about dried pomegranate arils using different technologies and to link the consumer data with descriptive and volatile composition data. The results might be used by pomegranate derivative or dried fruits companies to improve their procedures and make more successful products that meet consumers' demands.

## Materials and Methods

### Samples

The selection of the samples was based in the previous study conducted by Cano-Lamadrid et al. (2017), which concluded that the technology used in some of the samples (coded as B, C, and D in the present study) enhanced the functionality and therefore the healthy characteristics of the product. The present study was conducted using the 4 following samples:

- Sample A: commercial sample (unknown variety and drying conditions).
- Sample B: "Mollar de Elche" pomegranate arils dried using combined drying (CPD-VMFD: predrying at temperature 60 °C for 2 hr with an air velocity of 0.6 m/s and followed by vacuum microwave finish drying with microwave power reduced from the initial 360W to 120W).
- Sample C: "Mollar de Elche" arils pretreated by osmodehydration (OD) submerged at 45 °C for 90 min in 40 °C Brix 100% "Wonderful" pomegranate juice and then combined drying (same CPD-VMFD conditions as in sample B).
- Sample D: "Mollar de Elche" arils pretreated by osmodehydration (OD) submerged at 45 °C for 90 min in 40 °C Brix 50% "Wonderful" pomegranate and 50% chokeberry (*Aronia melanocarpa* L.) fruits juice, and then combined drying (same CPD-VMFD conditions than sample B).

### Extraction and chromatographic analysis of volatile compounds

The volatile composition of the samples was determined using headspace solid phase micro-extraction (HS-SPME) and the samples were processed as described below. Ground sample (5 g), ultrapure water (10 mL), NaCl (15 % w/v, weight/volume), and 1-octanol (10 µL of 1000 mg/L, internal standard) were placed into a 50 mL vial with polypropylene caps and PTFE/silicone septa. The vial was placed in a water bath with controlled temperature (40 °C) and automatic stirring. After 5 min of equilibration time, a 50/30 µm DVB/CAR/PDMS fiber was exposed to the sample headspace for 50 min at 40 °C.

The chromatographic set up and conditions were the same as reported by Cano-Lamadrid et al. (2018), and the column used was a Restek Rxi-1301 Sil MS (Restek Corporation, Palo Alto, USA) of 30 m  $\times$  0.25 mm i.d., 0.25 µm film thickness. The identification of compounds was conducted by GC (Shimadzu GC-17A), coupled with a Mass Spectrometer detector (Shimadzu GCMS QP-5050A; Shimadzu Corporation, Kyoto, Japan), and

the semi-quantification of compounds was conducted using the same conditions in and GC coupled to a Flame Ionization Detector (GC-FID Shimadzu GC17; Shimadzu Corporation, Kyoto, Japan). The analyses were run in triplicate, and results were expressed as percentage of the total area.

### Descriptive sensory analysis

Eight trained panelists from the department of Agro-Food Technology at Univ. Miguel Hernández (aged 26 to 55 years old, 4 females and 4 males), and with more than 600 h of training and experience in sensory testing, participated in this study. The panel was selected and trained following the ISO standard 8586-1 (1993), and it is specialized in descriptive sensory evaluation of fruits and vegetables, including pomegranate products. For the present study, the panel worked during two orientation sessions (90 min each one) discussing about the main appearance, flavor, and texture characteristics of dried pomegranate arils. The lexicon used for describing the flavor attributes was developed by Koppel and Chambers (2010). The lexicon used for describing the texture attributes was reported by Vázquez-Araújo et al. (2014). Both lexicons were adapted for dried arils during the orientation sessions. Samples were served into odor-free, disposable 90 mL covered plastic cups at room temperature, and coded using 3 digit numbers. Unsalted crackers and distilled water were provided to panelists to clean their palates between samples. The panel used an 11 points numerical scale with 0.5 increments for quantifying the intensity of the attributes (0 = absence, 10 = extremely strong). Samples were evaluated in duplicate in 2 different sessions.

### Consumer study

A consumer panel of 100 volunteers recruited from Senso-Food Solution, the consumer database at Department of Agrofood Technology (UMH), evaluated the samples. The consumer profile was as follows: 35% male and 65% female; 31% belonging to the 18 to 24 years old group, 25% to the 25 to 35 years old group, 24% to the 36 to 45 years old group, and 20% older than 45 years old. Also, information about fruit consumption was requested, because the sample should represent the general population but looking for a majority of regular fruit consumers:

- i) frequency of fresh fruit consumption (77% daily, 6% weekly, 16% 2 to 3 per month, and 1% <3 per month);
- ii) frequency of seasonal pomegranate consumption (17% daily, 53% weekly, 22% 2 to 3 per month, and 8% <3 per month); and,
- iii) frequency of dried fruit consumption (7% daily, 24% weekly, 26% 2 to 3 per month, and 43% <3 per month).

The consumer study was carried out in the testing room of UMH (individual booths, controlled temperature: 21 ± 1 °C; combined natural/artificial light), and each consumer evaluated all four samples in a single session. The presentation order of the samples was randomized to avoid biases. A 9-point hedonic intensity scale was used to determine liking, and 9-point Likert scale was used for the Just About Right (JAR) questions to determine possible improvements of the attributes: color tone, color homogeneity, appearance, fruity flavor, pomegranate flavor, sweetness, sourness, astringency, and adhesiveness. Unsalted crackers and distilled water were served in-between samples. A demographic questionnaire was presented at the end of sensory test, and a gift was given to encourage participation.

**Table 1–Retention indexes and sensory descriptors of the volatile compounds found in the samples.**

| Compounds | Material <sup>V</sup>          | RT (min) <sup>†</sup> | Retention Indexes <sup>‡</sup> |        |        |        | UMH  | Descriptors <sup>§</sup> |                           |
|-----------|--------------------------------|-----------------------|--------------------------------|--------|--------|--------|------|--------------------------|---------------------------|
|           |                                |                       | Exp. A                         | Exp. B | Exp. C | Exp. D |      |                          |                           |
| V1        | Hexanal                        | A,B,C,D               | 6.08                           | 830    | 832    | 831    | 832  | 835                      | Fatty, green, rancid      |
| V2        | 2,3-Butanediol                 | A,B,D                 | 7.87                           | 869    | 869    | -      | 869  | 873                      |                           |
| V3        | Furfural                       | A,B,C,D               | 7.82                           | 891    | 894    | 892    | 893  | 899                      | Woody, burnt, spice       |
| V4        | 1-Hexanol                      | A,C                   | 9.21                           | 911    | -      | 912    | -    | 912                      | Acidic, fruity, fermented |
| V5        | Furfuryl alcohol               | A,B,C,D               | 9.43                           | 917    | 918    | 917    | 918  | 919                      | Caramel                   |
| V6        | 5-Methyl-2(3H)-furanone        | A,B,C,D               | 9.95                           | 931    | 930    | 928    | 928  | -                        | Oily, nutty               |
| V7        | $\alpha$ -Thujene              | A,B,C,D               | 10.57                          | 946    | 948    | 946    | 949  | 946                      | Almond, apple, grape      |
| V8        | 2-Acetylfuran                  | A,C,D                 | 10.62                          | 964    | -      | 964    | 964  | -                        | Almond, caramel, coffee   |
| V9        | Ethyl hexanoate + Benzaldehyde | A,B,C,D               | 13.48                          | 1018   | 1018   | 1019   | 1019 | 1018                     | Almond, cherry, sweet     |
| V10       | 5-Methyl furfural              | A,B,C,D               | 13.50                          | 1025   | 1025   | 1026   | 1026 | -                        | Caramel, butter           |
| V11       | Linalool                       | A,B,C,D               | 14.59                          | 1042   | 1043   | 1043   | 1043 | 1042                     | Lemon, orange, sweet      |
| V12       | Limonene                       | A,B,C,D               | 14.75                          | 1046   | 1046   | 1046   | 1046 | 1046                     | Lemon, orange, sweet      |
| V13       | Nonanal                        | A,B,C,D               | 19.31                          | 1143   | 1143   | 1143   | 1144 | 1145                     | Citrus, lime, lemon       |
| V14       | 2-Phenylethyl alcohol          | A,B,D                 | 21.34                          | 1185   | 1185   | -      | 1182 | -                        | Honey, rose, floral       |
| V15       | 5-(Hydroxymethyl)-2-furfural   | A,B,C,D               | 29.87                          | 1361   | 1360   | 1361   | 1361 | 1362                     | Woody, burnt              |
| V16       | 3-Hydroxy-methyl hexanoate     | A,B,C,D               | 31.62                          | 1398   | 1397   | 1397   | 1398 | -                        | Fruity                    |
| V17       | Bornyl acetate                 | A,B,C,D               | 32.57                          | 1419   | 1418   | 1418   | 1419 | 1421                     | Sweet, fruity, pineapple  |
| V18       | Ethyl decanoate                | A,B,C,D               | 33.48                          | 1439   | 1438   | 1439   | 1439 | 1437                     | Grape, pear               |
| V19       | Bornyl butyrate                | A,B,C,D               | 36.75                          | 1511   | 1511   | 1511   | 1511 | 1512                     | Herb                      |

<sup>V</sup> A, commercial; B, CD-VMD; C, OD(POM)-CD-VMD; and D, OD(POM-Ch)-CD-VMD;

<sup>†</sup> RT, retention time;

<sup>‡</sup> Exp., experimental; UMH, The UMH research group (Universidad Miguel Hernández) has created their own library of standards to have proper retention indexes for the identification of the volatile compounds found in different food matrices. All 19 compounds found in the dehydrated pomegranate arils have been identified by using Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) standards;

<sup>§</sup> References (Merck KGaA, 2018).

### Statistical analysis

One-factor (samples) analysis of variance was performed using StatGraphics Plus 5.0 software (Manugistics, Inc, Rockville, MD) and post hoc mean separation was conducted using Tukey's multiple range test. Consumers' data and its relationship with descriptive and volatile composition were analyzed using XLSTAT Premium 2016 (Addingsoft, Barcelona, Spain). Partial least-square regression (PLS) analysis was carried out to study the relationship of the volatile composition and the descriptive analysis (x: independent variables) with the overall liking data (y: dependent variable). Also, penalty analysis was conducted to provide extra information about the possible improvements of some samples.

## Results and Discussion

### Volatile composition

A total of 19 compounds were identified in the dried arils samples. Table 1 shows the retention indexes used for the identification of compounds, retention time, and the main sensory descriptors of each compound. The five most abundant volatile compounds (content above 3%) found in sample A (the commercial sample) were: furfural (59.9%), 5-(hydroxymethyl)-2-furfural (7.84%), linalool (7.65%), 5-methyl furfural (7.64%), and 3-hydroxy-methyl hexanoate (3.06%; Table 2). The three main compounds belonged to the furan derivatives family, similar as the ones reported in previous studies conducted with dried fruits such as raisins, dried plums, dried pineapple, and dried banana (Fromberg, Mariotti, Pedreschi, Fagt, & Granby, 2014). Furfural and 5-methyl furfural (also present, but in lower concentrations, in samples B–D) have been associated with smoky, woody, burnt, and caramel flavor notes, being undesirable attributes in fresh fruit products when the concentration is high (Merck KGaA, 2018). However, the compound 5-(hydroxymethyl)-2-furfural was present in samples B–D in significantly higher concentrations than in sample A, which can also limit their consumer's acceptance. In general, furan compounds can come from a variety of precursors including ascorbic

acid, amino acids, and carbohydrates through the Maillard reaction (van Boekel, 2006), and might be indicators of an incorrect processing or uncontrolled drying conditions.

Although the compounds found in the different samples were the same, their relative amount varied among samples. 5-(Hydroxymethyl)-2-furfural was the predominant compound in the optimized dehydrated pomegranate arils (samples B–D). But the new developed samples highlighted by having less furfural and higher relative amounts of esters such as 3-hydroxymethyl hexanoate (fruity) or ethyl decanoate (grape, pear), and terpenes such as linalool (lemon, orange, sweet; sample C) or  $\alpha$ -thujene (almond, apple, grape; samples B and D). These volatile compounds have been previously found in pomegranate juices (Cano-Lamadrid et al., 2018). The volatile profile of the juices included aldehydes (such as the compounds also found in the present study: hexanal and nonanal), esters (bornyl acetate, ethyl decanoate, and bornyl butyrate), aliphatic alcohols (1-hexanol), monoterpenes ( $\alpha$ -thujene), and monoterpeneoids (linalool and limonene). In accordance with other authors, our findings confirmed that combined drying techniques such as convective predrying with vacuum microwave finish drying (sample B–D) seemed to retain a higher content of the original product volatile compounds if compared with traditional (for example, convective drying) and modern techniques (for example, combined drying method) applied as single treatments.

### Descriptive sensory analysis

Table 3 shows the significant differences found in the main sensory descriptors among samples. Off-flavors were evaluated because previous studies reported that drying of pomegranate might lead to a significant increase on the intensity of off-flavors (Calín-Sánchez et al., 2013). However, just the commercial sample evaluated in the present study (sample A) presented a slight intensity of off-flavors. This undesirable flavor might have come from furfural (>50% of total volatile compounds), which sensory



**Table 2-Volatile compounds (percentage of total area) in each analyzed sample.**

| No. | Compounds                      | ANOVA <sup>†</sup> | Content (%) <sup>‡</sup> |          |         |        |
|-----|--------------------------------|--------------------|--------------------------|----------|---------|--------|
|     |                                |                    | A                        | B        | C       | D      |
| V1  | Hexanal                        | **                 | 1.08 a                   | 0.50 b   | 0.80 ab | 0.27 b |
| V2  | 2,3-butanediol                 | **                 | 0.66 a                   | 0.25 cab | 0.00 b  | 0.07 b |
| V3  | Furfural                       | ***                | 59.9 a                   | 2.56 d   | 24.2 b  | 16.8 c |
| V4  | 1-hexanol                      | ***                | 0.27 a                   | 0.00 b   | 0.39 a  | 0.00 b |
| V5  | Furfuryl alcohol               | ***                | 0.85 a                   | 1.15 a   | 0.29 b  | 1.65 a |
| V6  | 5-Methyl-2(3H)-furanone        | **                 | 1.90 a                   | 0.73 b   | 0.17 c  | 0.24 c |
| V7  | $\alpha$ -Thujene              | ***                | 0.91 c                   | 24.8 a   | 0.08 c  | 15.4 b |
| V8  | 2-Acetyl furan                 | ***                | 1.90 a                   | 0.00 c   | 1.15 a  | 0.52 b |
| V9  | Ethyl hexanoate + Benzaldehyde | ***                | 1.36 b                   | 2.11 a   | 1.16 b  | 0.47 c |
| V10 | 5-Methyl furfural              | ***                | 7.64 a                   | 0.13 c   | 2.27 b  | 0.90 c |
| V11 | Linalool                       | ***                | 7.65 a                   | 4.10 b   | 8.16 a  | 1.33 c |
| V12 | Limonene                       | ***                | 2.89 a                   | 1.39 b   | 1.38 b  | 0.56 c |
| V13 | Nonanal                        | ***                | 0.48 c                   | 2.18 a   | 2.79 a  | 1.56 b |
| V14 | 2-Phenylethyl alcohol          | ***                | 0.54 a                   | 0.79 a   | 0.00 b  | 0.47 a |
| V15 | 5-(Methoxymethyl)-2-furfural   | ***                | 7.84 b                   | 37.1 a   | 39.3 a  | 31.8 a |
| V16 | 3-Hydroxy-methyl hexanoate     | ***                | 3.06 b                   | 12.6 a   | 10.9 a  | 12.4 a |
| V17 | Bornyl acetate                 | ***                | 0.25 c                   | 1.56 a   | 1.45 a  | 0.84 b |
| V18 | Ethyl decanoate                | ***                | 0.34 d                   | 5.46 b   | 2.89 c  | 11.3 a |
| V19 | Bornyl butyrate                | ***                | 0.49 b                   | 2.69 a   | 2.59 a  | 3.33 a |

<sup>†</sup> NS, not significant at  $P < 0.05$ ;

\*, \*\*, and \*\*\* significant at  $P < 0.05$ , 0.01, and 0.001, respectively; Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test;

<sup>‡</sup> A, commercial; B, CD-VMD; C, OD(POM)-CD-VMD; and D, OD(POM-Ch)-CD-VMD.

**Table 3-Descriptive sensory attributes of pomegranate dehydrated arils (A, B, C, and D).**

| Attributes         | ANOVA <sup>†</sup> | A <sup>‡</sup> | B      | C      | D     |
|--------------------|--------------------|----------------|--------|--------|-------|
| <b>Appearance</b>  |                    |                |        |        |       |
| Brightness         | ***                | 0.0 c          | 0.6 bc | 1.7 a  | 1.1 a |
| Color uniformity   | ***                | 6.7 b          | 5.5 b  | 8.0 a  | 9.0 a |
| Size uniformity    | ***                | 4.3 c          | 7.0 b  | 9.0 a  | 9.0 a |
| <b>Basic taste</b> |                    |                |        |        |       |
| Sweetness          | **                 | 3.0 c          | 7.3 a  | 6.1 b  | 6.1 b |
| Sourness           | ***                | 2.9 a          | 0.5 b  | 3.2 a  | 2.6 a |
| Bitterness         | *                  | 0.5 ab         | 0.2 b  | 0.4 b  | 1.3 a |
| Astringent         | *                  | 0.4 a          | 0.0 b  | 0.2 b  | 0.6 a |
| <b>Flavor</b>      |                    |                |        |        |       |
| Pomegranate ID     | **                 | 0.6 b          | 2.0 ab | 2.9 a  | 3.1 a |
| Chokeberry ID      | **                 | 0.0 b          | 0.0 b  | 0.0 b  | 1.5 a |
| Apple ID           | ***                | 0.2 b          | 1.1 b  | 2.3 a  | 0.9 b |
| Fruity             | ***                | 0.3 b          | 3.9 a  | 4.3 a  | 4.5 a |
| Caramel            | **                 | 0.6 c          | 3.0 a  | 1.5 b  | 2.8 a |
| Citric             | NS                 | 0.2 b          | 0.0 b  | 0.5 ab | 1.0 a |
| Woody              | **                 | 0.7 a          | 0.0 b  | 0.0 b  | 0.0 b |
| Off-Flavor         | **                 | 0.7 a          | 0.0 b  | 0.0 b  | 0.0 b |
| <b>Texture</b>     |                    |                |        |        |       |
| Crispiness         | ***                | 9.0 a          | 1.3 c  | 6.2 b  | 5.3 b |
| Adhesiveness       | ***                | 1.9 b          | 5.0 a  | 5.4 a  | 4.6 a |
| Solubility         | *                  | 0.0 b          | 1.0 a  | 1.2 a  | 1.1 a |
| Seed hardness      | ***                | 9.0 a          | 2.4 b  | 2.8 b  | 2.1 b |

<sup>†</sup> NS, not significant at  $P < 0.05$ ;

\*, \*\*, and \*\*\* significant at  $P < 0.05$ , 0.01, and 0.001, respectively; Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test;

<sup>‡</sup> A, commercial; B, CD-VMD; C, OD(POM)-CD-VMD; and D, OD(POM-Ch)-CD-VMD.

descriptors are burnt, woody, and spicy aromas (Merck KGaA, 2018). Sample A was also characterized by slight woody notes probably coming from the woody part of the aril (Mayuoni-Kirshinbaum & Porat, 2014) and because of its general low aromatic intensity.

Sample B was characterized by having the highest sweetness and lower acidity, as well as higher pomegranate ID, fruity, and caramel notes than sample A. When osmotic dehydration pretreatment (OD) was used (samples C and D), the sensory profile slightly changed; the OD treatment enriched the sensory

complexity of the “Mollar de Elche” dried arils using CD-VMFD (fruity, sourness and pomegranate ID). Both C and D samples had also higher sweetness and caramel notes than sample A, but lower sweetness than B. Also, the sourness was high as compared to sample B, while the intensities of pomegranate ID and fruity notes were the highest ones. The pretreatment with Wonderful cultivar pomegranate juice (sample C), significantly increased apple ID. Using pomegranate and chokeberry juices 1:1 during the OD stage (sample D), significantly increased the bitterness, caramel, chokeberry ID, and citric flavors.

**Table 4–Mean scores and ANOVA for color, appearance, flavor notes, basic taste, and overall liking for consumers.**

| Attribute          | ANOVA <sup>†</sup> | A <sup>‡</sup> | B      | C     | D      |
|--------------------|--------------------|----------------|--------|-------|--------|
| Color              | ***                | 3.4 c          | 5.8 a  | 6.3 a | 5.0 b  |
| Homogeneity color  | ***                | 4.5 c          | 5.5 b  | 6.1 a | 5.1 b  |
| Appearance         | ***                | 3.5 c          | 5.6 ab | 6.3 a | 5.1 b  |
| Fruity flavor      | ***                | 4.7 c          | 5.5 b  | 6.2 a | 5.6 ab |
| Pomegranate flavor | ***                | 4.2 c          | 4.9 b  | 5.7 a | 5.3 ab |
| Sweet              | ***                | 4.3 b          | 5.6 a  | 5.9 a | 5.6 a  |
| Sour               | ***                | 4.6 b          | 5.4 a  | 5.6 a | 5.3 a  |
| Astringency        | ***                | 4.8 b          | 5.7 a  | 5.4 a | 5.4 a  |
| Adhesiveness       | ***                | 4.7 b          | 5.3 ab | 5.7 a | 4.9 b  |
| Liking score       | ***                | 4.3 b          | 5.4 a  | 6.0 a | 5.7 a  |

<sup>†</sup> NS, not significant at  $P < 0.05$ ;

\*, \*\*, and \*\*\*, significant at  $P < 0.05$ , 0.01, and 0.001, respectively; Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test;

<sup>‡</sup> A, commercial; B, CD-VMD; C, OD(POM)-CD-VMD; and D, OD(POM-Ch)-CD-VMD.

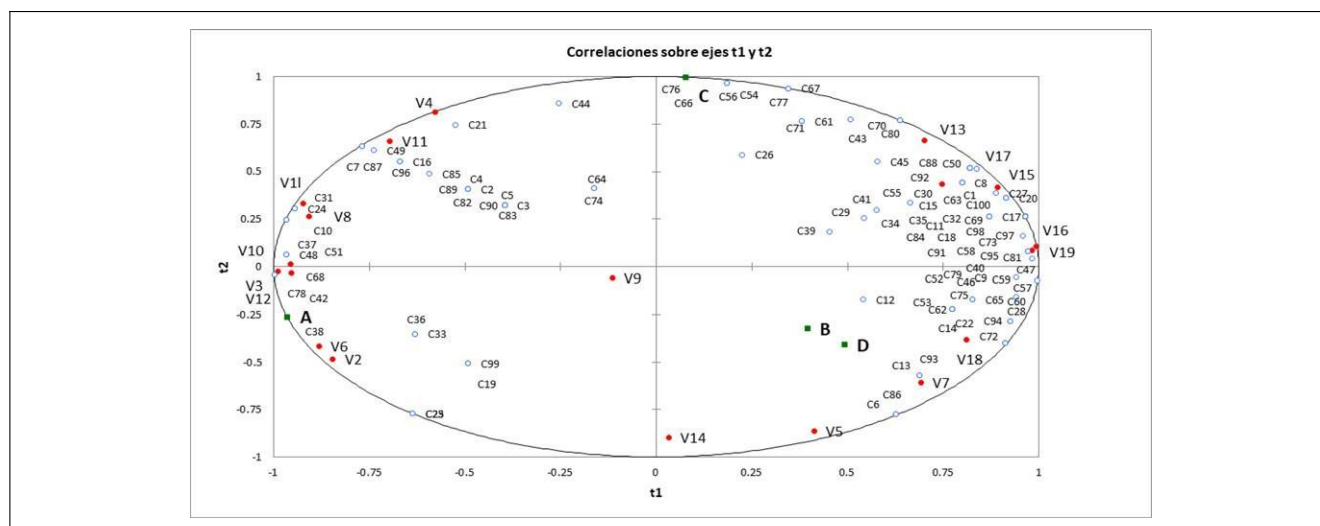


Figure 1–Partial least squares regression (PLS) of the volatile compounds (x) and consumers' overall liking (Y) of the samples (square, samples; unfilled circle unfilled, consumers; filled circle, volatile compounds). V1, hexanal; V2, 2,3-butanediol; V3, furfural; V4, 1-hexanol; V5, furfuryl alcohol; V6, 5-methyl-2(3H)-furanone; V7,  $\alpha$ -thujene; V8, 2-acetylfuran; V9, ethyl hexanoate benzaldehyde; V10, 5-methyl furfural; V11, linalool; V12, limonene; V13, nonanal; V14, phenylethyl alcohol; V15, 5-(hydroxymethyl)-2-furfural; V16, 3-hydroxy-methyl hexanoate; V17, bornyl acetate; V18, ethyl decanoate; V19, bornyl butyrate.

Appearance was improved by using the new developed drying treatments (B–D), especially in last two samples, in which brightness, color uniformity, and size uniformity were higher. Texture attributes showed a relationship between crispiness and seed hardness, being the highest values found in sample A; the high seed hardness indicated that the commercial sample could be made with Wonderful cultivar. Medium values of crispiness were also present in samples C and D, and might be due to an outside crust development because of the higher presence of sugars. Adhesiveness to teeth (amount of product adhering on/in the teeth after mastication of product; reference material: sticky rice, intensity 2.0) was higher in samples B–D, probably because of the difference in the sugar content of the samples.

#### Consumer acceptability and driving sensory attributes

Once the volatile and sensory properties of the samples had been characterized, the consumer study was conducted to determine the drivers of liking for this kind of product, and therefore to provide industry with relevant information to decide about processing conditions.

Mean scores for liking of color, appearance, some flavor notes, and overall liking of samples are shown in Table 4. In general

terms, sample C was the most liked by consumers (all evaluated attributes received the highest scores), and sample A was the significantly less liked. Appearance was better scored in samples B and C, both characterized by having a 100% pomegranate composition; sample D was significantly less liked maybe because the pomegranate:chokeberry juice used in the OD-pretreatment provided a less attractive color. Flavor attributes liking varied among samples, for example, fruity flavor and pomegranate flavor were less liked in sample A, but for all the evaluated attributes sample C was the best scored.

PLS regression analysis was conducted to determine the drivers of liking for dehydrated pomegranate arils (Calín-Sánchez et al., 2011). Data are represented by two principal components and was represented in two different maps to provide separate information useful to link correlations mappings of consumers' overall liking with the 19 volatile compounds, and 19 descriptive attributes using analytical techniques: CG-MS and descriptive sensory techniques (Figure 1 and 2, respectively). Figure 1 shows how the first dimension (horizontal axis) divided the consumers in two main groups, being the majority of consumers grouped close to volatiles corresponding to the ester and terpenes families (V16, V17, V13, and V7, among others). These compounds

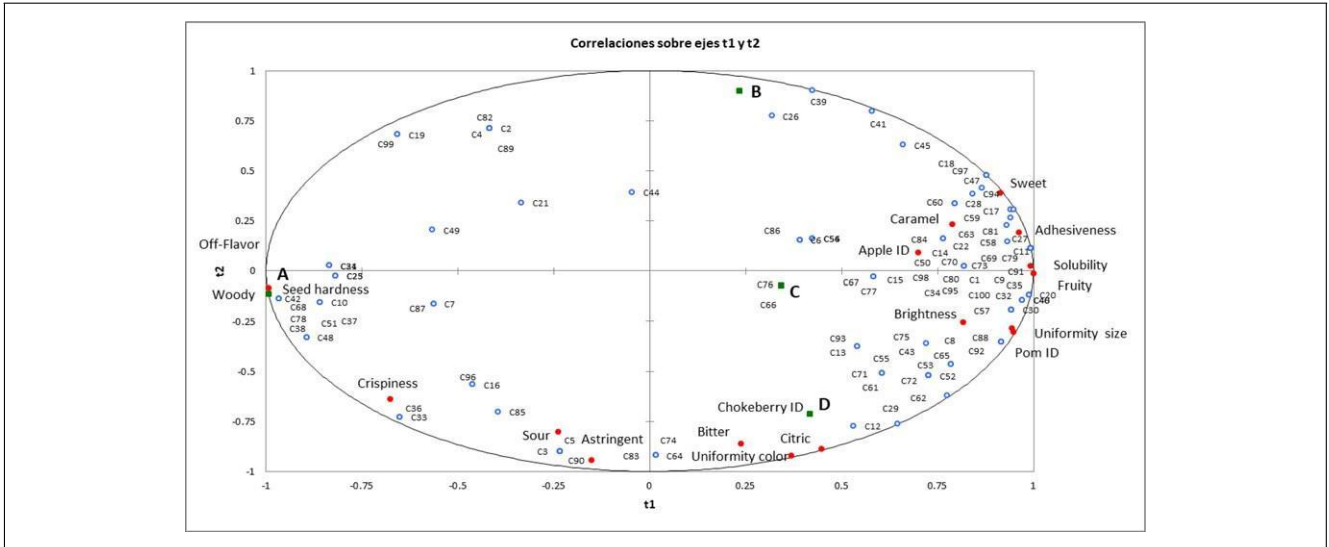


Figure 2—Partial least squares regression (PLS) of the descriptive sensory profile (X) and consumers' overall liking (Y) of the samples (squares, samples; unfilled circle, consumers; filled circle, descriptive attributes).

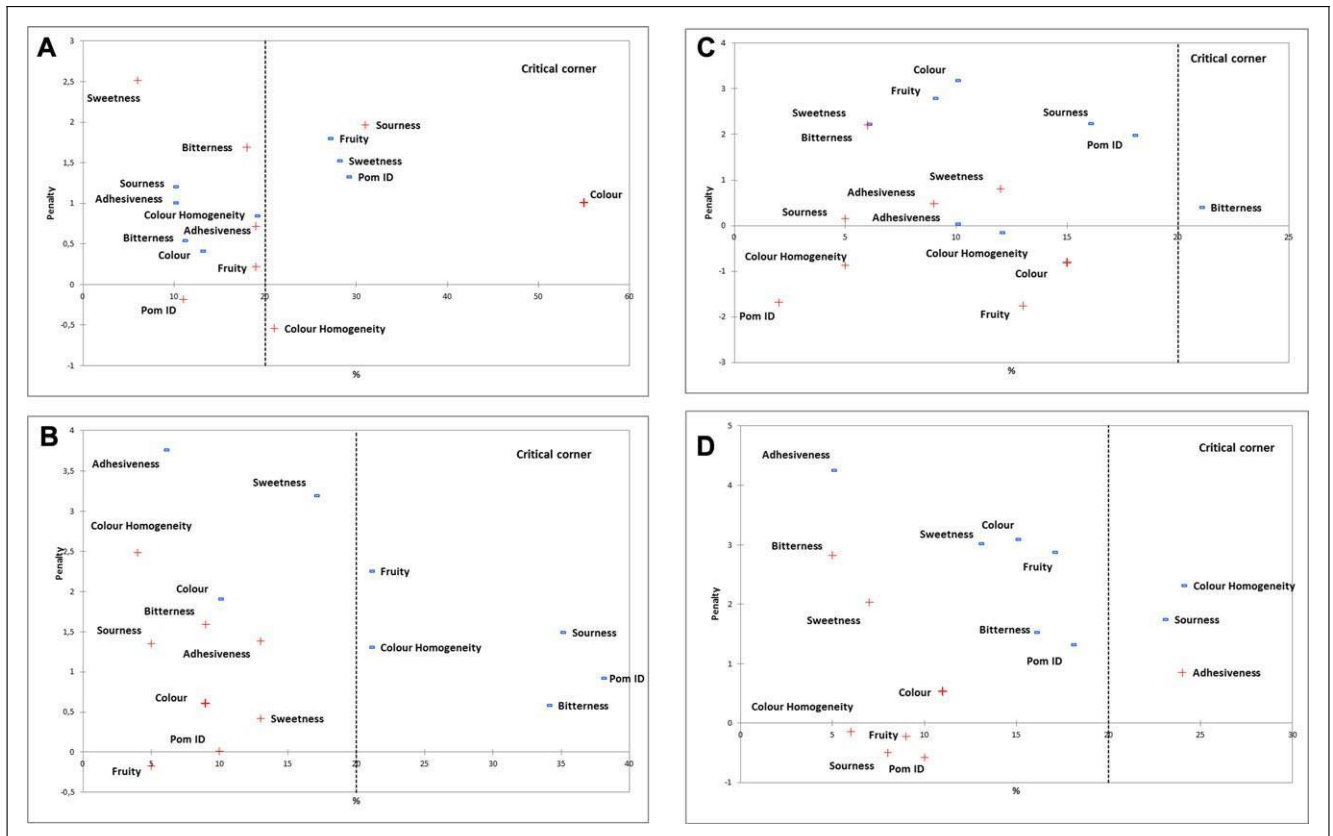


Figure 3—Penalty analysis of samples (sample code indicated on the top left of each figure; “too low intensity” indicated with “-”, and “too high intensity” indicated with “+”).

were characterized by having fruity, pineapple, apple, grape, and so on, descriptors. Knowing that consumers preferred this aromatic composition could be useful to industry in predicting liking of new products developed because of the processing optimization. On the other side of the map, fewer consumers were grouped close to 1-hexanol (V4: acidic, fruity) and linalool (V11: lemon, orange, sweet), indicating that a possible cluster of consumers could prefer these aromatics. A higher num-

ber of consumers could be used in future studies to favor the possibility of segmenting different clusters. The volatile compounds not acting as liking drivers were those belonging to the furan family (furfural, 5-methyl furfural, 5-methyl-2(3H)-furanone: V3, V10, and V6 respectively) coming from Maillard reaction (van Boekel, 2006), probably because consumers did not relate the descriptors associated to these compounds with fruit derivatives.

Figure 2 shows consumers' overall liking and the relationship with the different sensory descriptors. Regarding texture attributes, adhesiveness and solubility were the main drivers of liking, while seed hardness and crispiness seemed to be less liked. Also, a higher brightness and size uniformity was positively valued by consumers. Sweetness, fruity, and pomegranate ID were the most liked flavors, probably because of reminding to the original fresh fruit (Alcaraz-Mármol et al., 2015). On the contrary, woody, chokeberry ID, astringency, bitterness and sourness were not appreciated flavors by consumers. As expected, the off-flavor was not among the liked attributes (Butler, 2018).

In general, consumers' overall liking was positively linked with specific volatile compounds and sensory descriptors: aldehydes, esters, aliphatic alcohols and terpenes, and a higher intensity of pomegranate ID, fruity, and sweet, respectively. These results indicated that most of consumers would choose samples B-D. Industry could use these liking drivers as quality indicators for improving their commercial products. A small group of consumers preferred different sensory attributes and volatile compounds (for example, woody, off-flavor, seed hardness, compounds belonging to the furan family). Further studies with a higher number of volunteers could be conducted to study different consumer segments and adapt a diversity of products to consumers' demands.

### Penalty analysis

In addition to overall liking and liking of specific attributes, some JAR questions were asked during the consumer study. For a better understanding of the relationships between JAR scores and consumers linking, penalty analysis was conducted (Narayanan, Chinnasamy, Jin, & Clark, 2014). Figure 3 shows the proportion of consumers' opinion plots against the mean Penalty. The attributes susceptible of improvement were those that had the greatest negative impact on the sample liking for at least 20% of consumers and caused a drop of at least 1 point for liking.

Results of the penalty analysis indicated the clear need of improvement of the commercial sample (A). Sample A needed improvement of its sourness because of the excessive intensity. It was also shown that fruity, pomegranate ID, and sweetness needed to be increased in this sample. Sample B (pomegranate arils after CD-VFD) was penalized by presenting low intensities of fruity notes, color homogeneity, and sourness. The pretreatment with osmotic dehydration (sample C and D) improved the consumer acceptance of dehydrated pomegranate arils, and results of the penalty analysis indicated that just an increment on the color homogeneity and sourness in sample D was needed. According to consumers, no improvement was necessary in sample C.

### Concluding Remarks

Results showed consumers' opinion on the improvement of the volatile profile and sensory quality of developed dehydrated pomegranate arils if compared with a commercial product. The study of consumers' data and its relationship with analytical and descriptive sensory data showed the main liking drivers: high esters and low furan compounds content, high fruity and sweet attributes, and low seed-hardness. Application of penalty analysis showed that a possible improvement of the commercial products would be using "Mollar de Elche" cultivar and an osmotic dehydration pretreatment using 100% concentrated "Wonderful" pomegranate juice.

### Acknowledgments

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### Author Contributions

Cano-Lamadrid collected the physico-chemical, sensory data and conducted part of the statistical analysis; Vázquez-Araújo helped with the statistical analysis and the manuscript writing; Sánchez-Rodríguez and Wodyło prepared the samples and were the experts in drying technologies; Carbonell-Barrachina designed and coordinated the study.

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## **7.6 Publication 6**

### **Publication 6 (Literal transcription):**

**Phytochemical composition of smoothies combining pomegranate juice (*Punica granatum* L) and Mediterranean minor crop purées (*Ficus carica*, *Cydonia oblonga*, and *Ziziphus jujube*)**

**Cano-Lamadrid, M.**, Nowicka, P., Hernández, F., Carbonell-Barrachina, A.A., and Wojdyło, A.

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# Phytochemical composition of smoothies combining pomegranate juice (*Punica granatum* L) and Mediterranean minor crop purées (*Ficus carica*, *Cydonia oblonga*, and *Ziziphus jujube*)

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## Abstract

**BACKGROUND:** Daily intake of fruits and vegetables as suggested by the World Health Organization is lower than the recommended dietary intake (RDI). A good option to increase the intake of fruit and vegetables is the consumption of smoothies. This work evaluated the effect of adding fig, jujube or quince purée to pomegranate juice (cultivars 'Wonderful' and 'Mollar de Elche') in preparing smoothies at two ratios of purée:juice (40:60 and 60:40) on the composition of minerals, sugars, organic acids, vitamin C, antioxidant activity and polyphenols.

**RESULTS:** Smoothies composition was mainly affected by the addition of the fruit purée. Twenty-eight polyphenolic compounds were found in the pomegranate smoothies (quadrupole time-of-flight liquid chromatography–mass spectrometry). The highest total content of polyphenolic compounds (ultra-performance liquid chromatography with photodiode array and fluorescence detection) was found in smoothies with quince purée, 501 mg 100 g<sup>-1</sup> fresh weight (FW), followed by jujube and figs, with 374 and 320 mg 100 g<sup>-1</sup> FW, respectively. Fig smoothies were rich in anthocyanins, while the jujube ones had high content of flavonols and vitamin C; finally, the quince smoothies were rich in hydroxycinnamic acids.

**CONCLUSION:** A positive effect of the addition of minor crops (fig, jujube and quince) was observed on the nutritional and functionality of the novel pomegranate smoothies. Moreover, the addition of jujube contributed to an enrichment of the final smoothies in vitamin C and organic acids, while an increase of pectin content was found in fig and quince pomegranate based smoothies. Therefore, the blend of minor Mediterranean crop purées with pomegranate juice to produce smoothies is a good strategy to promote the consumption of these healthy but underutilized fruits.

**Keywords:** anthocyanins; quinces; figs; flavan-3-ols; flavonols; jujubes

## INTRODUCTION

The World Health Organization suggested daily intake of fruits and vegetables is lower than the recommended dietary intake (RDI).<sup>1</sup> The international recommendation for improving health and preventing chronic diseases such as diabetes and obesity is a minimum of 400 g of fruit and vegetables per day.<sup>2</sup> Consumers today are demanding new and healthy ready-to-eat products that resemble their natural fresh raw materials.

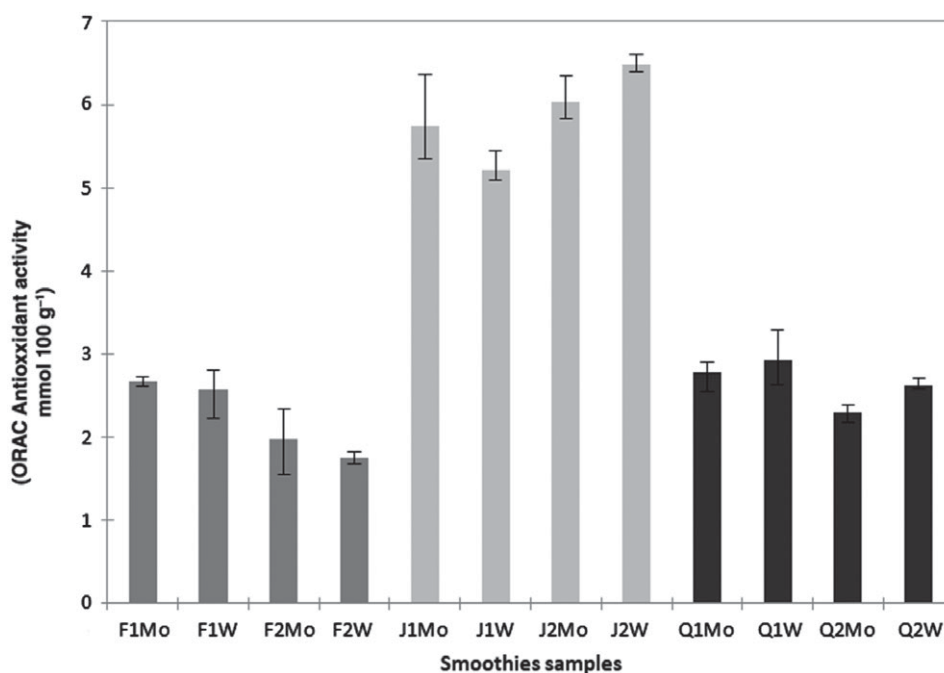
A good option to benefit from the nutrients and bioactive compounds responsible for healthy effects of fruits and vegetables is the consumption of smoothies containing carrot,<sup>3</sup> broccoli, tomato,<sup>4</sup> sour cherry,<sup>5</sup> apple<sup>6</sup> and *Prunus* fruits.<sup>7</sup> A 'smoothie', from the English word 'smooth' (tender, creamy) is a creamy drink (the texture is thick, similar to that of milkshakes) made from blended fruit (purée) together with fruit juice, and perhaps yoghurt or other dairy products and/or crushed ice cubes.<sup>8</sup>

The southeastern part of Spain is one of the most intensively Mediterranean agricultural areas, dominated by fruit orchards and vegetables. Some of the minor crops from this area are fruits

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**Figure 1.** Antioxidant activity (ORAC method) as affected by smoothie formulation; see Table 1 for specific formulation.

with huge potential because of their healthy properties, such as figs,<sup>9</sup> jujubes<sup>10</sup> and quinces;<sup>11,12</sup> all of these fruits are rich in bioactive compounds with high antioxidant capacity. Another interesting fruit in this area is pomegranate (*Punica granatum* L.), whose positive health effect is due to its unique bioactive profile.<sup>13</sup> A blend of pomegranate juice with the purée from these minor crops (figs, jujubes and quinces) seems a great opportunity to promote these minor crops, which are disappearing from Spanish farms due to their low consumption rates, and the lack of knowledge by consumers of their health benefits.

Consequently, the aim of this study was to determine the effect of minor Mediterranean crops (figs, jujubes or quinces) on the quality and functional properties of smoothies based on pomegranate juice, cultivars (cvs) 'Mollar de Elche' and 'Wonderful'. The parameters under study were: (i) physicochemical parameters (color); (ii) chemical composition (minerals, sugars and organic acids); (iii) phytochemical contents (vitamin C, anthocyanins, flavan-3-ols, flavonols, hydroxycinnamic acids and polymeric procyanidins); and (iv) antioxidant capacity (oxygen radical absorbance capacity (ORAC) assay) (Fig. 1).

## MATERIALS AND METHODS

### Plant material and smoothie preparation

Pomegranate shrubs (*Punica granatum* L., cvs 'Mollar de Elche' and 'Wonderful') were cultivated on a farm located in Murcia (Spain) under regulated deficit irrigation (RDI).<sup>14–17</sup> Pomegranate cvs 'Mollar de Elche' and 'Wonderful', figs (*Ficus carica*, cv. 'Colar'), jujubes (*Ziziphus jujube*, cv. 'Grandes de Albaterra') and quinces (*Cydonia oblonga*, cv. 'Gigante de Vranja') were hand-harvested between mid-August and mid-October 2016 at a commercial maturity stage, and immediately posted to Poland.

The different stages of the smoothie preparation process were: (i) purée preparation; (ii) pomegranate juice preparation; and (iii) obtaining of semi-products in appropriate proportions:

- i Figs (F), jujubes (J) or quinces (Q) were peeled, ground and heated at 80 °C in a Thermomix device (Vorwerk, Wuppertal, Germany); 10 mL rhubarb juice (prepared previously from rhubarb plant) per 1 kg of fruit was added to prevent enzymatic browning of the fruit. The particle size of the mixture was then reduced in a blender (Symbio, Zelmer, Rzeszów, Poland) until a thin purée was obtained. Then, the purées were cooled to room temperature.
- ii The pomegranate fruits ('Mollar de Elche' (Mo) and 'Wonderful' (W)) were cut into halves, and arils were manually separated from the husk and ground in a Thermomix to obtain the pomegranate juices.
- iii Immediately after preparation, purée and juice samples were mixed in proportions of 40/60 and 60/40, respectively, obtaining 12 samples (Table 1). Then, the products were heated to 100 °C, placed in glass jars (130 mL) and pasteurized (10 min at 90 °C).

### Physicochemical parameters

Titrate acidity (TA) was determined by titrating sample aliquots using 0.1 mol L<sup>-1</sup> NaOH to pH 8.1 using an automatic pH titration system (Schott Titroline 7500 KF volumetric KF titrator, Mainz, Germany) and results were expressed as grams of malic acid in 100 g. Total soluble solids (TSS, °Brix) were measured using a refractometer (PAL-88S; Atago Co., Tokyo, Japan). Color coordinates (*L\**, *a\**, *b\**) were determined by reflectance measurement with a Color Quest XE Hunter Lab colorimeter (Illuminant D65 and 10° observer angle).

### Chemical composition

#### Mineral content

Approximately 1 g of each smoothie (after appropriate homogenization) was digested using a START D medium microwave digestion system (SK-10) with 8 mL concentrated HNO<sub>3</sub> and 2 mL

**Table 1.** Formulation of smoothies consisting of pomegranate juice and figs, jujubes or quince purées

| No. | Code <sup>a</sup> | Formulation <sup>b</sup> |
|-----|-------------------|--------------------------|
| 1   | F1Mo              | 40% F + 60% Mo           |
| 2   | F1W               | 40% F + 60% W            |
| 3   | F2Mo              | 60% F + 40% Mo           |
| 4   | F2W               | 60% F + 40% W            |
| 5   | J1Mo              | 40% J + 60% Mo           |
| 6   | J1W               | 40% J + 60% W            |
| 7   | J2Mo              | 60% J + 40% Mo           |
| 8   | J2W               | 60% J + 40% W            |
| 9   | Q1Mo              | 40% Q + 60% Mo           |
| 10  | Q1W               | 40% Q + 60% W            |
| 11  | Q2Mo              | 60% Q + 40% Mo           |
| 12  | Q2W               | 60% Q + 40% W            |

<sup>a</sup> Mo, 'Mollar de Elche' pomegranate juice; W, 'Wonderful' pomegranate juice; F, fig purée; J, jujube purée; Q, quince purée.

<sup>b</sup> The percentage of each component was expressed as weight (w/w).

H<sub>2</sub>O<sub>2</sub> (30%). Determination of macronutrients (Ca, Mg and K) and micronutrients (Zn) in the previously mineralized samples was performed using a Unicam Solaar 969 atomic absorption– emission spectrometer (Unicam Ltd, Cambridge, UK). All minerals were analyzed using atomic absorption, except K, which was measured using atomic emission. Calibration curves were prepared for the quantification of minerals and showed good linearity ( $R^2 \geq 0.999$ ).

#### Pectin and vitamin C content

Pectin content was analyzed according to the Morris method, as previously described.<sup>7</sup> Vitamin C was analyzed using high-performance liquid chromatography (HPLC), as described by Wojdyto *et al.*<sup>12</sup>

#### Sugar and organic acid content

Each sample was mixed with distilled water (50 mL) and boiled for 30 min, then centrifuged at 20 000 × *g* for 10 min. The supernatant was filtered using SEP-PAK C18 (1 g; Waters, Milford, MA, USA). HPLC-ELSD (HPLC with evaporative light scattering detection) conditions used in the analysis of the sugars in smoothies were the same as reported by Nowicka *et al.*<sup>7</sup> Sugars were identified by comparison with the standards of fructose, sorbitol, glucose and sucrose. Calibration curves were prepared in the range 0.5 – 5 mg mL<sup>-1</sup> and linearity was good ( $R^2 \geq 0.9998$ ).

HPLC-PDA (HPLC with photodiode array detector) conditions used in the analysis of the organic acids in smoothies were as reported by Nowicka *et al.*<sup>7</sup> Organic acids were identified by comparison with standards of oxalic, malic, citric, tartaric, quinic, shikimic, succinic and formic acids. Calibration curves were prepared in the range 0.05 – 0.5 mg mL<sup>-1</sup> ( $R^2 \geq 0.9998$ ).

#### Phytochemical composition and antioxidant capacity

##### Polyphenols and procyanidins

The extraction of polyphenols was conducted using the conditions reported by Wojdyto *et al.*<sup>12</sup> Compounds were identified using fast liquid chromatography quadrupole time-of-flight mass spectrometry (LC-MS QToF) and quantified using ultra-performance liquid chromatography with photodiode array and fluorescence

detection (UPLC-PDA-FL). The chromatographic conditions for the identification and quantification of anthocyanin, flavan-3-ols, flavonol and hydroxycinnamic acids have been previously reported by Wojdyto *et al.*<sup>18</sup> The analysis of polymeric procyanidins by the phloroglucinol method was performed according to the protocol described previously by Kennedy and Jones.<sup>19</sup>

#### ORAC assay

Extraction of compounds with antioxidant activity was conducted according to a previous study.<sup>9</sup> The ORAC assay was prepared as described by Ou *et al.*<sup>20</sup> and an RF-5301 PC spectrofluorometer (Shimadzu, Kyoto, Japan) was used.

#### Statistical analysis

A three-way analysis of variance (ANOVA) was performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD, USA), and means were separated by Tukey's multiple range test. Instrumental parameters correlated with sensory descriptors were used for establishing a principal component analysis (PCA) regression map using XLSTAT Premium 2016. All analyses were performed in triplicate.

## RESULTS AND DISCUSSION

### Physicochemical parameters

Table 2 shows the main physicochemical parameters of the smoothies under evaluation. TSS ranged between 14.5 and 18.6 °Brix, the type of fruit purée being the only factor causing significant differences ( $P < 0.001$ ), with fig and quince purées leading to the highest and lowest TSS values, 18.0 and 15.8 °Brix, respectively. Results agreed with those of previous studies, showing that TSS increased in the order quince < jujube < fig.<sup>21–23</sup> pH and TA ranges were 3.31 – 4.52 and 0.28 – 1.77 g malic acid 100 g<sup>-1</sup>, respectively. Two factors – pomegranate cultivar and type of fruit purée – significantly affected the pH and TA values ( $P < 0.01$ ). The use of pomegranate 'Wonderful' resulted in reduced pH (3.47) and increased TA (1.23 g malic acid 100 g<sup>-1</sup>) as compared to 'Mollar de Elche' fruits (4.09 and 0.53 g malic acid 100 g<sup>-1</sup>, respectively). These experimental findings agreed with previous data for fresh pomegranate fruits.<sup>24</sup>

Moreover, CIE  $L^*a^*b^*$  color coordinates were significantly affected by all factors under study (purée:juice ratio, pomegranate cultivar and type of fruit purée). Taking into account that one of the most valued quality parameters of pomegranate-based products is the intensity of green–red coordinate ( $a^*$ ), all research regarding the development of novel pomegranate products<sup>25–27</sup> has focused on avoiding the loss of red color. Regarding color, the most important conclusion in the current study was that the combination leading to the most intense red color of the smoothies was that consisting of 60% pomegranate juice from cv. 'Wonderful' combined with quince purée.

### Chemical composition

#### Minerals

Calcium (Ca), magnesium (Mg), and zinc (Zn) were mainly affected by the fruit purée (Table 3). An enrichment of Ca content was noted in smoothies prepared using figs (mean value 13.8 mg 100 g<sup>-1</sup> FW). Results agreed with the values of the United States Department of Agriculture (USDA) database, which indicates that figs present higher Ca content (35 mg 100 g<sup>-1</sup>) than jujubes and quinces (21 and 11 mg 100 g<sup>-1</sup>, respectively).<sup>28</sup> Regarding Mg and



**Table 2.** Physicochemical parameters (total soluble solids, TSS ( $^{\circ}$ Brix); pH; titratable acidity, TA (g malic acid 100 g $^{-1}$ ); and instrumental color) of the smoothies under analysis

| Sample   | TSS ( $^{\circ}$ Brix) | pH    | TA (g malic acid 100 g $^{-1}$ ) | CIE $L^*a^*b^*$ coordinates |       |       |
|--|------------------------|-------|----------------------------------|-----------------------------|-------|-------|
|  |                        |       |                                  | $L^*$                       | $a^*$ | $b^*$ |
| F1Mo   | 17.6                   | 4.51  | 0.66                             | 36.1                        | 8.12  | 5.30  |
| F1W  | 18.1                   | 3.56  | 0.92                             | 35.6                        | 15.3  | 3.85  |
| F2Mo   | 17.8                   | 4.52  | 0.28                             | 36.3                        | 8.39  | 6.39  |
| F2W  | 18.6                   | 3.62  | 1.77                             | 35.6                        | 12.8  | 4.82  |
| J1Mo   | 16.4                   | 4.06  | 0.35                             | 47.3                        | 8.56  | 9.20  |
| J1W  | 17.4                   | 3.40  | 0.97                             | 42.7                        | 19.0  | 4.58  |
| J2Mo   | 16.9                   | 4.18  | 0.35                             | 51.4                        | 7.26  | 12.7  |
| J2W  | 18.1                   | 3.57  | 1.72                             | 45.6                        | 16.4  | 7.09  |
| Q1Mo   | 16.4                   | 3.70  | 0.44                             | 50.8                        | 14.9  | 6.70  |
| Q1W  | 16.9                   | 3.31  | 1.04                             | 45.1                        | 24.3  | 2.71  |
| Q2Mo   | 14.5                   | 3.58  | 1.07                             | 55.8                        | 13.4  | 8.54  |
| Q2W  | 15.5                   | 3.38  | 0.98                             | 51.5                        | 20.4  | 4.90  |
| <b>ANOVA test<sup>a</sup></b>                  |                        |       |                                  |                             |       |       |
| Purée:juice ratio                              | NS                     | NS    | NS                               | NS                          | *     | *     |
| Pomegranate cultivar                           | NS                     | **    | **                               | NS                          | **    | **    |
| Purée fruit                                    | **                     | **    | NS                               | ***                         | **    | **    |
| <b>Tukey's multiple range test<sup>b</sup></b> |                        |       |                                  |                             |       |       |
| <b>Purée:juice ratio (% w/w)</b>               |                        |       |                                  |                             |       |       |
| 40:60  | 17.1                   | 3.76  | 0.73                             | 42.9                        | 15.0a | 5.39b |
| 60:40  | 16.9                   | 3.80  | 1.03                             | 46.0                        | 13.1b | 7.41a |
| <b>Pomegranate cultivar</b>                    |                        |       |                                  |                             |       |       |
| 'Mollar de Elche'                              | 16.6                   | 4.09a | 0.53b                            | 46.3                        | 10.1b | 8.13a |
| 'Wonderful'                                    | 17.4                   | 3.47b | 1.23a                            | 42.7                        | 18.3a | 4.66b |
| <b>Purée fruit</b>                             |                        |       |                                  |                             |       |       |
| Fig  | 18.0a                  | 4.05a | 0.91                             | 35.9c                       | 11.2b | 5.09b |
| Jujube   | 17.2a                  | 3.80b | 0.85                             | 46.8b                       | 12.8b | 8.39a |
| Quince   | 15.8b                  | 3.49b | 0.88                             | 50.8a                       | 18.3a | 5.71b |

<sup>a</sup> NS, not significant at  $P < 0.05$ ; asterisks indicate significance at \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

<sup>b</sup> Values (mean of three replications) followed by the same letter, within the same factor (purée:juice ratio, pomegranate cultivar and purée fruit), were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test.

Zn, the highest contents were obtained when using quince purée. A previous study indicated the lower content of Mg and Zn in figs than jujube.<sup>29</sup>

On the other hand, the content of the predominant mineral, potassium (K), was affected by all three studied factors (Table 3). The amount of K was higher in smoothies with 60% purée (191 mg 100 g $^{-1}$ ), with 'Wonderful' pomegranate juice (205 mg 100 g $^{-1}$ ) and with fig purée (197 mg 100 g $^{-1}$ ).

#### Pectin and vitamin C

Pectin – soluble dietary fiber – has been associated with anti-cancer activity and with a reduction of cholesterol and serum glucose.<sup>30</sup> Pectin content increased when using the high-est content of fruit purée, as previously concluded by Nowicka *et al.*<sup>7</sup> On the other hand, the use of jujube purée led to the lowest pectin content (0.39 g 100 g $^{-1}$ ). Thus the use of both fig and quince purées would be a good option for consumers with diabetes and obesity. Moreover, pectin presents good techno-functional properties contributing to proper texture of the smoothies by the reaction of certain water-soluble pectic substances with Ca to form calcium pectates.

With respect to vitamin C, the results indicated a higher content when using a purée:juice ratio of 60:40. But the most relevant

factor controlling the vitamin C content of smoothies was the type of purée fruit, with jujubes leading to the highest content: 190 mg 100 g $^{-1}$  FW. This value is equivalent to more than three times the dietary reference value (DRV; 60 mg d $^{-1}$ ).<sup>31</sup> This experimental finding is based on the high vitamin C content of jujubes (387 – 555 mg 100 g $^{-1}$  FW).<sup>32</sup>

#### Sugars

Four sugars (fructose, sorbitol, glucose and sucrose) were identified and quantified in the smoothies under evaluation (Table 4). A significant increase ( $P < 0.05$ ) of all mentioned sugars was observed when the purée:juice ratio was 60:40; i.e. the higher the content of the fruit purée, the higher was the vitamin C content. Using 'Mollar de Elche' pomegranates significantly increased the content of fructose and glucose, which agreed with previous data.<sup>24</sup> The glucose:fructose ratio indicated previously for pomegranate juice (0.80 – 0.95) was maintained in the pomegranate-based smoothies (mean value: 0.85).<sup>13</sup>

According to previous studies,<sup>11</sup> the use of quince purée enriched the smoothies in fructose; this is important because fructose has a strong sweetening power (1.2 – 1.5 times that of sucrose). Moreover, sorbitol (a non-nutritive polyol) was found only in the quince-based smoothies. Although functional

**Table 3.** Mineral elements, pectin, and vitamin C contents in the smoothies under analysis

| Sample   | Minerals                    |        |       |       | Pectin<br>(g 100 g <sup>-1</sup> FW) | Vitamin C<br>(mg 100 g <sup>-1</sup> FW) |
|--|-----------------------------|--------|-------|-------|--------------------------------------|--|
|  | Ca                          | Mg     | K     | Zn    |                                      |  |
|  | (mg 100 g <sup>-1</sup> FW) |        |       |       |                                      |  |
| F1Mo   | 12.2                        | 3.73   | 174   | 0.08  | 1.08                                 | 1.02                                     |
| F1W  | 12.7                        | 6.26   | 205   | 0.14  | 1.08                                 | 1.66                                     |
| F2Mo   | 13.8                        | 4.06   | 175   | 0.08  | 1.21                                 | 1.18                                     |
| F2W  | 17.6                        | 9.76   | 235   | 0.18  | 1.11                                 | 1.18                                     |
| J1Mo   | 3.36                        | 5.84   | 149   | 0.22  | 0.06                                 | 148                                      |
| J1W  | 8.59                        | 9.53   | 199   | 0.22  | 0.55                                 | 157                                      |
| J2Mo   | 8.33                        | 9.60   | 170   | 0.22  | 0.23                                 | 230                                      |
| J2W  | 8.47                        | 6.73   | 193   | 0.15  | 0.70                                 | 223                                      |
| Q1Mo   | 3.33                        | 8.59   | 166   | 0.14  | 0.88                                 | 1.02                                     |
| Q1W  | 7.59                        | 7.62   | 187   | 0.22  | 1.31                                 | 1.01                                     |
| Q2Mo   | 4.87                        | 12.2   | 166   | 0.24  | 1.19                                 | 0.70                                     |
| Q2W  | 6.02                        | 14.6   | 209   | 0.22  | 1.33                                 | 1.06                                     |
| <b>ANOVA test<sup>a</sup></b>                  |                             |        |       |       |                                      |  |
| Purée:juice ratio                              | NS                          | NS     | *     | NS    | *                                    | **                                       |
| Pom. cultivar                                  | NS                          | NS     | ***   | NS    | NS                                   | NS                                       |
| Purée fruit                                    | ***                         | **     | *     | ***   | **                                   | ***                                      |
| <b>Tukey's multiple range test<sup>b</sup></b> |                             |        |       |       |                                      |  |
| <b>Ratio purée: juice (%:%, w:w)</b>           |                             |        |       |       |                                      |  |
| Ratio 40:60                                    | 7.96                        | 6.93   | 180b  | 0.17  | 0.83b                                | 51.6b                                    |
| Ratio 60:40                                    | 9.67                        | 9.49   | 191a  | 0.18  | 0.96a                                | 79.2a                                    |
| <b>Pomegranate cultivar</b>                    |                             |        |       |       |                                      |  |
| 'Mollar de Elche'                              | 7.46                        | 7.33   | 167 b | 0.16  | 0.90                                 | 63.7                                     |
| 'Wonderful'                                    | 10.2                        | 9.09   | 205 a | 0.19  | 1.01                                 | 64.2                                     |
| <b>Purée fruit</b>                             |                             |        |       |       |                                      |  |
| Fig  | 13.8a                       | 5.95b  | 197a  | 0.12b | 1.12a                                | 1.26b                                    |
| Jujube   | 7.19b                       | 7.92ab | 178b  | 0.20a | 0.39b                                | 190a                                     |
| Quince   | 5.45b                       | 10.8a  | 182ab | 0.21a | 1.18a                                | 0.95b                                    |

<sup>a</sup> NS, not significant at  $P < 0.05$ ; asterisks indicate significance at \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

<sup>b</sup> Values (mean of three replications) followed by the same letter, within the same factor (purée:juice ratio, pomegranate cultivar and purée fruit), were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test.

gastrointestinal problems can appear in fructose malabsorption and sorbitol-intolerant consumers, the content of these two sugars did not reach critical values, and were always well below the thresholds established in previous studies reporting severe symptoms after sugar intake (25 and 5 g, respectively).<sup>33</sup> On the other hand, fig and jujube purées increased the content of glucose and sucrose, respectively.

#### Organic acids

Eight organic acids were identified and quantified in the smoothie samples: oxalic, malic, citric, tartaric, quinic, shikimic, succinic and formic acids (Table 4). The occurrence of different organic acids will significantly influence the fruits' color (depending on pH) and their taste, through the sugar:organic acid ratio.<sup>34</sup>

The factor with the most important influence on the organic acid profile was the type of fruit used to prepare the purées; also the pomegranate cultivar affected the content of two acids. The use of jujube led to the highest content of citric, tartaric, shikimic and formic acids, while quince led to high content of oxalic and quinic acids. The most relevant effect of the pomegranate cultivar was that the use of 'Wonderful' fruits significantly increased the content of tartaric acid, as previously shown in fresh fruits.<sup>35</sup>

Regarding the antinutrient character of some organic acids, it is worth mentioning that phytic acid was not found in any of the smoothies under analysis; this acid has strong chelating ability associated with cations such as Ca<sup>2+</sup> and Mg<sup>2+</sup>.<sup>36</sup> Moreover, oxalic acid, which inhibits the mineral bioavailability and forms calcium oxalates in urinary stones,<sup>37</sup> was found in all samples but in low quantities (0.06–0.18 g 100 g<sup>-1</sup> FW), none presenting antinutrient activity.

#### Identification and quantification of phenolic compounds

##### Anthocyanins (ACNs)

Table 5 shows the identification and quantification of ACNs (five compounds): delphinidin-3,5-di-O-glucoside (A1; [M-H]<sup>-</sup> at  $m/z = 627.16$ ; MS/MS fragment at  $m/z = 303.06$ , and retention time,  $R_t = 2.68$  min); cyanidin-3,5-di-O-glucoside (A2; 611.17; 287.06; 3.20 min); delphinidin-3-O-glucoside (A3; 465.11; 303.06; 3.77 min); cyanidin-3-O-glucoside (A4; 449.11; 287.06; 4.26 min); and pelargonidin-3-O-glucoside; (A5; 433.11; 271.06; 4.73 min).

Previously, ACN profiles were studied in smoothies based on berry fruits, such as blackcurrant, cranberry and chokeberry (representing 9 – 45% of total polyphenolic content)<sup>38</sup> and based on *Prunus* fruits, such as peach, apricot and plum (representing 0–26% of total polyphenolic content).<sup>7</sup> In the smoothies under

**Table 4.** Sugar and organic acid content in the smoothies under analysis

| Sample   | Sugars                     |          |         |         | Organic acids |       |        |          |        |          |          |        |
|--|----------------------------|----------|---------|---------|---------------|-------|--------|----------|--------|----------|----------|--------|
|  | Fructose                   | Sorbitol | Glucose | Sucrose | Oxalic        | Malic | Citric | Tartaric | Quinic | Shikimic | Succinic | Formic |
|  | (g 100 g <sup>-1</sup> FW) |          |         |         |               |       |        |          |        |          |          |        |
| F1Mo   | 3.87                       | nd       | 5.28    | nd      | 0.06          | nd    | 0.08   | 0.30     | 0.88   | nd       | 0.07     | 0.33   |
| F1W  | 4.30                       | nd       | 6.48    | nd      | 0.06          | nd    | 0.07   | 1.56     | 0.66   | nd       | 0.05     | 0.24   |
| F2Mo   | 7.38                       | nd       | 9.66    | nd      | 0.06          | nd    | 0.10   | 0.33     | 0.87   | nd       | 0.07     | 0.36   |
| F2W  | 3.84                       | nd       | 6.04    | nd      | 0.06          | nd    | 0.08   | 1.34     | 0.74   | nd       | 0.07     | 0.24   |
| J1Mo   | 3.92                       | nd       | 5.02    | 0.42    | 0.08          | 0.01  | 0.14   | 0.36     | 0.79   | 0.02     | 0.01     | 0.37   |
| J1W  | 3.08                       | nd       | 4.85    | 0.28    | 0.06          | 0.01  | 0.17   | 1.66     | 0.65   | 0.03     | 0.01     | 0.23   |
| J2Mo   | 5.35                       | nd       | 7.49    | 0.89    | 0.06          | 0.01  | 0.20   | 0.52     | 0.77   | 0.04     | 0.01     | 0.38   |
| J2W  | 3.83                       | nd       | 5.79    | 0.68    | 0.05          | 0.01  | 0.20   | 1.48     | 0.69   | 0.03     | 0.01     | 0.27   |
| Q1Mo   | 5.56                       | 0.36     | 5.95    | 0.00    | 0.14          | 0.01  | nd     | 0.11     | 1.11   | nd       | 0.01     | nd     |
| Q1W  | 3.71                       | 0.45     | 3.85    | 0.04    | 0.14          | 0.01  | nd     | 1.31     | 0.95   | nd       | 0.01     | nd     |
| Q2Mo   | 7.96                       | 0.62     | 3.41    | 0.00    | 0.18          | 0.01  | nd     | 0.09     | 1.38   | nd       | 0.02     | nd     |
| Q2W  | 8.32                       | 0.67     | 7.73    | 0.09    | 0.18          | 0.01  | nd     | 0.89     | 1.14   | nd       | 0.01     | nd     |
| <b>ANOVA test<sup>a</sup></b>                  |                            |          |         |         |               |       |        |          |        |          |          |        |
| Purée:juice ratio                              | **                         | *        | **      | *       | NS            | NS    | NS     | NS       | NS     | NS       | NS       | NS     |
| Pom. cultivar                                  | *                          | NS       | *       | NS      | NS            | NS    | NS     | ***      | NS     | NS       | NS       | ***    |
| Purée fruit                                    | ***                        | ***      | **      | **      | ***           | ***   | ***    | *        | ***    | ***      | ***      | ***    |
| <b>Tukey's multiple range test<sup>b</sup></b> |                            |          |         |         |               |       |        |          |        |          |          |        |
| <b>Purée:juice ratio (% w/w)</b>               |                            |          |         |         |               |       |        |          |        |          |          |        |
| Ratio 40:60                                    | 4.07b                      | 0.41b    | 5.24b   | 0.19b   | 0.09          | 0.01  | 0.12   | 0.88     | 0.84   | 0.03     | 0.03     | 0.29   |
| Ratio 60:40                                    | 6.11a                      | 0.65a    | 6.69a   | 0.42a   | 0.10          | 0.01  | 0.15   | 0.78     | 0.93   | 0.04     | 0.03     | 0.31   |
| <b>Pomegranate cultivar</b>                    |                            |          |         |         |               |       |        |          |        |          |          |        |
| 'Mollar de Elche'                              | 5.67a                      | 0.49     | 6.14a   | 0.33    | 0.10          | 0.01  | 0.13   | 0.29b    | 0.97   | 0.03     | 0.03     | 0.36a  |
| 'Wonderful'                                    | 4.51b                      | 0.56     | 5.79b   | 0.27    | 0.09          | 0.01  | 0.13   | 1.37a    | 0.81   | 0.03     | 0.03     | 0.25b  |
| <b>Purée fruit</b>                             |                            |          |         |         |               |       |        |          |        |          |          |        |
| Fig  | 4.85b                      | 0.00b    | 6.87a   | 0.00b   | 0.06b         | 0.00b | 0.08b  | 0.88ab   | 0.79b  | 0.00b    | 0.07a    | 0.29a  |
| Jujube   | 4.05b                      | 0.00b    | 5.79ab  | 0.57a   | 0.06b         | 0.01a | 0.18a  | 1.01a    | 0.73b  | 0.03a    | 0.01b    | 0.31a  |
| Quince   | 6.39a                      | 0.41a    | 5.24b   | 0.03b   | 0.16a         | 0.01a | 0.00c  | 0.60b    | 1.15a  | 0.00b    | 0.01b    | 0.00b  |

<sup>a</sup> NS, not significant at  $P < 0.05$ ; asterisks indicate significance at  $*P < 0.05$ ,  $**P < 0.01$  and  $***P < 0.001$ .

<sup>b</sup> Values (mean of 3 replications) followed by the same letter, within the same factor (ratio purée: juice, pomegranate cultivar, and purée fruit), were not significantly different ( $P < 0.05$ ), according to Tukey's least significant difference test.

study, the content of total ACNs ranged from 2.20 mg 100 g<sup>-1</sup> FW in the smoothie sample Q1Mo to 103 mg 100 g<sup>-1</sup> FW in the sample F1W. ACNs were significantly affected by the type of fruit purée, with fig smoothies having the highest content in all ACNs. In partial agreement with previous data,<sup>9</sup> the compounds delphinidin-3-*O*-glucoside (A3) and pelargonidin-3-*O*-glucoside (A5) were only detected when fig purée was included in the formulation. The current study is the first to state that ACNs were found in quince- and jujube-based products.<sup>38</sup> On the other hand, the factor of pomegranate cultivar only affected the content of two out of five ACNs, with 'Wonderful' fruits increasing the content of delphinidin-3,5-di-*O*-glucoside (A1) and cyanidin-3,5-di-*O*-glucoside (A2). According to other authors,<sup>35</sup> the ACNs identified are common compounds of pomegranate juices and their pomegranate-based products, but with the profiles being different for 'Mollar de Elche' and 'Wonderful' fruits. Moreover, the purée:juice ratio of 40:60 increased cyanidin-3,5-di-*O*-glucoside (A2), while the 60:40 ratio increased delphinidin-3-*O*-glucoside (A3).

Several biological activities of the ACNs have been reported in *in vivo* and *in vitro* studies: improvement of blood lipid metabolism, prevention of the formation and development of atherosclerosis, anti-inflammatory response, prevention of DNA damage, etc.<sup>39–41</sup>

It is worth mentioning, however, that a recent review<sup>42</sup> indicated that the chemical structure of ACNs, food matrix and the personal inter-individual changes in the microbiota influenced the bio-accessibility and bio-availability of ACNs; thus the beneficial health effects of ACNs not only depend on their content but also on many other factors.

Values of  $a^*$  were not directly correlated with ACN content but were well correlated with the pH of the smoothies. These results agreed with previous data,<sup>26</sup> which indicated that the pH in a food matrix is the main factor controlling the changes in ACN color.<sup>43</sup>

#### Flavonols (Fvs)

Table 5 shows the identification and quantification of Fvs (11 compounds): quercetin-3-*O*-galactoside (Fv1; [M-H]<sup>-</sup> at  $m/z = 601.06$ ; MS/MS fragment at  $m/z = 463.16$ , and  $R_t = 6.30$  min); quercetin-3-*O*-rutoside (Fv2; 609.14; 300.03;  $R_t = 6.60$  min); quercetin-3-*O*-glucoside (Fv3; 433.17; 301.03; 6.84 min); kaempferol-3-*O*-galactoside (Fv4; 447; 284; 8.04 min); kaempferol-3-*O*-rutoside (Fv5; 593.15; 285.05; 7.32 min); quercetin-3-*O*-rutoside-7-*O*-hexoside (Fv6; 771; 301.02; 4.41 min); quercetin-3-*O*-rutoside-7-*O*-pentoside (Fv7; 741.19; 301.02; 5.82 min); quercetin-3-*O*-robinobioside (Fv8; 609.14; 447.09/301.02; 7.02 min); kaempferol-3-*O*-deoxyhexoside-*O*-

**Table 5.** Concentration of anthocyanins and flavonols (mg 100 g<sup>-1</sup> FW) in the smoothies under analysis

| Sample                           | Anthocyanins <sup>a</sup>                |       |       |       |       | Flavonols <sup>b</sup>                   |       |       |       |       |       |       |       |       |       |       |
|----------------------------------|--|-------|-------|-------|-------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                  | A1                                       | A2    | A3    | A4    | A5    | Fv1                                      | Fv2   | Fv3   | Fv4   | Fv5   | Fv6   | Fv7   | Fv8   | Fv9   | Fv10  | Fv11  |
|                                  | (mg 100 g <sup>-1</sup> FW)              |       |       |       |       | (mg 100 g <sup>-1</sup> FW)              |       |       |       |       |       |       |       |       |       |       |
| F1Mo                             | 2.69                                     | 22.6  | 2.71  | 6.52  | 30.5  | nd                                       | 1.72  | 14.4  | Nd    | 0.74  | nd    | nd    | nd    | nd    | 13.6  | 3.28  |
| F1W                              | 9.46                                     | 34.7  | 3.46  | 11.5  | 44.8  | nd                                       | 1.87  | 17.9  | Nd    | 0.79  | nd    | nd    | nd    | nd    | 10.3  | 4.28  |
| F2Mo                             | nd                                       | 10.8  | nd    | 9.42  | 55.7  | nd                                       | 2.67  | 20.8  | Nd    | 1.30  | nd    | nd    | nd    | nd    | 19.7  | 4.58  |
| F2W                              | 11.3                                     | 23.8  | 0.38  | 6.96  | 13.1  | nd                                       | 2.20  | 19.8  | nd    | 1.26  | nd    | nd    | nd    | nd    | 17.2  | 5.06  |
| J1Mo                             | nd                                       | 7.24  | nd    | 3.63  | nd    | 11.7                                     | 12.3  | nd    | nd    | nd    | 0.97  | 5.35  | 2.62  | 11.2  | nd    | nd    |
| J1W                              | 3.72                                     | 16.6  | nd    | 2.64  | nd    | 30.5                                     | 6.37  | nd    | nd    | nd    | 1.46  | 5.41  | 2.17  | 9.54  | nd    | nd    |
| J2Mo                             | nd                                       | nd    | nd    | 4.18  | nd    | 42.4                                     | 9.02  | nd    | nd    | nd    | nd    | 8.11  | 1.64  | 6.23  | nd    | nd    |
| J2W                              | 5.06                                     | 11.3  | nd    | 5.01  | nd    | 41.4                                     | 8.63  | nd    | nd    | nd    | 1.57  | 8.43  | 1.26  | 6.01  | nd    | nd    |
| Q1Mo                             | nd                                       | nd    | nd    | 2.20  | nd    | 3.52                                     | 16.6  | 12.5  | 0.62  | 0.74  | nd    | nd    | nd    | nd    | nd    | nd    |
| Q1W                              | 7.46                                     | 18.5  | nd    | 5.80  | nd    | 3.58                                     | 16.6  | 11.4  | 0.77  | 1.02  | nd    | nd    | nd    | nd    | nd    | nd    |
| Q2Mo                             | nd                                       | nd    | nd    | 2.68  | nd    | 5.72                                     | 23.0  | 12.6  | 0.91  | 1.18  | nd    | nd    | nd    | nd    | nd    | nd    |
| Q2W                              | 4.55                                     | 5.01  | nd    | 3.24  | nd    | 4.79                                     | 22.3  | 10.4  | 1.05  | 1.19  | nd    | nd    | nd    | nd    | nd    | nd    |
|                                  | ANOVA test <sup>c</sup>                  |       |       |       |       | ANOVA test <sup>c</sup>                  |       |       |       |       |       |       |       |       |       |       |
| Purée:juice ratio                | NS                                       | ***   | **    | NS    | NS    | **                                       | NS    | NS    | NS    | NS    | NS    | **    | NS    | *     | *     | NS    |
| Pom. cultivar                    | ***                                      | ***   | NS    | NS    | NS    | **                                       | NS    | NS    | NS    | NS    | NS    | NS    | NS    | NS    | *     | NS    |
| Purée fruit                      | *  | ***   | ***   | **    | **    | ***                                      | ***   | ***   | ***   | ***   | *     | ***   | ***   | ***   | ***   | ***   |
|                                  | Tukey's multiple range test <sup>d</sup> |       |       |       |       | Tukey's multiple range test <sup>d</sup> |       |       |       |       |       |       |       |       |       |       |
| <b>Purée:juice ratio (% w:w)</b> |  |       |       |       |       |  |       |       |       |       |       |       |       |       |       |       |
| Ratio 40:60                      | 3.89                                     | 16.6a | 1.03b | 5.38  | 12.6  | 12.3b                                    | 9.24  | 14.1  | 0.70  | 0.82  | 1.22  | 5.38b | 2.40  | 10.4a | 12.0b | 3.78  |
| Ratio 60:40                      | 3.49                                     | 8.49b | 0.06a | 5.24  | 11.5  | 23.6a                                    | 11.3  | 15.9  | 0.98  | 1.23  | 0.79  | 8.27a | 1.45  | 6.12b | 18.5a | 4.82  |
| <b>Pomegranate cultivar</b>      |  |       |       |       |       |  |       |       |       |       |       |       |       |       |       |       |
| 'Mollar de Elche'                | 0.45b                                    | 6.77b | 0.45  | 4.77  | 9.65  | 15.8b                                    | 9.66  | 15.1  | 0.77  | 0.99  | 0.49  | 6.73  | 2.13  | 8.72  | 16.7a | 3.93  |
| 'Wonderful'                      | 6.93a                                    | 18.3a | 0.64  | 5.86  | 14.4  | 20.1a                                    | 10.9  | 14.9  | 0.91  | 1.07  | 1.52  | 6.92  | 1.72  | 7.78  | 13.8b | 4.67  |
| <b>Purée fruit</b>               |  |       |       |       |       |  |       |       |       |       |       |       |       |       |       |       |
| Fig                              | 5.86a                                    | 22.9a | 1.64a | 8.60a | 36.0a | 0.00c                                    | 2.12c | 18.2a | 0.00b | 1.02a | 0.00b | 0.00b | 0.00b | 0.00b | 15.2a | 4.03a |
| Jujube                           | 2.20b                                    | 8.79b | 0.00b | 3.86b | 0.00b | 31.5a                                    | 9.08b | 0.00c | 0.00b | 0.00b | 1.00a | 6.83a | 1.92a | 8.25a | 0.00b | 0.00b |
| Quince                           | 3.00ab                                   | 5.88b | 0.00b | 3.48b | 0.00b | 4.40b                                    | 19.6a | 11.7b | 0.84a | 1.03a | 0.00b | 0.00b | 0.00b | 0.00b | 0.00b | 0.00b |

<sup>a</sup> A1, delphinidin-3,5-di-O-glucoside; A2, cyanidin-3,5-di-O-glucoside; A3, delphinidin-3-O-glucoside; A4, cyanidin-3-O-glucoside; A5, pelargonidin-3-O-glucoside.

<sup>b</sup> Fv1, quercetin-3-O-galactoside; Fv2, quercetin-3-O-rutinose; Fv3, quercetin-3-O-glucoside; Fv4, kaempferol-3-O-galactoside; Fv5, kaempferol-3-O-rutinose; Fv6, quercetin-3-O-rutinoside-7-O-hexoside; Fv7, quercetin-3-O-rutinoside-7-O-pentoside; Fv8, quercetin-3-O-robinobioside; Fv9, kaempferol-3-O-deoxyhexoside-O-pentoside; Fv10, quercetin-3-O-malonyl-glucoside; Fv11, apigenin-hexoside-pentoside.

<sup>c</sup> Asterisks indicate significance at \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001.

<sup>d</sup> Values (mean of two replicates) followed by the same letter, within the same factor, were not significantly different (*P* < 0.05), according to Tukey's least significant difference test.

pentoside (Fv9; 725.12; 593.9/447.9; 6.48 min); quercetin-3-O-malonyl-glucoside (Fv10; 549.13; 505.10/463.01/301.03; 7.48 min); and apigenin-hexoside-pentoside (Fv11; 563.14; 503.01/473.11/443.09/383.07/269.05; 5.98 min).

In a previous study, the Fv profile was analyzed in smoothies based on different fruits, such as *Prunus* (ranging between 25 and 97 mg 100 g<sup>-1</sup>).<sup>7</sup> The total Fv content of the current smoothies ranged from 33.4 mg 100 g<sup>-1</sup> in sample Q1W to 67.4 mg 100 g<sup>-1</sup> in the sample J2Mo. The profile of Fv content was significantly affected by the type of fruit purée. The only compound detected in all samples was quercetin-3-O-rutinose (Fv2), the highest value being found in the quince smoothies (mean value of 19.6 mg 100 g<sup>-1</sup>), followed by the jujube and fig samples, with values of 9.08 and 2.12 mg 100 g<sup>-1</sup>, respectively. The presence of certain Fvs can be used as an indicator of the type of fruit used in the purée; the compounds Fv6, Fv7, Fv8 and Fv9 were specific to jujube, while Fv10 and Fv11 were specific to fig, and finally Fv4 was specific to

quince. The reported results for the smoothies agreed quite well for the profiles of the fresh fruits (jujubes, figs and quinces).<sup>9,12,32</sup> On the other hand, the factor pomegranate cultivar only affected the content of 2 out of 11 Fvs, with 'Wonderful' juice increasing the content of Fv1, and 'Mollar de Elche' that of Fv10. Finally, a higher content of the fruit purée (60:40 ratio) increased the content of Fv1, Fv7 and Fv10. Probably, the changes were due to different pH values in the final product.

The importance of the different flavonol profiles in the described smoothies is that they can affect their functionality. For instance, quercetin-3-O-rutinose (Fv2) is used in the treatment of various human disorders, such as diabetes mellitus, cancer insurgence, oxidative stress and cardiovascular disorders.<sup>44</sup>

#### Flavan-3-ols (F3os)

Table 6 shows the identification and quantification of F3os (seven compounds): procyanidin B1, PB1 ([M-H]<sup>-</sup> at *m/z* = 577; MS/MS

**Table 6.** Concentrations of flavan-3-ols and hydroxycinnamic acids in the analyzed products

| Sample                           | Flavan-3-ols <sup>a</sup>                |       |       |       |       |       |       | Hydroxycinnamic acids <sup>b</sup>       |                            |       |       |       |       |
|----------------------------------|--|-------|-------|-------|-------|-------|-------|--|----------------------------|-------|-------|-------|-------|
|                                  | C  | PB1   | E     | PB2   | P3    | P4    | PP    | NCh                                      | 4op                        | Ch    | 4cq   | cq    | Dcq   |
|                                  | (mg 100g <sup>-1</sup> FW)               |       |       |       |       |       |       | (g 100g <sup>-1</sup> FW)                | (mg 100g <sup>-1</sup> FW) |       |       |       |       |
| F1Mo                             | 49.7                                     | nd    | 128   | nd    | nd    | nd    | 1.40  | nd                                       | nd                         | 2.23  | nd    | nd    | nd    |
| F1W                              | 33.4                                     | nd    | 154   | nd    | nd    | nd    | 1.36  | nd                                       | nd                         | 1.52  | nd    | nd    | nd    |
| F2Mo                             | 26.8                                     | nd    | 194   | nd    | nd    | nd    | 1.80  | nd                                       | nd                         | 1.35  | nd    | nd    | nd    |
| F2W                              | 29.4                                     | nd    | 194   | nd    | nd    | nd    | 1.85  | nd                                       | nd                         | 1.38  | nd    | nd    | nd    |
| J1Mo                             | 96.0                                     | 51.0  | nd    | 23.8  | 43.0  | 8.28  | 28.8  | nd                                       | nd                         | nd    | nd    | nd    | nd    |
| J1W                              | 93.5                                     | 70.4  | nd    | 41.1  | 71.2  | 7.36  | 28.8  | nd                                       | nd                         | nd    | nd    | nd    | nd    |
| J2Mo                             | 162                                      | 115   | nd    | 27.3  | 34.7  | 9.86  | 34.8  | nd                                       | nd                         | nd    | nd    | nd    | nd    |
| J2W                              | 199                                      | 72.7  | nd    | 34.6  | 32.8  | 7.87  | 38.2  | nd                                       | nd                         | nd    | nd    | nd    | nd    |
| Q1Mo                             | 31.7                                     | 192   | nd    | nd    | nd    | nd    | 36.1  | 53.5                                     | 3.63                       | 89.1  | 3.35  | 1.92  | 6.27  |
| Q1W                              | 32.4                                     | 192   | nd    | nd    | nd    | nd    | 38.7  | 60.3                                     | 3.93                       | 98.6  | 3.83  | 2.31  | 7.20  |
| Q2Mo                             | 22.7                                     | 279   | nd    | nd    | nd    | nd    | 50.6  | 88.5                                     | 2.94                       | 108   | 1.80  | 1.41  | 6.36  |
| Q2W                              | 52.2                                     | 258   | nd    | nd    | nd    | nd    | 49.3  | 82.2                                     | 3.72                       | 105   | 1.66  | 1.82  | 6.16  |
|                                  | ANOVA test <sup>c</sup>                  |       |       |       |       |       |       | ANOVA test <sup>c</sup>                  |                            |       |       |       |       |
| Purée:juice ratio                | *  | *     | *     | NS    | NS    | NS    | *     | *  | NS                         | *     | *     | NS    | NS    |
| Pom. cultivar                    | NS                                       | NS    | NS    | *     | *     | NS    | NS    | NS                                       | NS                         | NS    | NS    | NS    | NS    |
| Purée fruit                      | **                                       | ***   | ***   | ***   | ***   | ***   | ***   | ***                                      | ***                        | ***   | ***   | ***   | ***   |
|                                  | Tukey's multiple range test <sup>d</sup> |       |       |       |       |       |       | Tukey's multiple range test <sup>d</sup> |                            |       |       |       |       |
| <b>Purée:juice ratio (% w/w)</b> |  |       |       |       |       |       |       |  |                            |       |       |       |       |
| Ratio 40:60                      | 56.1b                                    | 126b  | 141b  | 32.5  | 57.1  | 7.82  | 22.5b | 56.9b                                    | 3.78                       | 47.9b | 3.59a | 2.12  | 6.74  |
| Ratio 60:40                      | 82.0a                                    | 181a  | 194a  | 31.0  | 33.8  | 8.87  | 29.4a | 85.4a                                    | 3.33                       | 53.9a | 1.73b | 1.62  | 6.26  |
| <b>Pomegranate cultivar</b>      |  |       |       |       |       |       |       |  |                            |       |       |       |       |
| 'Mollar de Elche'                | 64.8                                     | 159   | 161   | 25.6b | 38.9b | 9.07  | 25.6  | 71.0                                     | 3.29                       | 50.2  | 2.58  | 1.67  | 6.32  |
| 'Wonderful'                      | 73.3                                     | 148   | 174   | 37.9a | 52.0a | 7.62  | 26.4  | 71.3                                     | 3.83                       | 51.6  | 2.75  | 2.07  | 6.68  |
| <b>Purée fruit</b>               |  |       |       |       |       |       |       |  |                            |       |       |       |       |
| Fig                              | 34.8b                                    | 0.00c | 168a  | 0.00b | 0.00b | 0.00b | 1.60c | 0.00b                                    | 0.00b                      | 1.62b | 0.00b | 0.00b | 0.00b |
| Jujube                           | 138a                                     | 77.3b | 0.00b | 31.7a | 45.4a | 8.34a | 32.7b | 0.00b                                    | 0.00b                      | 0.00c | 0.00b | 0.00b | 0.00b |
| Quince                           | 34.8b                                    | 230a  | 0.00b | 0.00b | 0.00b | 0.00b | 43.7a | 71.1a                                    | 3.56a                      | 100a  | 2.66a | 1.87a | 6.50a |

<sup>a</sup> C, (+) catechin; PB1, procyanidin B1; E, (-)-epicatechin; PB2, procyanidin B2; 3, procyanidin trimer; P4, procyanidin tetramer; PP, polymeric procyanidins.

<sup>b</sup> NCh, neochlorogenic acid (3-O-caffeoylquinic cid); 4op, 4-O-p-coumaroylquinic acid; Ch, chlorogenic acid (5-O-caffeoylquinic acid); 4cq, cryptochlorogenic acid (4-O-caffeoylquinic acid); Cq, caffeoylquinic acid; Dcq, 3,5-dicaffeoylquinic acid.

<sup>c</sup> Asterisks indicate significance at \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001.

<sup>d</sup> Values (mean of two replicates) followed by the same letter, within the same row, were not significantly different (*P* < 0.05), according to Tukey's least significant difference test.

fragment at *m/z* = 289, and *R*<sub>t</sub> = 3.41 min), (+)-catechin, C (289.07; 3.27 min), (-)-epicatechin, E (289.07; 4.79 min), procyanidin B2, PB2 (577.13; 289.19; 3.50 min), procyanidin trimer, P3 (865.19; 577.13/289.07; 3.73/5.89 min), procyanidin tetramer, P4 (1153.26; 577.13/289.07; 4.16 min). In particular, the catechins have been well established in various *in vitro* and *in vivo* systems as potential agents against lipid peroxidation in low-density lipoprotein (LDL).<sup>45</sup>

The content of all F3os was significantly affected by purée fruit. Jujubes were the fruits leading to the highest total content of F3os, and the main compound in these fruits was (+)-catechin, at 138 mg 100 g<sup>-1</sup>. On the other hand, quinces and figs contributed to high content of procyanidin B1 and (-)-epicatechin, with content of 230 and 168 mg 100 g<sup>-1</sup>, respectively. Previously, (+)-catechin and (-)-epicatechin were the predominant F3os in smoothies based on peach, apricot and plum.<sup>7</sup> Only the compound (+)-catechin was found in all smoothies. On the other hand, the presence of certain F3os can be used as an indicator of the type of fruit used in

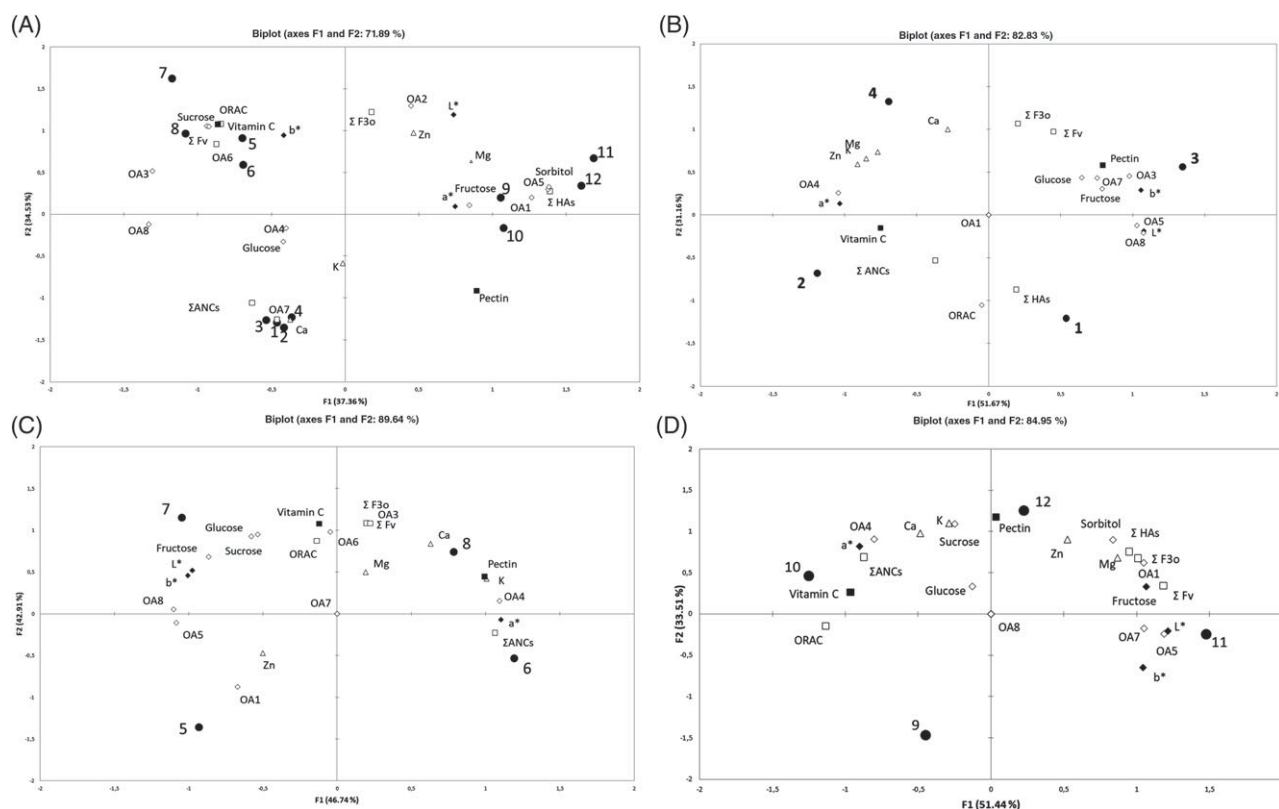
the purée; the compounds PB2, P3 and P4 were specific to jujube, while the compound (-)-epicatechin was specific to figs.

Only the content of two compounds was affected by the pomegranate cultivar, with 'Wonderful' juice leading to the highest content of procyanidin B2 and procyanidin trimer, probably the main reason the value of pH. The previously discussed result for the smoothies agreed quite well with previous data for fresh fruits (jujubes, figs and quinces).<sup>9,12,32</sup>

Additionally, the concentration of polymeric procyanidins (PP) was also evaluated, and it represented the highest contribution to the total content of the flavan-3-ols. This same trend was reported in quinces,<sup>12,46</sup> jujubes,<sup>32,47</sup> and some fig cultivars.<sup>9</sup> The content of PP followed the order quince > jujube >> fig.

#### Hydroxycinnamic acids (HAs)

The last group of polyphenols identified by LC-MS analysis was HAs (six compounds): neochlorogenic acid (NCh; [M-H]<sup>-</sup> at



**Figure 2.** PCA scores plot showing the relationships among color coordinates, chemical composition, antioxidant capacity (ORAC) and phenolic profile of the smoothies under study: (A) all smoothies; (B) fig smoothies; (C) jujube smoothies; and (D) quince smoothies. ♦ : color coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ); ◯ : sugar profile (fructose, sorbitol, glucose, and sucrose) and organic acid profile (OA1, oxalic; OA2, malic; OA3, citric; OA4, tartaric; OA5, quinic; OA6, shikimic; OA7, succinic; OA8, formic) △ : mineral content (Ca, Mg, K, Zn) ■ : pectin and vitamin C content; □ : antioxidant capacity (ORAC assay) and phenolic compounds ( $\Sigma$  ANCs, anthocyanins;  $\Sigma$  Fv, flavonols;  $\Sigma$  F3o, flavan-3-ols;  $\Sigma$  HAs, hydroxycinnamic acids).

$m/z = 353.09$ ; MS/MS fragment at  $m/z = 191.06$  and  $R_t = 3.23$  min), chlorogenic acid (Ch; 353.10; 191.06; 4.14 min), cryptochlorogenic acid (4qc; 353.10; 173.05; 4.42 min), caffeoylquinic acid (cq; 353.14; 137.01; 5.33 min), 3,5-dicaffeoylquinic acid (Dcq; 515; 353/136/182; 8.21 min) and, 4-*O-p*-coumaroylquinic acid (4op; 337; 173/136; 3.13 min) (Table 6).

The identified HAs were significantly affected by the factor purée fruit. Quince smoothies contained all six compounds, with fig samples having only chlorogenic acid, and jujube products having no HAs at all;<sup>32</sup> these profiles are supported by the profiles of fresh quinces and figs.<sup>9,12</sup> Although the absorption of HAs when passing through the human digestive tract depends on the esterase activity on intestine mucosa and the microflora,<sup>48</sup> several healthy properties were shown such as improvements in diabetes, cardiovascular disease, obesity and cancer.<sup>49</sup>

### Antioxidant capacity (AC)

The AC determined by the ORAC assay ranged from 1.75 to 6.48 mmol Trolox 100 g<sup>-1</sup>, with quince purée leading to the highest values of AC (5.78 mmol Trolox 100 g<sup>-1</sup>) as compared to quince and fig purées, at 2.61 and 2.34 mmol Trolox 100 g<sup>-1</sup>, respectively. Pearson's correlation coefficient showed that the ORAC values were positively correlated with the total flavonol content ( $R = 0.77$ ;  $P$ -value < 0.01), flavan-3-ols without PP ( $R = 0.60$ ;  $P$ -value < 0.05), and vitamin C ( $R = 0.98$ ;  $P$ -value < 0.001). No significant ( $P > 0.05$ ) correlation was observed among the ORAC values and the total content of ANCs, PPs and HAs. Moreover, it has been reported that compounds from the flavan-3-ol and flavonol families presented

higher ORAC activity than those from other phenolic families, such as anthocyanins and phenolic acids.<sup>50,51</sup> Contrarily, these authors concluded that vitamin C did not present ORAC activity.<sup>50</sup> In summary, it can be concluded that the ORAC activity of the assayed smoothies was mainly linked to the flavan-3-ols and flavonols.

### Principal component analysis (PCA)

For an easy visualization of all the studied variables of the different smoothies (three factors: purée:juice ratio, pomegranate cultivar and fruit purée), a PCA was run for all 12 smoothies (labelled in the figures as 1 – 12) (Fig. 2A), and for each type of fruit purée: fig (Fig. 2B), jujube (Fig. 2C) and quince (Fig. 2D).

Figure 2(A) shows that the first principal component (F1) explained 37.36% of the total data variance in all smoothies under evaluation, while the second principal component (F2) explained 34.53% of the total variance. The addition of fig purée to the pomegranate smoothies was positively linked with the content of total anthocyanins, succinic acid, and Ca (negative F1 and F2 coordinates). The addition of jujube purée to the pomegranate smoothies was associated with the content of vitamin C, flavonols and sucrose, as well as ORAC activity, and values of the blue–yellow coordinate,  $b^*$  (negative F1 and positive F2 coordinates). Finally, the addition of quince purée was linked to the content of sorbitol, hydroxycinnamic acid, oxalic acid and quinic acid, and values of the green–red coordinate,  $a^*$  (positive values of both F1 and F2 coordinates).

In Fig. 2(B–D), the F1 axis explained 51.61%, 46.14% and 51.44% of the total data variance in the fig (samples 1–4), jujube (samples

5–8) and quince (samples 9–12) smoothies, respectively; while the axis F2 explained 31.16%, 42.31% and 33.51% of the total variance, respectively.

Considering F1 as the dimension explaining the highest percentage of the differences among the samples, here are some comments on the positioning of the samples as affected by the F1 axis:

- In the smoothies prepared with fig purée (Fig. 2B), the 'Mollar de Elche' samples (1 and 3) were positively correlated with the content of fructose, glucose and formic acid, while the 'Wonderful' smoothies (samples 2 and 4) were associated with the content of total anthocyanins, vitamin C and tartaric acid, as well as  $a^*$  values. On the other hand, and considering the F2 axis, the highest percentage of purée in the formulation (samples 3 and 4) showed a positive correlation with the content of flavonols and flavan-3-ols, while a higher percentage of pomegranate juice was linked to higher values of the ORAC activity, and total content of anthocyanins and hydroxycinnamic acids.
- In the jujube smoothies (Fig. 2C), the use of juice of 'Wonderful' pomegranates was linked to high values of the coordinate  $a^*$  and the total anthocyanin content, while the use of 'Mollar de Elche' fruits was related to high fructose and glucose content. On the other hand, and considering the F2 axis, the highest percentage of purée in the formulation (samples 7 and 8) showed a positive correlation with the content of vitamin C, flavonols and flavan-3-ols.
- Finally, Fig. 2(D) shows the main differences among smoothies prepared with quince purée. The use of a higher percentage of the quince purée, 60% (samples 11 and 12), was linked to high content of total flavonols, flavan-3-ols and hydroxycinnamic acids, and high values of both coordinates  $L^*$  and  $b^*$ . Also, the use of 'Wonderful' juice (samples 10 and 12) was linked again to high values of the green– red coordinate,  $a^*$ , and the total content of anthocyanins.

## CONCLUSIONS

A positive effect of the addition of minor crops (fig, jujube and quince) was observed on the nutritional and functionality of novel pomegranate smoothies. The antioxidant capacity of the smoothies (ORAC assay) was mainly controlled by flavan-3-ol and flavonols. Moreover, the addition of jujube contributed to an enrichment of the final smoothies in vitamin C and organic acids, while an increase of pectin content was found in fig and quince pomegranate-based smoothies. On the other hand, the most valued quality parameter of pomegranate products, red color ( $a^*$  coordinate), was not negatively affected at all, and even it was improved in quince pomegranate-based smoothies and when 'Wonderful' pomegranate juice was used as compared to the 'Mollar de Elche' fruits. Therefore, after knowing the nutritional and functional information of the developed products, it is worth continuing research in this area to elucidate their sensory profiles, their behavior during storage and their consumer acceptance. Moreover, the blend of minor Mediterranean crop purées with pomegranate juice to produce smoothies is a good strategy to promote the consumption of these healthy but underutilized fruits.

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## **7.7 Publication 7**

### **Publication 7 (Literal transcription):**

**Formulation and storage effects on pomegranate smoothie phenolic composition, antioxidant capacity and colour**

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# Formulation and storage effects on pomegranate smoothie phenolic composition, antioxidant capacity and color

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## ABSTRACT

Smoothies are an increasingly popular way of consuming fruits and the industry is focusing on the increment of shelf life and the maintenance original color and the content of bioactive compounds. The aim of the present study was to evaluate how formulation and storage conditions (6 months at 4 or 20 °C) of different pomegranate smoothies affected on functional compounds. Phenolic compounds, antioxidant capacity (ABTS, FRAP) and color of 12 different smoothies were studied. The study was completed evaluating the effect of ratio purée:juice (60:40 or 40:60), pomegranate cultivar (*Mollar de Elche* or *Wonderful*) and fruit purée (quince, jujube, or fig) on studied smoothies. The smoothies before storage presented high values of total polyphenolic content (TPC): 247–314 mg/100 g fresh weight (fw), 2939–3920 mg/100 g fw, and 3809–5324 mg/100 g fw, in fig, jujube and quinces pomegranate smoothies, respectively. A positive effect of the 40:60 ratio purée:juice, the *Wonderful* pomegranate juice storing at 4 °C was found on total polyphenolic content [sum of anthocyanins, flavanols, flavan-3-ols (as monomeric and dimeric), polymeric procyanidins and phenolic acids] and quality of smoothies ( $a^*$  coordinate) being only a reduction of 30.1%, 13.1% and 9.5% in fig, jujube and quinces smoothies, respectively.

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*Mollar de Elche*

*Wonderful*

Anthocyanins

Polymeric procyanidins

FRAP

ABTS

## 1. Introduction

In the last years, smoothies are an increasingly popular way of consuming fruits. They are a non-alcoholic creamy drink made from fruit purée and fruit juice (or less commonly vegetables), and optionally ice, yoghurt and/or milk. Fruits and vegetables are rich in polyphenols, which do not only play physiological roles in plants but also act as antioxidants by donating a hydrogen atom or an electron to other compounds, scavenging free radicals, quenching singlet oxygen, and maintaining a balance between oxidants and antioxidants to improve human health (Wolfe, Wu, & Liu, 2003). Various studies concluded that high consumption of fruits and vegetables promotes health, and it is associated with reduced risk of degenerative diseases (Miller et al., 2017). Therefore, smoothies are one of the many ways that consumers have to include fruits and vegetables in their diets (Castillejo, Martínez-Hernández, Gómez, Artés, & Artés-Hernández, 2016).

The shelf life of non-pasteurized smoothies is relatively short due to microbial growth as a result of the minimal level of processing associated with these products. These are normally consumed fresh or

preserved for short periods (1–3 weeks) by storing them under refrigeration. Besides, storage time can affect the color and polyphenolic composition of the smoothies. Some researchers suggested that it could be worth to use a mild thermal pasteurization (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2010) or a thermal and high hydrostatic pressure processing to increase their shelf life and for better color retention, polyphenols and other quality attributes, guaranteeing consumers acceptance and food safety (Keenan, Brunton, Gormley, & Butler, 2011; Tiwari, 2018, pp. 261–278).

There are some pomegranate cultivars which extreme sourness precludes their fresh consumption, although they are rich in functional ingredients; similarly, several quince cultivars, especially the most known cannot be used as fresh fruits because of their excessive astringency (Szychowski, Munera-Picazo, Szumny, Carbonell-Barrachina, & Hernández, 2014). However, these non-edible-as-fresh fruits could be used as fruit purée to develop novel products, such as smoothies, and will have intense and interesting flavor and taste, and will help in getting a high consumer acceptance. Pomegranate fruit contains many phenolic compounds including flavonoids - anthocyanins, and other

complex flavanoids and hydrolyzable tannins (punicalagin, gallic acid, and ellagic acid), which have high antioxidant capacity that may offer beneficial health properties (Aloqbi et al., 2016).

Therefore, the aim of the present research was to study how storage conditions (6 months at 4 or 20 °C) affected color, polyphenolic profile, and antioxidant activity of pomegranate smoothies. The study was completed by evaluating the effect of ratio purée:juice (60:40 and 40:60), pomegranate cultivar (*Mollar de Elche* and *Wonderful*), and fruit purée (quinces, jujubes, and figs) on the quality of the studied smoothies.

## 2. Materials and methods

### 2.1. Sample preparation

Pomegranate trees (*Punica granatum* L., cultivar *Mollar de Elche* and *Wonderful*) were cultivated in a farm located in Murcia (Spain) under regulated deficit irrigation (RDI) (Cano-Lamadrid et al., 2018). Pomegranate (cv. *Mollar de Elche* and *Wonderful*), figs (*Ficus carica*, cv. *Colar*), jujubes (*Ziziphus jujube*, cv. *Grande de Albaterra*) and quinces (*Cydonia oblonga*, cv. *Gigante de Vranja*) were hand-harvested at a commercial maturity stage.

The different stages of the smoothie preparation were:

**i) Purée preparation:** The figs (F), jujubes (J), or quinces (Q) were peeled, ground, and heated at 80 °C in a Thermomix device (Vorwerk, Wuppertal, Germany). Rhubarb juice (5%) was added to prevent enzymatic browning. Purées were cooled to room temperature. The purees were subjected to analyses right after preparation and after 6 months of storage at 4 or 20 °C. These same formulation ratio purée:juice and storage conditions used were based on previous smoothies studies (Nowicka, Wojdyło, Teleszko, & Samoticha, 2016). Rhubarb juice was added in the same amount to all smoothies, the effect for preserve polyphenolic compounds before oxidation (Oszmiański & Wojdyło, 2008).

**ii) Juice preparation:** Pomegranate fruits [*Mollar de Elche* (Mo) and *Wonderful* (W)] were cut in halves, and arils were manually separated and juices were prepared using only arils.

**iii) Smoothies preparation:** Purée and juices samples, immediately after their preparation, were mixed in the proportions 40/60 and 60/40, respectively, to obtain 12 treatments/samples (Table 1). Then, the products were heated to 100 °C and pasteurized (10 min at 90 °C).

Table 1  
Formulation of smoothies consisting of pomegranate juice and figs, jujubes, or quinces purées.

| N° | Code <sup>a</sup> | Formulation <sup>b</sup> |
|----|-------------------|--------------------------|
| 1  | F1Mo              | 40% F + 60% Mo           |
| 2  | F1W               | 40% F + 60% W            |
| 3  | F2Mo              | 60% F + 40% Mo           |
| 4  | F2W               | 60% F + 40% W            |
| 5  | J1Mo              | 40% J + 60% Mo           |
| 6  | J1W               | 40% J + 60% W            |
| 7  | J2Mo              | 60% J + 40% Mo           |
| 8  | J2W               | 60% J + 40% W            |
| 9  | Q1Mo              | 40% Q + 60% Mo           |
| 10 | Q1W               | 40% Q + 60% W            |
| 11 | Q2Mo              | 60% Q + 40% Mo           |
| 12 | Q2W               | 60% Q + 40% W            |

<sup>a</sup> Mo, Mollar de Elche pomegranate juice; W, Wonderful pomegranate juice; F, fig purée; J, jujube purée; Q, quince purée.

<sup>b</sup> The percentage of each component was expressed in weight:weight, w:w.

### 2.2. Color parameters

Color coordinates ( $L^*$ ,  $a^*$ , and  $b^*$ ) were determined by reflectance measurement with a Color Quest XE Hunter Lab colorimeter. The samples were filled in a 1 cm cell, and  $L^*$ ,  $a^*$ ,  $b^*$  values were determined using Illuminant D65 and 10° observer angle. Samples were measured against a white ceramic reference plate ( $L^* \frac{1}{4} 93.92$ ;  $a^* \frac{1}{4} 1.03$ ;  $b^* \frac{1}{4} 0.52$ ). Measurements were run in triplicate.

### 2.3. Extraction, LC-PDA/MS and UPLC-PDA-FL analysis of polyphenolic compounds

The extract of polyphenols were performed as previously described (Wojdyło, Oszmiański, & Bielicki, 2013). The compound identification was done using fast liquid chromatography quadrupole time-of-flight mass spectrometry (LC/MS QToF), and the compound quantification was done using ultra-performance liquid chromatography-photodiode array (UPLC-PDA). The analysis of polymeric procyanidins was done using UPLC-FL by phloroglucinol method was performed according to the protocol described previously by Kennedy and Jones (2001). All measurements were run triplicate, and results were expressed as mg/100 g of product.

### 2.4. Antioxidant capacity (ABTS<sup>+</sup> and FRAP)

Each sample (~1 g) was mixed with 5 mL of extractant solution (MeOH/water 80:20 v/v, 1% HCl), sonicated at 20 °C for 10 min, and left for 24 h at 4 °C. Then, the extract was again sonicated for 15 min, and centrifuged until the separation of the supernatant. The antioxidant capacity was determined using ABTS<sup>+</sup> and FRAP assays, previously described by Re et al. (1999) and Benzie and Strain (1996), respectively. Calibration curves within the range 0.50–5.00 mmol Trolox L<sup>-1</sup> were used for the quantification using all of the two methods; these calibration curves showed good linearity ( $R^2 \geq 0.998$ ). Determinations were performed using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). Analyses were run in triplicate and results were expressed as mmol TE/100 g.

### 2.5. Statistics

A four-way ANOVA (factor 1: Ratio purée:juice, factor 2: pomegranate cultivar, factor 3: fruit purée, and factor 4: storage conditions) was performed using XLSTAT Premium 2016 (Microsoft Corporation, Redmond, WA, USA), and means were separated by Tukey's multiple range test.

## 3. Results and discussion

### 3.1. Polyphenolic composition

Fig. 1 shows the quantification of the polyphenols found in fig (A), jujube (B) and quince (C) pomegranate smoothies before (T0) and after 6 months of storage at 4 °C (T1) and 20 °C (T2). The values of total polyphenolic content (TPC) in fig, jujube, and quince pomegranate smoothies at the beginning of the storage period (T0) ranged between 247 and 314 mg/100 g fresh weight (fw), 2939–3920 mg/100 g fw, and 3809–5324 mg/100 g fw, respectively. During storage, changes on TPC were noticed. Regarding fig pomegranate smoothies, the best formulation was F2W, which only experienced a TPC decrease of 13.3% after T1 and 52.2% after T2. The reduction of TPC content the rest of fig pomegranate smoothies ranged between 30.2 and 34.3% after T1 and 64.2–64.7% after T2, respectively. On the other hand, jujube pomegranate smoothies stored at 4 °C, suffered a TPC reduction between 24.71 and 24.9%, except when *Wonderful* cultivar was used, with a drastically lower reduction of only 6.30% or even an increase of 11.8% in the treatment J2W. During storage at 20 °C, the TPC was reduced

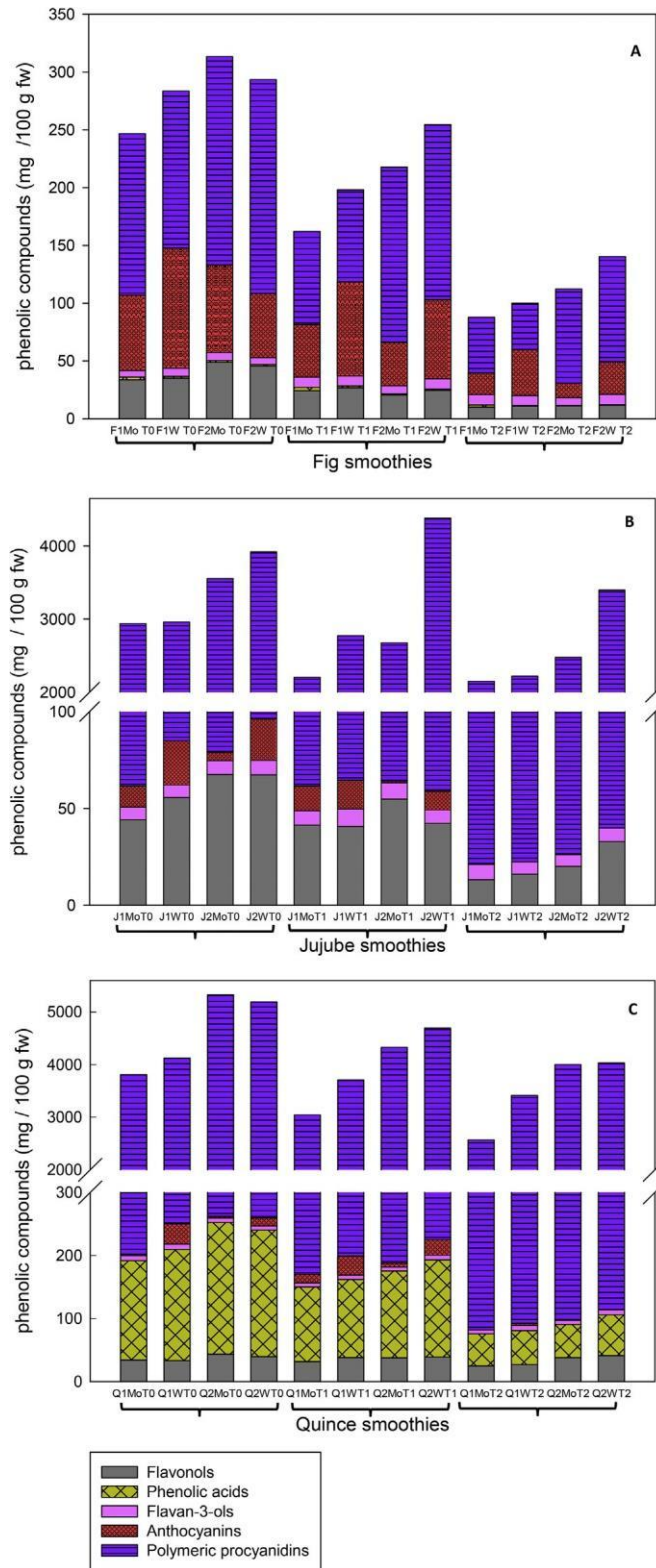


Fig. 1. Content of flavonols, phenolic acids, flavan-3-ols (as monomeric, dimeric and polymeric procyanidins), and anthocyanins, in mg/100 g fw (fresh weight) of fig (A), jujube (B), and quince (C) smoothies before (T0) and after 6 months of storage time at 4 °C (T1) and 20 °C (T2).

Table 2

Flavonols, phenolic acid, flavan-3-ols and antioxidant capacity (ABTS<sup>+</sup> and FRAP) in pomegranate smoothies during storage as affected by: (i) ratio purée:juice (40:60 and 60:40), (ii) pomegranate cultivar (W, Wonderful, and Mo, Mollar de Elche), (iii) fruit purée (fig, jujube, and quince), and, (iv) storage temperature during 6 months (4 and 20 °C).

| Parameter                   | ANOVA <sup>†</sup> | Smoothies <sup>‡</sup> |         |        |        |        |        |        |        |        |        |         |        |
|-----------------------------|--------------------|------------------------|---------|--------|--------|--------|--------|--------|--------|--------|--------|---------|--------|
|                             |                    | F1Mo                   | F1W     | F2Mo   | F2W    | J1Mo   | J1W    | 2Mo    | J2W    | Q1Mo   | Q1W    | Q2Mo    | Q2W    |
| Flavonols <sup>‡</sup>      | ***                | 22.7                   | 24.3c   | 26.9bc | 27.3bc | 32.9b  | 37.3ab | 47.4a  | 47.5a  | 30.2b  | 32.8b  | 39.8ab  | 40.1ab |
| Phenolic acids <sup>‡</sup> | ***                | 2.32c                  | 1.15d   | 0.84d  | 0.96d  | nd     | nd     | nd     | nd     | 109b   | 118b   | 133a    | 140a   |
| Flavan-3-ols <sup>‡</sup>   | ***                | 7.82                   | 8.20a   | 7.00b  | 7.91ab | 7.32b  | 7.39b  | 7.18b  | 7.17b  | 6.45c  | 8.07a  | 6.54c   | 7.36b  |
| PP <sup>‡</sup>             | ***                | 89.5e                  | 85.2e   | 138d   | 143d   | 2383c  | 2593bc | 2846b  | 3835ab | 2989b  | 3566ab | 4368a   | 4440a  |
| FRAP ±                      | ***                | 0.30e                  | 0.64cde | 0.45de | 0.45de | 1.85b  | 2.52a  | 2.24ab | 1.89b  | 0.76cd | 1.01c  | 0.68cde | 0.76cd |
| ABTS ±                      | ***                | 0.68c                  | 1.01bc  | 0.72c  | 0.66c  | 1.34bc | 2.88a  | 3.03a  | 3.09a  | 1.82b  | 1.07bc | 1.31bc  | 1.12bc |

| Parameter                 | ANOVA <sup>†</sup> | Ratio purée:juice |       | ANOVA <sup>†</sup> | Pomegranate cultivar |      | ANOVA <sup>†</sup> | Fruit purée |        |        | ANOVA <sup>†</sup> | Storage temperature |       |       |
|---------------------------|--------------------|-------------------|-------|--------------------|----------------------|------|--------------------|-------------|--------|--------|--------------------|---------------------|-------|-------|
|                           |                    | 40:60             | 60:40 |                    | Mo                   | W    |                    | Fig         | Jujube | Quince |                    | T0                  | T1    | T2    |
| Flavonols <sup>‡</sup>    | **                 | 30.0b             | 38.2a | NS                 | 33.3                 | 34.9 | ***                | 25.3c       | 41.3a  | 35.7b  | ***                | 45.7a               | 35.2b | 21.4c |
| Phenolic ac. <sup>‡</sup> | **                 | 38.4b             | 45.8a | NS                 | 40.9                 | 43.3 | ***                | 1.32b       | nd     | 125a   | ***                | 62.5a               | 35.2b | 18.7c |
| Flavan-3-ols <sup>‡</sup> | NS                 | 7.64              | 7.19  | NS                 | 7.15                 | 7.68 | NS                 | 7.76        | 7.26   | 7.23   | NS                 | 6.99                | 7.73  | 7.53  |
| PP <sup>‡</sup>           | NS                 | 1951              | 2628  | NS                 | 2135                 | 2443 | ***                | 114c        | 2914b  | 3840a  | NS                 | 2597                | 2270  | 2002  |
| FRAP ±                    | NS                 | 1.18              | 1.04  | NS                 | 0.97                 | 1.25 | ***                | 0.46b       | 2.14a  | 0.46b  | ***                | 1.19a               | 1.25a | 0.88b |
| ABTS ±                    | NS                 | 1.47              | 1.64  | NS                 | 1.42                 | 1.67 | ***                | 0.77c       | 2.59a  | 1.36b  | ***                | 1.85a               | 1.69a | 1.07b |

<sup>‡</sup>expressed in followed units: mg/100 g fw smoothie.

<sup>±</sup> expressed in followed units: mmol Trolox/100 g fw smoothie.

<sup>†</sup>NS = not significant F ratio ( $p < 0.05$ ); \*, \*\*, and \*\*\*, significant at  $p < 0.05$ , 0.01, and 0.001, respectively.

<sup>‡</sup>Values followed by the same letter, within the same factor (ratio purée:juice, pomegranate cultivar, fruit purée and storage time), were not significant different ( $p > 0.05$ ), Tukey's multiple-range test.

<sup>†</sup>T0: freshly made; T1: storage 6 months 4 °C; and, T2: storage 6 months 20 °C.

between 25.0 and 30.3%, with the exemption of the J2W samples, which reduction was significantly lower, only 13.3%. Finally, the TPC reduction of quince pomegranate smoothies ranged between 9.5 and 20.1% after storage at 4 °C, and between 17.2 and 32.6% at 20 °C. The fact that the higher the storage temperature, the higher the loss of TPC in smoothies was previously reported in sour cherry smoothies (Nowicka et al., 2016). These authors indicated that after storing samples 6 months samples at 4 °C maintained more TPC, especially anthocyanins, than at 30 °C. It is worth mentioning that the ratio purée:juice 60:40 and *Wonderful* cultivar positively influenced the maintenance of TPC during storage in all types of smoothies.

Table 2 shows the contents of flavonols, phenolic acids, flavan-3-ols, and polymeric procyanidins as affected by ratio purée:juice, pomegranate cultivar, type of fruit purée, and storage conditions on smoothies. Although a positive effect was observed on flavonols and phenolic acids at the ratio purée:juice 60–40, each purée had a different behavior. Regarding flavonols, jujube pomegranate smoothies presented the highest content, followed by the quince ones. However, no phenolic acids were detected on jujube pomegranate smoothies, whilst quince pomegranate smoothies presented the highest content of these compounds. Previous studies indicated that jujubes and quinces are rich sources of flavonols and phenolic acids, respectively (Wojdyto et al., 2013, 2016). Finally, the content of the monomer and dimeric forms of the flavan-3-ols was not significant affected by any of the studied factors.

As to polymeric procyanidin (PP), a huge difference among all smoothies was found. The values of PP in fig, jujube and quince pomegranate smoothies in T0 ranged between 136 and 185, 2875–3823, and 3607–5062 mg/100 g fw, respectively. The PP content was only affected by the fruit purée. It is very important to know which treatment was the best in keeping high PP content due to their healthy properties; although the PP has been reported that is not absorbed, unabsorbed PP are directly linked with colon cancer (Gossé et al., 2005). Fig. 1 clearly shows how a reduction of PP after storage was noticed. Among fig pomegranate smoothies, a reduction of 41.9% was found after 6 months at 4 °C, except smoothies based on the 60:40 purée:juice ratio, which only decreased 17.0%. Moreover, after

6 months at 20 °C a mean reduction of 44.1% was observed. Among jujube pomegranate smoothies, the most relevant factors were pomegranate cultivar and storage conditions. The combination *Wonderful* cultivar and storage at 4 °C maintained the PP content, or even increased its content by 13.1% (J2W); while after storage at 20 °C, *Mollar de Elche* smoothies had a better maintenance (mean reduction of 3.5%) than *Wonderful* smoothies (mean reduction of 20.6%). Finally, pomegranate cultivar was the factor which affected the reduction of PP content on quinces pomegranate smoothies, with reduction values being 9.1% and 14.4% in *Wonderful* and *Mollar de Elche* smoothies, respectively.

Table 3 shows significant effects of (i) ratio purée:juice, (ii) pomegranate cultivar, (iii) fruit purée, and, (iv) storage time, on the contents of individual and total anthocyanins. Specifically, the anthocyanins (ANCs) profile was significantly affected by fruit purée and storage time, while the factors ratio purée:juice and pomegranate cultivar only affected the contents of A1 and A2 out of 5 ANCs. As expected, the highest ANCs were observed in smoothies with a ratio purée:juice of 40:60, with ANCs mostly coming from the pomegranate juice. Moreover, it was noticed that depending on the pomegranate cultivar, ANC profile and  $\Sigma$ ANCs content were different presenting higher values those smoothies based on *Wonderful* fruits as compared to those of *Mollar de Elche*; the differences were mainly due to the contents of delphinidin-3,5-di-O-glucoside (A1) and cyanidin-3,5-di-O-glucoside (A2). These results agreed with previous studies showing that the *Wonderful* fruits had higher ANCs content than the *Mollar de Elche* ones (Mena, Martí, & García-Viguera, 2014). According to previous studies (Mena et al., 2014), the major component in all smoothies was cyanidin-3,5-di-O-glucoside (A2) coming from pomegranate. On the other hand, cyanidin-3-O-glucoside (A4) and pelargonidin-3-O-glucoside (A5) were mostly detected when fig purée was included in the formulation (Wojdyto, Nowicka, Carbonell-Barrachina, & Hernández, 2016). Moreover, as expected fig smoothies presented 6.5 and 5.0 higher times of ANCs than jujube and quinces smoothies, respectively due to the presence of ANCs in figs.

Although further research is needed to identify the mechanisms responsible for anthocyanin degradation during storage, several studies

Table 3

Anthocyanins contents (ACNs) in pomegranate smoothies during storage as affected by: (i) ratio purée:juice (40:60 and 60:40), (ii) pomegranate cultivar (W, Wonderful, and Mo, Mollar de Elche), (iii) fruit purée (fig, jujube, and quince), and, (iv) storage temperature during 6 months (4 and 20 °C).

| ACNs (mg/100 g fw) | ANOVA <sup>†</sup> | Smoothies <sup>‡</sup> |       |        |        |        |        |       |        |        |         |         |        |
|--------------------|--------------------|------------------------|-------|--------|--------|--------|--------|-------|--------|--------|---------|---------|--------|
|                    |                    | F1Mo                   | F1W   | F2Mo   | F2W    | J1Mo   | J1W    | J2Mo  | J2W    | Q1Mo   | Q1W     | Q2Mo    | Q2W    |
| A1 ±               | ***                | 0.90b                  | 9.73a | 0.00b  | 8.87a  | 0.00b  | 1.64b  | 0.00b | 1.98 b | 0.00b  | 4.50ab  | 0.000 b | 2.50b  |
| A2                 | ***                | 14.9abc                | 31.9a | 10.8bc | 21.2ab | 5.47bc | 3.31bc | 0.00d | 6.32bc | 3.25bc | 14.0abc | 1.46c   | 7.68bc |
| A3                 | NS                 | 3.28a                  | 3.55a | 0.74a  | 1.48   | 1.17   | 1.75   | 0.23  | 0.35   | 0.82   | 1.32    | 0.59    | 1.15   |
| A4                 | ***                | 13.9a                  | 12.5a | 11.8ab | 15.0ab | 1.21a  | 3.88b  | 1.39b | 1.67b  | 0.73b  | 1.93b   | 0.89b   | 1.08b  |
| A5                 | ***                | 10.2b                  | 17.4a | 18.6a  | 4.37c  | 0.00d  | 3.00d  | 0.00d | 0.00d  | 0.38d  | 0.00d   | 0.00d   | 0.00d  |
| ΣA                 | ***                | 43.1ab                 | 75.0a | 41.9ab | 50.8ab | 7.85b  | 12.6b  | 1.63b | 10.3b  | 5.18b  | 21.8b   | 2.93b   | 12.4b  |

| ACNs(mg/100 g fw) | ANOVA <sup>†</sup> | Ratio purée:juice |       | ANOVA <sup>†</sup> | Pomegranate cultivar |       | ANOVA | Fruit purée |        |        | ANOVA | Storage temperature |        |       |
|-------------------|--------------------|-------------------|-------|--------------------|----------------------|-------|-------|-------------|--------|--------|-------|---------------------|--------|-------|
|                   |                    | 40:60             | 60:40 |                    | Mo                   | Wond  |       | Fig         | Jujube | Quince |       | T0                  | T1     | T2    |
| A1 ±              | NS                 | 2.79              | 2.22  | ***                | 0.15b                | 4.87a | ***   | 4.87a       | 0.90b  | 1.75ab | **    | 3.69a               | 2.61ab | 1.23b |
| A2                | **                 | 13.1a             | 7.91b | **                 | 5.98b                | 15.1a | ***   | 19.7a       | 5.27b  | 6.61b  | ***   | 12.5ab              | 14.5a  | 4.56b |
| A3                | NS                 | 1.82              | 0.76  | NS                 | 1.14                 | 1.43  | **    | 2.26a       | 0.63b  | 0.97ab | ***   | 0.55b               | 2.96a  | 0.36b |
| A4                | NS                 | 5.19              | 5.30  | NS                 | 4.98                 | 5.51  | ***   | 13.3a       | 1.29b  | 1.16b  | ***   | 5.32b               | 7.98a  | 2.43c |
| A5                | NS                 | 4.65              | 3.82  | NS                 | 4.85                 | 3.63  | **    | 12.6a       | 0.00a  | 0.09a  | ***   | 12.0a               | 0.71a  | 0.00a |
| ΣA                | **                 | 27.6a             | 20.0b | ***                | 17.1b                | 30.5a | ***   | 52.7a       | 8.09b  | 10.6b  | ***   | 34.1a               | 28.7b  | 8.58c |

<sup>‡</sup>A1: Delphinidin-3,5-di-O-glucoside; A2: cyanidin-3,5-di-O-glucoside; A3: Delphinidin-3-O-glucoside; A4: Cyanidin-3-O-glucoside; A5: Pelargonidin-3-O-glucoside.

<sup>†</sup> NS = not significant F ratio ( $p < 0.05$ ); \*, \*\*, and \*\*\*, significant at  $p < 0.05$ , 0.01, and 0.001, respectively.

<sup>‡</sup> Values followed by the same letter, within the same factor (ratio purée:juice, pomegranate cultivar, fruit purée and storage time), were not significant different ( $p > 0.05$ ), Tukey's multiple-range test.

<sup>¶</sup>T0: freshly made; T1: storage 6 months 4 °C; and, T2: storage 6 months 20 °C.

reported that is due to the polymerization of the monomeric compounds, leading to an undesirable brown color (Sinela et al., 2017). The temperature is a key factor during storage to avoid the loss of ANCs and their linked color. The higher the temperature, the higher the ANCs degradation. Regarding ANCs stability in the samples under study, after 6 months of storage the total content of ANCs was reduced only 15.8% when smoothies were stored at 4 °C (T1), but this content was reduced by 74.8% when stored at 20 °C; these results agreed with those from the literature (Sinela et al., 2017). Besides, results also agreed with an almost complete reduction, 94% and 98% for delphinidin-3,5-di-O-glucoside and cyanidin-3,5-di-O-glucoside, when samples were stored at a higher temperature, 37 °C. It was observed that figs smoothies maintained more the ANCs content than jujube and quinces ones at the end of the storage period (Fig. 1). Besides the initial highest content of ΣACN, the high pectin content found in previous studies in fig fruits might be the reason of the anthocyanin pigment stability and extension of pigment shelf-life (Wojdyło et al., 2016). An anthocyanin and pectin interaction might influence the above mentioned trend (Fernandes, Brás, Mateus, & de Freitas, 2014). Recently, two mechanisms for the binding of pectin fractions and ANCs were described in blueberry: i) ionic interaction between anthocyanin flavylium cations and free pectic carboxyl groups, and/or, ii) anthocyanin aromatic stacking on bound anthocyanins (Lin, Fischer, & Wicker, 2016).

### 3.2. Color coordinates

Table 4 shows the values of instrumental color coordinates as affected by the 4 factors under study, ratio purée:juice, pomegranate cultivar, fruit purée, and, storage time. A bright and intense red color is one of the most valued quality attribute of pomegranate products, which influences consumer acceptance and commercial value. The green-red coordinate,  $a^*$ , was significantly affected by all factors described above. As expected, the highest  $a^*$  values (more intense red color) were found in smoothies with ratio 40:60 purée:juice, coming mostly from pomegranate juice. Moreover, the pomegranate cultivar selected to prepare the smoothies also affected the  $a^*$  values, having smoothies based on Wonderful pomegranate a more intense red color than those of the cultivar Mollar de Elche; these results agreed with previous studies dealing with fresh hydroSOSTainable pomegranate

fruits (Cano-Lamadrid et al., 2018). Adding the different types of fruit purée significantly affected  $L^*$ ,  $a^*$  and  $b^*$  coordinates. The highest lightness ( $L^*$ ) values were found in the quince- and jujube-pomegranate smoothies, followed by the fig ones. The values of  $a^*$  coordinate in quince-pomegranate smoothies were higher than those found in the fig and jujube samples. Finally, the highest  $b^*$  values (blue-yellow coordinate) were found in jujube-pomegranate smoothies, followed by those found in the quince and fig products. On the other hand, after 6 months of storage at two temperatures, the intensity of the red color ( $a^*$ ) significantly decreased, being greater the final color more reddish in samples stored at 4 °C (T1) as compared to those stored at 20 °C. Results agreed with previous studies, which pointed that the enzymatic browning of phenolic compounds was the main reason of the decrease of red color during storage in smoothies based on sour cherry (Nowicka et al., 2016). In a similar trend, the original blue color notes ( $b^*$ ) of the initial pomegranate smoothies experienced more intense changes when stored at 20 °C as compared to those at 4 °C. As a summary, it can be concluded that ratio purée:juice 40:60, Wonderful pomegranate juice, quince purée and storage 6 months at 4 °C of the assayed smoothies were the conditions leading to a more intense red smoothies.

Total anthocyanin content is well-known as the main parameter behind the red color of pomegranate products. Pearson's correlation coefficient showed that the total anthocyanin content was positively and significantly ( $p < 0.05$ ) correlated in fig, jujube and quince based smoothies with the  $a^*$  coordinate values ( $R^2 = 0.84$ , 0.86, and 0.59 respectively). As seen, the type of smoothies with lower correlation was that of quinces, perhaps because of the pH of the smoothies (Cano-Lamadrid, Trigueros, Wojdyło, Carbonell-Barrachina, & Sendra, 2017); these authors concluded that the food matrix pH controlled the changes in the ANCs color. Besides, a low pH is a favorable environment for the formation of anthocyanin-procyanidin polymers which are more stable than their anthocyanins precursors, maintaining the color during storage (Li & Sun, 2017). For all mentioned above, although fig pomegranate smoothies were more enriched on ANCs after storage, a higher red color was found in quince-pomegranate smoothies.

### 3.3. Antioxidant capacity by ABTS and FRAP assay

Table 2 shows the results of antioxidant capacity (AC) measured by

Table 4

CIEL<sup>\*</sup>a\*b\* coordinates in smoothies during storage time as affected by: (i) ratio purée:juice (40:60/60:40), (ii) pomegranate cultivar (W, Wonderful, and Mo, Mollar de Elche), (iii) fruit purée (fig, jujube and quinces), and, (iv) storage temperature during 6 months (4 and 20 °C).

| Parameter | ANOVA <sup>†</sup> | Smoothies <sup>†</sup> |       |       |        |        |        |        |         |         |        |        |         |
|-----------|--------------------|------------------------|-------|-------|--------|--------|--------|--------|---------|---------|--------|--------|---------|
|           |                    | F1Mo                   | F1W   | F2Mo  | F2W    | J1Mo   | J1W    | J2Mo   | J2W     | Q1Mo    | Q1W    | Q2Mo   | Q2W     |
| L*        | ***                | 35.1d                  | 35.5d | 34.1d | 34.9d  | 48.8bc | 46.7c  | 53.5ab | 49.4abc | 50.8abc | 47.7bc | 54.8a  | 52.1abc |
| a*        | ***                | 7.45d                  | 12.1b | 7.01d | 9.73cd | 5.90e  | 12.1b  | 3.89f  | 9.19cd  | 10.4c   | 16.2a  | 9.10cd | 13.2b   |
| b*        | ***                | 4.61b                  | 4.35b | 4.98b | 4.63b  | 13.1ab | 11.0ab | 17.6a  | 14.1ab  | 9.54ab  | 7.04ab | 9.19ab | 9.69ab  |

| Parameter | ANOVA <sup>†</sup> | Ratio purée:juice |       | ANOVA <sup>†</sup> | Pomegranate cultivar |        | ANOVA | Fruit purée |        |        | ANOVA | Storage temperature <sup>α</sup> |       |       |
|-----------|--------------------|-------------------|-------|--------------------|----------------------|--------|-------|-------------|--------|--------|-------|----------------------------------|-------|-------|
|           |                    | 40:60             | 60:40 |                    | Mo                   | W      |       | Fig         | Jujube | Quince |       | T0                               | T1    | T2    |
| L*        | NS                 | 44.1              | 46.5  | NS                 | 46.2                 | 44.4   | ***   | 34.9b       | 49.6a  | 51.3a  | NS    | 44.5                             | 46.1  | 45.3  |
| a*        | ***                | 10.7a             | 8.69b | ***                | 7.30b                | 12.08a | ***   | 9.08b       | 7.76c  | 12.2a  | ***   | 14.1a                            | 8.53b | 6.45c |
| b*        | ***                | 8.26b             | 10.6a | NS                 | 10.4                 | 8.46   | ***   | 4.64c       | 13.9a  | 9.70b  | ***   | 6.4c                             | 10.0b | 11.8a |

<sup>†</sup> NS = not significant F ratio ( $p < 0.05$ ); \*, \*\*, and \*\*\*, significant at  $p < 0.05$ , 0.01, and 0.001, respectively.

<sup>‡</sup> Values followed by the same letter, within the same factor (ratio purée:juice, pomegranate cultivar, fruit purée and storage time), were not significant different ( $p > 0.05$ ), Tukey's multiple-range test.

<sup>α</sup>T0: freshly made; T1: storage 6 months 4 °C; and, T2: storage 6 months 20 °C.

the ABTS<sup>+</sup> and FRAP assays. The AC was significantly affected by fruit purée and storage time, while no significant effect of ratio purée:juice and pomegranate cultivar were found. There were no drastic changes in the values of the antioxidant capacity after 6 months of storage, with this trend not being correlated with those previously discussed for the main bioactive compounds. Similar results were found in smoothies based on other fruits as apple, quince, orange, and pear at 4 and 30 °C (Andrés, Villanueva, & Tenorio, 2016; Nowicka et al., 2016); these authors explained this trend by the fact that some degradation products also have antioxidant properties, such as Maillard compounds. On the other hand, a higher loss of antioxidant capacity was noticed in smoothies stored at 20 °C as compared to those stored at 4 °C. Recently, this trend was reported in the storage of red and green smoothies (Di Cagno, Minervini, Rizzello, De Angelis, & Gobbetti, 2011) and commercial ones (Nunes et al., 2016).

Pearson's correlation coefficient showed that PP were significantly and positively correlated with FRAP ( $R = 0.40$ ;  $p < 0.05$ ) and ABTS<sup>+</sup> ( $R = 0.44$ ;  $p < 0.05$ ) data. Besides, anthocyanin content was significantly but negatively correlated with FRAP ( $R = -0.47$ ;  $p < 0.05$ ) and ABTS<sup>+</sup> ( $R = -0.40$ ;  $p < 0.05$ ) data. No significant ( $p < 0.05$ ) correlation was observed among flavonols and flavan-3-ols with FRAP and ABTS<sup>+</sup> data.

#### 4. Conclusions

At the beginning (T0), all developed smoothies presented high values of total polyphenolic content (TPC): 247–314 mg/100 g fresh weight (fw), 2939–3920 mg/100 g fw, and 3809–5324 mg/100 g fw, in fig, jujube and quinces pomegranate smoothies, respectively. Regarding higher content of bioactive compounds and attractive color, the best formulation and storage conditions for pomegranate smoothies were ratio purée:juice 40:60, Wonderful pomegranate juice, and storage at 4 °C, being only a reduction of 30.1%, 13.1% and 9.5% in fig, jujube and quinces smoothies, respectively. Moreover, regarding red color, a more intense red color was found in quince-pomegranate smoothies after storage. On the other hand, the type of smoothie which maintained the highest total polyphenolic content [TPC: sum of anthocyanin, flavanols, flavan-3-ols (as monomeric and dimeric), polymeric procyanidins and phenolic acid] was the quince ones. On the other hand, fig pomegranate smoothies had higher contents of anthocyanins, after storage TPC presented a high reduction of them. After checking the quality and functionality of the smoothies developed (blend of minor Mediterranean crops purées with pomegranate juice) and their behavior during storage, the next logical step will be to evaluate their sensory profile and consumer acceptance.

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## **8. RESULTS AND DISCUSSION**

In this section the main results and discussions of the published articles included in this thesis are summarized in 4 blocks. For more detailed aspects the papers that constitute this thesis can be consulted.

### **7.1. Block I: Farming practices of hydrosustainable fruits** (publication 1)

#### **Objective 1**

**To assess the simultaneous influence of irrigation status and crop load on yield and fruit quality parameters.**

There were two *irrigation treatments* to study the effects on the plant water status: i) T0: control pomegranate fruits, and, ii) T1: sustained deficit irrigated fruits (SDI). There were also two *thinning treatments* to study the effects on the crop load: i) A0: pomegranate fruits were not thinned, and ii) A1 trees were manually thinned. Therefore, taking into account the interaction on SDI and thinning, four samples for each pomegranate cultivar (*Wonderful* and *Mollar de Elche*) were under study: T0A0, T0A1, T1A0 and T1A1.

The  $\Psi_{\text{stem}}$  (midday stem water potential) and  $g_{\text{leaf}}$  (midday leaf conductance) values for T0 trees in *Wonderful* (*Wond*) and *Mollar de Elche* (*ME*) cultivars were enough to avoid water deficit during the experimental period. The  $\Psi_{\text{stem}}$  and  $g_{\text{leaf}}$  values of T1 trees were characterized by a gradual reduction from day of the year (DOY) 109, reaching minimum values at DOY 230-279, indicating a significant water deficit situation. Additionally, the results from the present experiment indicated that  $\Psi_{\text{stem}}$  and  $g_{\text{leaf}}$  values in A0 plants were very similar to those of A1 trees in both T0 and T1.

Both cultivars showed similar response of the marketable yield and fruit morphology to SDI and thinning. Both SDI and thinning treatments significantly reduced the marketable yield, but the weight and size were not negatively affected, even after thinning higher values were observed as has been previously reported (Galindo *et al.*, 2014; Mellisho *et al.*, 2012).

The color density (CD) in *ME* fruits (2.18-4.08) was lower than in *Wond* fruits (2.69-5.89), due to the differences of compounds and content previously reported among monomeric anthocyanins (Türkyılmaz *et al.*, 2014). CD was reduced when water deficit was applied (T1) in *Wond*; while the opposite tendency was noticed in *ME* fruits. Thinning only increased CD in *Wond* fruits. Additionally, water stress increased the value of polymeric color in *Wond* and *ME* cultivars, indicating the polymerization and the variation of red color.

Thinning had negative effect on total phenolic content (TPC) of both *Wond* and *ME* (mean of 7 % of reduction). What is more, neither TPC nor punicalagin content were affected by water deficit, which did not agree with previous studies on pomegranate fruits produced under moderate and severe SDI (Galindo *et al.*, 2014; Galindo *et al.*, 2017); differences may be due to the higher level of water stress reached in the previous experiment as compared to the current one (Turfan *et al.*, 2012).

The glucose and fructose contents only increased in *Wond* fruits after water stress (T1), which will help in masking the intense sourness of this cultivar. It seemed that the degradation of sucrose was enhanced; thereupon, the level of water stress played a role in fruit maturity. Contrary, sugars from *ME* cultivar were not modified by water stress, because *ME* trees are better adapted to water stress than the *Wond* ones (Laribi *et al.*, 2013). The interaction on SDI and thinning (T1A1) in water deficit did not affect caused the greatest increase in glucose and fructose.

Regarding the volatile compounds, which are key in determining pomegranate odor, aroma and flavor, in *Wond* and *ME* fruits, the predominant chemical families were aliphatic alcohols (1-hexanol and 3-hexen-1-ol) and aldehydes (hexanal and nonanal), respectively, in agreement with previous data (Mayuoni-kirshinbaum *et al.*, 2012). Changes on volatile composition as affected by water stress have been previously found in table olive (Cano-Lamadrid *et al.*, 2015) and pistachio (Carbonell-Barrachina *et al.*, 2015), among other plant materials. A reduction of

aldehydes, especially hexanal (vegetal notes), was observed in both cultivars when water stress was applied, probably due to the effect on the synthesis of this chemical family by water stress. The synthesis starts with the C18 fatty acids, linolenic and linoleic acids (Klee, 2010; Osorio *et al.*, 2010). The reduction of hexanal was also observed in grapes produced under water deficit, and was associated with changes in fruit maturity (Song *et al.*, 2012). On the other hand, the reduction of the ethyl hexanoate content was observed after thinning in both cultivars.

### Summary

In *Wond* cultivar fruits, T0A0 (control fruits with no water restriction and not subject to thinning) was positively linked with yield, color, total polyphenolic content, total monoterpenoids, aldehydes, esters (citric, vegetable and fruity notes, respectively), and key sensory attributes such as fruity and pomegranate ID. The rest of treatments were positively correlated with fruit weight, diameter, polymeric color, aliphatic alcohols (vegetable notes), and specific sensory attributes (apple and pear notes).

In *ME* cultivar fruits, the treatment T1A1 (trees under water stress and subjected to thinning) was positively correlated with color, total content of aliphatic alcohols (vegetable notes), total monoterpenoids (citric notes), and key descriptive sensory parameters (fruity, floral, and pomegranate ID). The other treatments (T0A0, T0A1, and T1A0) were positively correlated with yield and citric acid content, aldehydes, monoterpenes and esters compounds (vegetable, citric and fruity notes, respectively).

**7.2. Block II: Overview of commercial pomegranate products** (publication 2 & 3)

**Objective 2**

**To analyze and compare the labelling information, total phenolic content, punicalagin content and antioxidant capacity of different commercial pomegranate based products available on the international market.**

**7.2.1. Study I** (publication 2)

**Objective 2.1**

**To analyze the phytochemical content and antioxidant capacity of pomegranate-based products: i) capsules and supplements, and ii) juices and concentrated drinks.**

Samples (n=22) from 2 pomegranate-based commercial product groups were analyzed: (i) capsules and supplements (n=11), and (ii) pure and concentrated juices (n=11). According to the phytochemicals content, there was a high variability among analyzed samples. Regarding punicalagin content of capsules and supplements, the range was from 4.23 up to 308 mg g<sup>-1</sup>; these contents demonstrated that these products mainly consisted of pomegranate peel and seeds. Among juices and concentrated drinks, the values ranged from 0.96 up to 10.4 mg g<sup>-1</sup>. Results agreed with previous studies which reported that the phenolic compounds are higher in the non-edible parts of the pomegranates (Akhtar *et al.*, 2015). Regarding the ellagic acid (EA) content, the ranges were 0.05 to 0.49 mg g<sup>-1</sup> and 0.11 to 13 mg g<sup>-1</sup> in juices/concentrated drinks and capsules/supplements, respectively. All these samples had a higher EA content as compared to juices prepared from just arils because several commercial manufacturers produce juice by squeezing whole fruit, including some nonedible parts of pomegranate, which

increases the phenolic content by maceration of the juice with the peel and carpelar membranes (Qu *et al.*, 2012). The variation among all these products can be explained by the use of different pomegranate varieties, manufacturing and storage conditions.

Among capsules and supplements, the TPC was highly correlated ( $R=0.88$ ;  $p<0.001$ ) with the punicalagin content, but it was not correlated ( $R=0.23$ ;  $p>0.05$ ) with the EA content. On the other hand, among pomegranate-based drinks, the TPC was not correlated with punicalagin ( $R=0.43$ ;  $p>0.05$ ) but was correlated with EA ( $R=0.62$ ;  $p<0.05$ ). These results showed that polyphenols are not the only substances having antioxidant activity (Rinaldi *et al.*, 2013) in pomegranate based products. Concentrated pomegranate juices presented the highest values of antioxidant capacity (DPPH, ABTS<sup>•+</sup> and FRAP) while the samples with the lowest antioxidant capacity were pomegranate juices prepared from concentrate juices and 100 % pomegranate juice after heat treatment. If the set of all the studied samples is considered, punicalagin content was positively correlated with DPPH<sup>•</sup> and TPC ( $R=0.94$  and  $0.88$ , respectively;  $p<0.001$ ). TPC was highly correlated ( $R=0.80$ ;  $p<0.001$ ) with DPPH<sup>•</sup> data. No significant correlation was observed among EA and any antioxidant capacity assays, probably due to the degradation of EA and generation of others compounds with antioxidant potential during processing and storage conditions, and/or even adulteration (Mphahlele *et al.*, 2016; Nuncio-Jáuregui *et al.*, 2014; Zhang *et al.*, 2009).

### Summary

A high variability on the contents of the main bioactive compound in pomegranate-based product was found because these contents can be affected by many factors, including formulation and conditions used in each of the unit operations of the industrial processing. More research and control are needed to fully adjust reality in composition and bioactivity and labelling; consumers are getting some wrong statements through inappropriate use of images (big pictures of pomegranates

when the juice only contains 1 % of the type of juice) and/or directly with unsupported health claims (Study II).

### **7.2.2. Study II** (publication 3)

#### **Objective 2.2**

**To compare the labelling information with the real phytochemical content, antioxidant capacity and quality parameters of commercial pomegranate juice-based drinks: i) 100 % pomegranate juices, and ii) <100 % pomegranate drinks.**

Samples under study were i) 100% pomegranate juices (n=12), and ii) pomegranate juice based drink (n=18), which consisted of blended juices containing pomegranate at different percentages. Color density (CD) of 100% pomegranate juices and pomegranate juice based drinks ranged from 1.74 and 3.73, and 0.76 to 5.27, respectively. The variability could be due to formulation, heat treatment and storage conditions. It has been noticed that the addition of different juices based on berries to the pomegranate juice increases CD values due to the large number of anthocyanins compounds present in berry juices (Abountiolas *et al.*, 2018). Higher CD values were found in non-heat-treated products (Cao *et al.*, 2011), being the main reaction the enzymatic browning. Regarding the percentage of polymeric color (fresh pomegranate juices should present values less than 10 %), it ranged from 13 up to 90.3 %. It has been previously determined that after High-Temperature-Short-Time (HTST) treatment, the condensation of anthocyanins can occurred, increasing PC (polymeric color) (Zou *et al.*, 2016). It is also demonstrated that these condensation reactions during refrigerated storage can also lead to increased PC values (Rein *et al.*, 2005).

Among 100 % pomegranate juices, the punicalagin content ranged from non-detectable (below limit of quantification) to 3.76 mg mL<sup>-1</sup>. The differences could be due, among other factors, to different cultivar, processing and/or storage conditions (Akhavan *et al.*, 2015; Mena *et al.*, 2013; Sánchez-Salcedo *et al.*, 2015; Tzulker *et al.*, 2007). It is well-known that the highest punicalagin content is located in pomegranate peel (Tzulker *et al.*, 2007); therefore, when juices are produced by pressing/squeezing whole fruits, the punicalagin content is higher (Akhavan *et al.*, 2015; Tezcan *et al.*, 2009). In non-100 % pomegranate juice based drinks, the punicalagin range was from non-detectable to 2.09 mg mL<sup>-1</sup>, with higher values being found in juices with less than 50% pomegranate juices as compared to products with pomegranate contents above 50 % (70, 65 and 55 %). Thus, current results indicated that in commercial juices the content of pomegranate juice was not directly correlated with high punicalagin content, as previous studies reported (Borges *et al.*, 2010); these differences can be due, among other factors, to pomegranate cultivar, type of extraction, and formulation (other fruit juices, sweeteners and preservatives). The content of EA in 100 % juices ranged from non-detectable up to 0.09 mg mL<sup>-1</sup>, while in non-pure pomegranate juice based drinks it was just detected in one sample (0.04 mg mL<sup>-1</sup>). As it was mentioned above, the squeezing process significantly affects the polyphenolic profile and composition of the final juice.

High values of TPC were found in 100 % juices, ranging from 9.0 to 70.9 mmol gallic acid L<sup>-1</sup>. Data was in agreement with previous report about 100 % commercial pomegranate juices from the Turkey (from 15.30 to 59.29 mmol gallic acid L<sup>-1</sup>) (Tezcan *et al.*, 2009). A previous study about pomegranate based drinks available in European market carried out by Borges *et al.*, (2010) indicated that TPC 100 % pomegranate juice ranged between 10.3 and 20.7 mmol gallic acid L<sup>-1</sup>. This current study reported 3.5-fold higher contents than previously shown. As to non-pure-pomegranate based drinks, current results ranged found from 2.75 up to



54.4 mmol gallic acid L<sup>-1</sup>, being by far higher than previous data (1.2 up to 9.7 mmol gallic acid L<sup>-1</sup>).

Because consumers are not aware of authorized nutrition and health European claims, there is a tendency to take fruit properties for granted, especially if pomegranate picture is shown in labelling. Additionally, there are not EU officially approved health claims related to punicalagins, anthocyanins, or antioxidants, or pomegranate (only in olive oil) (EFSA, 2018). Vázquez-Araújo *et al.*, (2010) showed that consumers are more likely to choose drinks with the highest content of pomegranate juice. Therefore, when labelling information (text, picture) is compared with the real content of bioactive substances in the pomegranate-based products, data are sometimes inconsistent. Several examples are shown here:

- i) sample with the claim "a new way of consuming health" had the highest TPC (66.7 mmol gallic acid L<sup>-1</sup>), but the lowest punicalagin content (0.97 mg mL<sup>-1</sup>);
- ii) "natural antioxidant" claim was found in the sample having the lowest value of TPC; and,
- iii) two samples which the content of punicalagin were below limit of quantification claimed "with all polyphenols from pomegranate" and "presence of pomegranate peel extract", respectively.

It is worth mentioning that some authorized health claims were found in non-pure-pomegranate drinks because they included vitamin C or E.

### **Summary**

Current results provides useful information to enhance the know-how on functional pomegranate juice based drinks and the wide variability among commercial products. An improvement of antioxidant capacity and total polyphenolic content was found when compared with previous studies about similar products available in the European market. The pomegranate juice percentage was highly correlated

with the pomegranate ID sensory attribute, suggesting that the development of a new sensory quality marker will be an interesting option. No correlation was found between TPC and punicalagin, confirming that punicalagin was not the only compound implied in the total polyphenolic content, and cultivar, processing and storage conditions, among other factors, influence their content. On the other hand, total polyphenolic content was positively correlated with polymeric color.

### **7.3. Block III: Dehydrated pomegranate arils** (publication 4 & 5)

#### **Objective 3**

**To develop novel hydrosustainable pomegranate-based products, such as dehydrated pomegranate arils.**

#### **7.3.1. Study I** (publication 4)

#### **Objective 4**

**To evaluate the physico-chemical of hydrosustainable *Mollar de Elche* pomegranate dried arils using first osmotic dehydration, with blends of selected fruit juice concentrates, and later a combined drying technique.**

“Mollar the Elche” arils first underwent an osmotic dehydration pre-treatment (OD) with concentrated juices: (i) chokeberry (Ch), (ii) apple (A), and/or (iii) pomegranate cultivar “Wonderful” (POM), and later the arils were dried by a combined method [convective pre-drying (CPD)+ vacuum microwave finish drying (VMFD)]. Moreover, samples after FD (Freeze drying) and CPD-VMFD without OD were study. Osmotic dehydration (OD) in different fruit solutions reduced the moisture content of *ME* arils down from 81.5 to 72.1 %. The mean value of water loss (WL) was 30.2 % during osmotic solution. Current results showed the highest solid gain (SG) increase (23.3 %) was achieved when POM was used in OD and the lowest SG increase (15.3 %) when POM-Ch (pomegranate:chokeberry 1:1). An

increase of 19 % in SG after osmotic pre-treatment with Ch in dried beetroot was reported (Lech *et al.*, 2015). Particle size, composition and viscosity of concentrated juice affect SG values (Kulling *et al.*, 2008; Kraemer-Schafhalter *et al.*, 1998; Juszczak *et al.*, 2009). The high values of  $R^2$  ( $>0.9822$ ) and low ones of root mean square error, RMSE ( $<4.2 \times 10^{-3}$  and  $5.0 \times 10^{-4}$  for CPD and VMFD, respectively) indicated the suitability of the modified Page Model to describe the drying kinetics of the current study (Horuz *et al.*, 2015; Kingsly *et al.*, 2007).

The duration of OD and CPD for all OD-CPD samples was 240 min and 90 min, respectively. Depending on the composition of osmotic solutions, different amounts of water were removed from pomegranate arils, being the highest when POM and POM-Ch were used as osmotic solution (the lowest value constant A of the equation, being  $A=1$  when no OD was applied). Among OD-samples, the highest water removal during CPD was noted for OD(POM), whereas the osmotic solutions consisting of combination of A and Ch [OD(A+Ch) and OD(AP+Ch)], caused a lower water removal. The VMFD (vacuum-microwave finish drying) step was significantly affected by the moisture content of the samples after CPD, which was linked with the water removal during the CPD step. The longest time of VMFD was noted for arils that were not OD. Sample after OD(POM)-CPD presented the lowest A constant, influencing the final dehydration time being the shortest. The composition of the osmotic solution affected the final drying time of samples, and the application of OD(POM) reduced the final drying time of arils in contrast to the other osmotic solutions and sample without OD. It is worth noting, that enriching pomegranate arils with solids originated from pomegranates increased the drying rate during CD-VMFD by increasing water diffusivity, which on other hand was decreased by gaining of solids from apple concentrate. It can be concluded that the particles present in apple juice delay water transport (Lech *et al.*, 2015), contributing to an increased internal pressure and less intensive water evaporation, linked with the highest maximal temperature reached during VMFD. The cooling effect due to its intensive water evaporation (Figiel, 2010) decreased the maximal

temperature of the samples with the highest moisture content at the beginning of VMFD.

The rehydration ratio (RR) was significantly affected by the drying method and composition of the osmotic solution (from 1.07 up to 3.14), agreeing with previous studies Megías-Pérez *et al.*, 2014). The lowest RR (1.07) was found in OD(POM+A) arils due to the amount of solids and composition of A osmotic solution. FD products had relatively low RR values (1.90) since probably destruction of the outer layer of arils. The electrical conductivity ( $\Sigma$ ) of rehydration solution ranged between  $200 \pm 1$  (CPD-VMFD, control sample) and  $284 \pm 2 \mu\text{S cm}^{-1}$  (FD sample, with porous structure). Because of solids were not irreversibly bonded to the aril matrix, OD increase  $\Sigma$  values, comparing to CPD-VMFD.

The lowest degradation of anthocyanins was observed after FD, followed by all-CPD-VMFD (a mean value of loss of 45 % comparing to FD). It seemed that the combined treatments were more suitable than previous treatments assayed such as cabinet and sun drying (61 %) (Jaiswal *et al.*, 2010). Among CDP-VMFD, OD treatment with POM-Ch (1:1) resulted in the highest total anthocyanins content ( $441 \text{ mg kg}^{-1} \text{ dm}$ ), followed by CD-VMFD with no OD step ( $358 \text{ mg kg}^{-1}$ ), and OD(POM)-CPD-VMFD ( $295 \text{ mg kg}^{-1}$ ). It is essential to highlight that the use of apple in the osmotic solution reduced migration of anthocyanins as compared to both POM and Ch.

The highest ABTS<sup>•+</sup>, and FRAP values were found in the OD samples with POM and POM-Ch. On the other hand, the lowest ABTS<sup>•+</sup> values were found in the samples osmotically treated with A and Ch at different ratios, presenting similar values to those of CPD-VMFD without OD. Current results did not agree with those of previous experiments using dried sour cherry, where the antioxidant capacity was reduced after OD (Nowicka *et al.*, 2015), probably due to the short drying time and the low formation of Maillard reaction products (Yilmaz *et al.*, 2005).

The values of coordinate  $L^*$  (lightness) decreased in all samples when compared to FD, indicating the presence of Maillard compounds or degradation of

anthocyanins after heat treatment. The darkest products were found in the samples which A was part of the osmotic solution. High  $a^*$  (green-red coordinate) values were found in OD samples with POM-Ch (value of 16.6) and POM (14.6), which are similar to FD (16.3) and CPD-VMFD (15.2). These values are equivalent to previous dried pomegranate arils developed by other authors (14.0) (Horuz & Maskan, 2015), but using "Hicaz" cultivar which is characterized by its very intense red colour, contrary to what happens with *ME* cultivar. The lowest  $\Delta E$  values were found in the CPD-VMFD, followed by OD-samples with POM. Anthocyanins play an important role in the final color of the dehydrated pomegranate arils because positive correlations were found with  $L^*$ ,  $a^*$ , and  $b^*$  coordinates ( $R=0.90$ ,  $0.77$ , and  $0.79$ , respectively).

All treatments were acceptable in terms of sensory parameters but with different characteristic flavor notes. Control samples (FD and CPD-VMFD) presented a plain sensory profile, prevailing sweetness (value of 8). When POM was used during OD, sourness (4.7), fruity notes (3.7) and pomegranate flavor (3.7) were increased. The use of chokeberry considerably decreased sweetness, but increased astringency, bitterness, sourness, and chokeberry flavor. On the other hand, the addition of A to osmotic solution resulted in lower color and pomegranate flavor of samples, while a higher intensity of apple flavor was found, as expected.

### Summary

The recommended osmotic dehydration methods were those using (i) pomegranate or (ii) pomegranate with chokeberry concentrates, because they improved total anthocyanin content (mean of  $368 \text{ mg kg}^{-1}$ ), red color ( $a^*$  coordinate 15.6), sensory attributes and antioxidant capacity (ABTS<sup>•+</sup> mean of  $5.7 \text{ mmol Trolox } 100 \text{ g}^{-1}$ ). Additionally, a reduction of the final drying time of arils was also found in these samples in contrast to the other osmotic solutions and sample without OD.

### **7.3.2. Study II** (publication 5)

#### **Objective 5**

**To determine consumer insights about dried hydrosustainable pomegranate arils prepared using different technologies and to link consumer data with descriptive sensory and volatile composition data.**

The selection of the samples was based on results from the Study I (publication 4), which concluded that the technology used in OD(POM)-CPD-VMFD and OD(POM+Ch)-CPD-VMFD enhanced the functionality and therefore the healthy characteristics of the product. These both samples plus the control CPD-VMFD and commercial samples were used in this study. Regarding the volatile profile, commercial sample was characterized by high contents of furfural (59.9 %), 5-(hydroxymethyl)-2-furfural, 5-HMF (7.84 %), linalool (7.65 %), 5-methyl furfural (7.64 %), and 3-hydroxy-methyl hexanoate (3.06 %). These compounds are responsible for smoky, woody, burnt and caramel flavor notes and may be markers of an incorrect processing or uncontrolled drying conditions. Results agreed with previous reported data, the furan derivatives family, was found in raisins, dried plums, and dried banana (Fromberg *et al.*, 2014); developed dehydrated pomegranate arils also presented furfural and 5-methyl furfural, but in lower concentrations. It is worth mentioning that the highest concentrations of 5-HMF were found in the optimized dehydrated samples than in commercial one. Optimized dried samples also had higher contents of esters (e.g. 3-hydroxymethyl hexanoate; fruity notes) and terpenes (e.g. linalool and  $\alpha$ -thujene; citric and fruity notes, respectively); some of these volatile compounds were recently detected in pomegranate juices (Cano-Lamadrid, *et al.*, 2018). Therefore, suitable drying techniques such as convective pre-drying with vacuum microwave finish drying are able to maintain characteristic flavor of raw product.

Previous studies indicated the presence of off-flavors in dried products, especially pomegranate (Calín-Sánchez *et al.*, 2013). In the current study, off-flavors were only detected in the commercial sample (probably from furfural which represented >50 % of total volatile compounds). Among developed samples, the CPD-VMFD sample, without osmotic dehydration step, were characterized by higher sweetness, pomegranate ID, fruity and caramel notes than the commercial one. The sensory profile was more complex in *ME* CPD-VMFD dehydrated arils when OD step was applied. The OD(POM) significantly increased their sourness, apple ID, pomegranate ID and fruity notes. When OD(POM-Ch) was used, it significantly increased the sourness, bitterness, caramel, chokeberry ID, pomegranate ID, fruity and citric flavors. The highest values of crispiness and seed-hardness were found in the commercial sample, indicating that the commercial sample seemed to be prepared using *Wond* cultivar fruits.

Regarding the consumer study, CPD-VMFD was the most liked by consumers, and commercial sample was the least liked one. Appearance was better scored in samples which were characterized by having 100 % pomegranate composition. The liking of the different flavor attributes varied among samples; for instance, fruity flavor and pomegranate flavor were less liked in commercial sample, but for all the evaluated attributes OD(POM)-CPD-VMFD was the best scored. In general, consumers overall liking was positively linked with specific volatile compounds and sensory descriptors: aldehydes, esters, aliphatic alcohols, terpenes, pomegranate ID, fruity and sweet notes. The furan chemical family was certainly not among the liking drivers (van Boekel, 2006). Further studies with a higher number of volunteers could be conducted to study. Commercial sample needed improvement of its sourness because of the excessive intensity; in this way, fruity, pomegranate ID, and sweetness attributes needed to be increased in this sample. Sample CPD-VMFD was penalized by presenting low intensities of fruity notes and sourness. Osmotic dehydration improved consumer acceptance of dehydrated pomegranate arils, and results of the penalty analysis indicated that just an increment on the

color homogeneity and sourness in sample OD(POM-Ch)-CPD-VMFD was needed. According to consumers, no improvement was necessary in OD(POM)-CPD-VMFD.

### **Summary**

The proposed drying techniques improved the volatile profile and sensory quality of developed dehydrated pomegranate arils when compared with a commercial product. Consumers overall liking was positively correlated with "pomegranate ID", "sweet", and "fruity" attributes, and also volatile compounds of the esters family. Overall liking was negatively correlated with the "off-flavor" and "burnt" attributes, related to the furan compounds family.

#### **7.4. *Block IV*: Smoothies based on pomegranate and minor Mediterranean fruits** (publication 6 & 7)

### **Objective 3**

**To develop novel hydrosustainable pomegranate-based products, such as smoothies.**

Pomegranate-based smoothies were developed by the addition of fig (F), jujube (J) and quince (Q) purée and pomegranate juices (*ME* or *Wond* cultivars) at different ratio of purée:juice (40:60 and 60:40).

#### **7.4.1. Study I** (publication 6)

### **Objective 6**

**To determine the effect on quality parameters and chemical and phytochemical composition of pomegranate-based smoothies by the addition of minor Mediterranean fruits purée, pomegranate cultivar and different ratio of purée:juice.**



Considering the  $a^*$  (green-red) coordinate, which is deemed as one of the most important quality parameters of pomegranate-based products (Berenguer *et al.*, 2016; Bursać Kovačević *et al.*, 2016; Cano-Lamadrid *et al.*, 2017), the most intense red color was achieved in the smoothie formulation including quince purée added to 60 % *Wond* pomegranate juice.

An enrichment of both Ca and K content were observed in the fig smoothies [13.8 mg (100 g)<sup>-1</sup> fw and 197 mg (100 g)<sup>-1</sup> fw, respectively]. This tendency was also found in all 60:40 (purée:juice) smoothies [191 mg (100 g)<sup>-1</sup> fw] and in *Wond* pomegranate smoothies [205 mg(100 g)<sup>-1</sup> fw].

An increase of the amount of pectin and vitamin C was seen when 60 % of fruit purée was used, in accordance with previous research (Nowicka *et al.*, 2016b). In addition, the type of fruit purée also affected the content of these two parameters, being jujube smoothies those which presented the lowest pectin content [0.39 g (100 g)<sup>-1</sup> fw] and the highest vitamin C content [190 mg (100 g)<sup>-1</sup> fw]. On the other hand, fig and quince smoothies presented the highest pectin content, being good alternative for diabetic and obese patients due to soluble dietary fiber, which has been associated with a reduction of both cholesterol and serum glucose (Naqash *et al.*, 2017). Besides, water-soluble pectins components link with Ca to obtain techno-functional properties enhancing texture properties.

Similarity, an increase of total sugar content was detected when the ratio purée:juice was 60:40. The use of different fruit purée modified sugar profile, with quince smoothies being the richest ones in fructose, agreeing with other authors (Szychowski *et al.*, 2014); while, jujube smoothies presented the highest amount of sucrose. The highest content of glucose was observed in both fig and jujube smoothies. When *ME* pomegranate juice was used, an increase in fructose and glucose contents was found. Although sorbitol has been reported as producing intolerance, it was just found in quince smoothies and below the thresholds established for severe symptoms after sugars intake, considering a threshold of 5 g (FAO/WHO, 1995).

On the other hand, both the type of fruit and pomegranate cultivar used to develop smoothies significantly affected the profile of organic acids. Jujube smoothies were characterized by the richest content of citric, tartaric, shikimic, and formic acids values. Similarly, fig smoothies had the highest formic, tartaric and succinic acids contents; while quince smoothies presented the highest oxalic and quinic acids contents. When *Wond* juices were used, the highest values of tartaric acid were found, as previous studies shown in fresh fruits (Cano-Lamadrid *et al.*, 2018). Oxalic acid was detected in all samples, but its content [0.06-0.18 g (100 g)<sup>-1</sup> fw] was below the thresholds established for anti-nutrient activity (inhibition of the mineral bioavailability and formation of urinary stones) (Teleszko *et al.*, 2014).

The anthocyanins (ACNs) were significantly affected by the type of fruit purée, fig smoothies presented the highest content in all ACNs. It is worth mentioning that the compounds delphinidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside were only detected when fig purée was used agreeing with results by Wojdyło *et al.*, (2016). As to the factor pomegranate cultivar, *Wond* smoothies increased the amount of delphinidin-3,5-di-*O*-glucoside and cyanidin-3,5-di-*O*-glucoside. Differences on the anthocyanin profile between *ME* and *Wond* fruits have been previously reported (Mena *et al.*, 2011). In agreement with Grzesik *et al.*, (2018), the values of *a*\* were not directly correlated with the anthocyanin content, but were well-correlated with the pH; pH values in a food matrix is the main factor controlling the changes in the anthocyanin forms and this affects the food color. Regarding flavonols, quercetin-3-*O*-rutinose was the only compound in common in all samples. The presence of some individual flavonols can be used as an indicator of the type of fruit purée used. Jujube smoothies presented specific flavonols such as quercetin-3-*O*-rutinoside-7-*O*-hexoside, quercetin-3-*O*-rutinoside-7-*O*-pentoside, quercetin-3-*O*-robinobioside, and keampferol-3-*O*-deoxyhexoside-*O*-pentoside. While, quercetin-3-*O*-malonyl-glucoside and apigenin-hexoside-pentoside were only identified on fig smoothies; and, keampferol-3-*O*-galactoside was exclusively detected on quince smoothies. Apart from that, an increase of quercetin-3-*O*-galactoside and

quercetin-3-*O*-malonyl-glucoside was noticed in *Wond* and *ME* pomegranate smoothies, respectively. Finally, the greatest percentage of fruit purée (60:40) used led to the highest content of quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside-7-*O*-pentoside and quercetin-3-*O*-malonyl-glucoside. Taking flavan-3-ols content into account, only the compound (+)-catechin was found in all smoothies. The highest content of flavan-2-ols was found in jujube smoothies being the main compound (+)-catechin [138 mg (100 g)<sup>-1</sup> fw]. Quince and fig smoothies were characterized by the highest amounts of procyanidin B1 [230 mg (100 g)<sup>-1</sup> fw] and (-)-epicatechin [168 mg(100 g)<sup>-1</sup> fw], respectively. It is worth mentioning that certain compounds can be used as an indicator of the fruit purée used in the smoothies. For instance, procyanidin B2, procyanidin trimer and procyanidin tetramer were singularly detected in jujube smoothies, whilst (-)-epicatechin was only identified of figs. Probably due to the fact the pH changes, the *Wond* pomegranate smoothies presented a great deal higher contents of procyanidin B2 and procyanidin trimer than *ME* smoothies. Additionally, the concentration of the polymeric procyanidins (PP) was also evaluated, being the highest values found in quinces, followed by jujube and figs; PP represented highest contribution to the total content of the flavan-3-ols. The identified hydroxycinnamic acids were only found in quince smoothies, while fig smoothies only presented chlorogenic acid; no presence of hydroxycinnamic acids was identified in jujube samples.

Quince purée led to the highest values of antioxidant capacity measured by ORAC assay [5.78 mmol Trolox (100 g)<sup>-1</sup>] as compared to quince and fig purées [2.61 and 2.34 mmol Trolox (100 g)<sup>-1</sup>, respectively]. Pearson's correlation coefficient showed that the ORAC values were positively correlated with the total flavonols content (R=0.77; *p*<0.01), flavan-3-ols without PP (R=0.60; *p*<0.05), and vitamin C (R=0.98; *p*<0.001). Contrary, recent study concluded that vitamin C did not present ORAC activity (Prior *et al.*, 2016). No significant correlation was observed among the ORAC values and the total contents of anthocyanin, polymeric procyanidins and hydroxycinnamic acid content.

### Summary

The addition of fig purée to the pomegranate smoothies was positively linked with the content of total anthocyanins, succinic acid, and Ca. The addition of jujube purée to the pomegranate smoothies was associated with the content of vitamin C, flavonols and sucrose, as well as ORAC activity, and values  $b^*$  (blue-yellow coordinate). The addition of quince purée was linked to the content of sorbitol, hydroxycinnamic acid, oxalic acid and quinic acid, and values of  $a^*$  (green-red coordinate). The use of *ME* cultivar increased glucose and fructose contents, whilst *Wond* cultivar enhanced anthocyanin content and  $a^*$  value. Finally, an improvement on the content of flavonols and flavan-3-ols were observed when the highest percentage of purée in the formulation was used.

#### **7.4.2. Study II** (publication 7)

##### Objective 7

**To evaluate how formulation and storage conditions of pomegranate-based smoothies affected on color, functional compounds and antioxidant capacity**

The values of total polyphenolic content (TPC) in fig, jujube, and quince pomegranate smoothies at the beginning of the storage period (T<sub>0</sub>) ranged between 247-314, 2939-3920 and 3809-5324 mg (100 g)<sup>-1</sup> fw (fresh weight), respectively. A reduction of 13.3-34.3 % of TPC was observed in fig smoothies after 6 months of storage at 4 °C, and of 52.2-64.7 % at 20 °C. The best result was obtained when 40 % of *Wond* juice was used in fig smoothies. On the other hand, the use of jujube in *ME* pomegranate smoothies stored at 4 °C, suffered a reduction of TPC between 24.71-24.9 %. When *Wond* cultivar was used, only 6.30 % was decrease and even an increase of 11.8 % in 60 % jujube smoothies was observed

(J2W). During storage at 20 °C, a decrease of 13.3-30.3 % of TPC was detected, being J2W the lowest one. Finally, the addition of quince purée reduced the TPC in 9.5-20.1 % after storage at 4 °C, and 17.2-32.6 % at 20 °C. This trend (the higher the storage temperature, the greatest the loss of TPC) was recently reported also in sour cherry smoothies (Nowicka *et al.*, 2016). It is worth mentioning that the highest percentage of purée and *Wond* cultivar, the higher maintenance of TPC during storage in all types of smoothies.

No drastic changes in the antioxidant capacity were observed after 6 months of storage. Similar results were found in apple, quince, and pear smoothies (Andrés *et al.*, 2016; Nowicka *et al.*, 2016). Some degradation products also present antioxidant properties, such as those from the Maillard compounds. Therefore, although some antioxidant compounds were destroyed during heat treatment, other compounds with bioactive capacity were simultaneously generated. The tendency of the highest the temperature, the highest the loss of antioxidant capacity was reported in previous studies about red and green smoothies (Di Cagno *et al.*, 2011).

On the other hand, the content of the total of monomer- and dimeric-forms of flavan-3-ols was not significant affected by storage conditions; whilst total flavonols content in fig, jujube and quince products were reduced 41.2, 23.6 and 2.2 %, and 45.6, 45.8 and 10.7 % after 6 months at 4 °C and 20 °C, respectively. As to total phenolic acids, 28.3 % and 58.4 % were decreased in quinces smoothies after storage at 4 °C and 20 °C, respectively. Fig smoothies after 6 months at 4 °C were not affected, but 57.7 % loss after 6 months at 20 °C was noticed. In jujube smoothies, phenolic acids were not detected.

It is very important to be aware of the stability of polymeric procyanidins (PP) during storage due to their well-known healthy properties (Gossé *et al.*, 2005). In fig pomegranate smoothies, a PP reduction of 41.9 % was found after 6 months at 4 °C, except when 60 % of purée was added decreasing just 17.0 %. Moreover, after 6 months at 20 °C a mean reduction of 44.1 % was observed. The jujube

*Wond* pomegranate smoothies after 6 months at 4 °C maintained the highest PP content, or even increased it by 13.1 % (J2W). After storage at 20 °C, jujube *ME* smoothies had a better maintenance (mean reduction of 3.5 %) than *Wond* smoothies (20.6 %). Finally, pomegranate cultivar was the factor which affected the least reduction on the PP content on quinces pomegranate smoothies, with reduction values being 9.1 % and 14.4 % in *Wond* and *ME* smoothies, respectively.

Several studies reported that the polymerization of the monomeric compounds produces an undesirable brown color (Sinela *et al.*, 2017). The temperature during storage could induce or avoid the polymerization (the loss of monomeric anthocyanins and their color). The lower the temperature used, the lower the ANCs degradation. After 6 months of storage at 4 °C, the total content of anthocyanins was reduced only by 15.8 %; but this content was reduced by 74.8 % when stored at 20 °C. The tendency observed was in agreement with previous study which indicated a loss of 94 % and 98 % for delphinidin-3,5-di-*O*-glucoside and cyanidin-3,5-di-*O*-glucoside, when smoothies were stored at 37 °C (Sinela *et al.*, 2017). It is worth mentioning that fig smoothies maintained the most total anthocyanins content at the end of the storability. The main reasons can be: (i) their initial high content and (ii) the large pectin content present in fig fruits and their based products, which can form ionic interactions among anthocyanin flavylium cations and free pectic carboxyl groups and/or anthocyanin aromatic stacking on bound anthocyanins (Fernandes *et al.*, 2014; Lin *et al.*, 2016; Wojdyło *et al.*, 2016).

The green-red coordinate ( $a^*$ ) significantly decreased during 6 month of storage, being smoothies stored at 4 °C redder than those stored at 20 °C. In sour-cherry-smoothies, a negative effect on red color was recently observed by enzymatic browning of phenolic compounds during storage (Nowicka *et al.*, 2016). The combination of 60 % of *Wond* pomegranate juice, quince purée and storage 6 months at 4 °C was the best formulation/conditions to avoid deterioration of red color.

The total anthocyanin content was positively correlated with the  $a^*$  coordinate values in fig and jujube smoothies. The quince smoothies presented a low correlation because of the pH, being important its value on the food matrix to control the changes in the anthocyanins color (Cano-Lamadrid *et al.*, 2018); In addition, a low pH enhances the linking and stability of anthocyanin and procyanidin polymers, improving color during storage (Li *et al.*, 2019). Although fig pomegranate smoothies were richer in anthocyanins after storage as it was above mentioned, a redder coloration was found in quince-pomegranate smoothies.

### **Summary**

After storage, the reddest color was found in quince-pomegranate smoothies although the richest in anthocyanins were fig smoothies. In addition, the highest total polyphenolic content [sum of anthocyanin, flavanols, monomeric and dimeric flavan-3-ols, polymeric procyanidins and phenolic acids] was found in the quince products. The best formulation and storage conditions for pomegranate smoothies were ratio purée:juice 40:60, *Wonderful* pomegranate juice, and storage at 4 °C, experiencing only a reduction of 30.1 %, 13.1 % and 9.5 % of bioactive compounds in fig, jujube and quince smoothies, respectively.

## **9. CONCLUSIONS / *CONCLUSIONES***



## Conclusions

### **Overview of the main contributions**

#### **Block I: Farming of hydroSOStainable pomegranate fruits (Objective 1)**

1. The application of a sustained deficit irrigation strategy (T1) caused water stress in pomegranate trees. Although a decrease of marketable yield was observed after deficit irrigation (T1) and thinning (A1), the fruits remaining on the tree were of higher weight and size, which is a positive fact and result in a higher price of the commercial fruits.
2. Thinning had negative effect on total phenolic content (TPC) of *Wonderful* and *Mollar de Elche* fruits. Besides, neither TPC nor punicalagin content were affected by the irrigation treatments.
3. *Wonderful* fruits were more sensitive than *Mollar de Elche* fruits to changes in the sugar profile, with the values of glucose and fructose being increased by deficit irrigation strategies (T1), which is important due to the strong sourness of these fruits. Besides, T1 caused a reduction of total aldehydes (mainly hexanal) and terpenoids in both cultivars, losing vegetables notes in their flavor.
4. **The best treatment for *Wonderful* and *Mollar de Elche* pomegranate cultivars was T1A1, which consisted of the simultaneous application of soft deficit irrigation during fruit growth and ripening (T1) and the application of thinning (A1).**

#### **Block II: Overview of commercial pomegranate products (Objective 2)**

5. A high variability in the content of bioactive compounds was observed among the pomegranate-based products available in the Spanish market and “unfortunately” the fact that pomegranate is present in the ingredient list of a product did not guarantee the presence of the “expected” contents of bioactive compounds.

6. There is a need for labelling “standardization” of commercial pomegranate products due to the wide variability of the key compounds (punicalagins, ellagic acid, and the total polyphenolic compounds) among “theoretically” similar pomegranate-based products.
7. **The “real and analyzed” contents of the key compounds should be declared on product labels, as indicators of the potential health benefits, and should replace subjective or expected functional descriptions.** This lack of correlation is certainly misinforming potential consumers.
8. Pomegranate juice and nectar producers need to evaluate the real content of punicalagins, ellagic acid, and the total polyphenolic compounds in their products to optimize formulation, heat treatment, and storage conditions to guarantee high levels of bioactive compounds during shelf life and to be able to label their products with the expected contents.

**Block III:** Preparation of dehydrated pomegranate arils (Objective 3, 4 & 5)

9. Osmotic dehydration using “Wonderful” pomegranate and chokeberry concentrated juices improved the quality of dried “Mollar de Elche” pomegranate arils in terms of rehydration rate, antioxidant capacity, color, and sensory profile.
10. An improvement of the volatile profile and sensory quality of developed dehydrated pomegranate arils was clearly noticeable when compared with those of the available commercial products.
11. **The main liking drivers of developed dehydrated arils were high content of esters, low content of furans, high fruity notes, sweet taste and low seed hardness.**

**Block IV: Preparation of pomegranate-based smoothies** (Objective 3, 6 & 7)

**12. A positive effect of the addition of minor crops (fig, jujube and quince) was observed on the nutritional and functionality of the novel pomegranate smoothies.**

13. The addition of jujube contributed to an enrichment in vitamin C, flavonols and organic acids, while an increase of pectin and anthocyanin content was found in fig and quince pomegranate based smoothies.
14. The best formulation and storage conditions was the addition of quince purée to *Wonderful* pomegranate juice at a ratio 40:60 purée:juice and stored at 4 °C. The main reasons were: i) the most valued quality parameter of pomegranate products (red color) was significantly improved and was maintained during storage; and ii) the highest total polyphenolic content [TPC: sum of anthocyanin, flavanols, flavan-3-ols, polymeric procyanidins and phenolic acid] was found after the smoothies preparation and was maintained during 6 months of storage.

**Implications in the agriculture and food industry**

1. HydroSOStainable agriculture can help, within its reach, in mitigating global climate change by reducing uncontrolled consumption of water resources establishing an optimized irrigation management. This farming strategy will produce clearly labeled pomegranate fruits with specific identity that can be used to produce novel products with added value and the highest possible quality by using hydroSOStainable pomegranate fruits.
2. Consumer preference is essential to improve processed food products quality, but small companies sometimes lack knowledge or tools to conduct consumer studies. Producers' organizations may benefit from harmonizing pomegranate products labelling, so they may fulfill consumer expectations and may be ready if health claims are finally authorized by EFSA for these

products. Authors suggest a possible improvement of the Commission Regulation (EU) N° 432/2012, reviewing the unregulated actions to improve labelling and avoid misleading the consumers, especially in the specific case of its application in pomegranate products.

3. Improving the quality of dried pomegranate arils and smoothies based on pomegranate and minor Mediterranean crops must increase the popularity of these fruits (some of them highly underutilized even in producing regions), especially in groups with reduced fruit consumption, such as teenagers and children, leading to higher consumer acceptance, consequently higher product demand, and finally higher benefits for the farmers and industry.
4. This dissertation also provides useful information to understand consumers' preference regarding pomegranate products such as dehydrated pomegranate arils. Also, the link of physico-chemical and sensory tools is clearly described and help in selecting proper sensory quality indicators.
5. Novel pomegranate-based products prepared using fruits grown under environmental friendly practices (hydroSOSustainability) should encourage both food industry and consumers to produce, buy and consume these high-quality products because they are safe, tasty and social-acceptable because use local (PDO, traditional cultivars) and environmental friendly (hydroSOSustainable farming) raw materials .

### **Future work**

1. A certification index of the hydroSOSustainable nature (including agronomic, chemical, functional, and sensory markers) should be developed for pomegranate fruits and their based products.
2. Further research is needed to fully optimize the combined drying treatments because the freeze-dried sample still had higher anthocyanin content and better instrumental color parameters. Additionally, the

improvement should be carried out avoiding undesirable compounds generated by the Maillard reaction. It can be worth studying other osmodehydration treatments increasing bioactive compounds such as using fortified concentrated juices with own-bioactive compounds from pomegranate peel co-product.

3. The energy consumption and cost of the drying treatment should be evaluated in future studies.
4. After knowing the nutritional and functional information of the smoothies, it is worth continuing the research in this area to elucidate their full sensory profiles and consumer acceptance.
5. The volatile composition of other types of smoothies and other types of pasteurization treatments should be used to avoid undesirable compounds and aromas; and then, it will be interesting to know their odor-active compounds. Moreover, consumer studies should be carried out to know the acceptance drivers of the new smoothies.
6. There is a need to assess the influence of the hydroSOStainable information (e.g. logotype) on consumers' acceptance and preference towards hydroSOStainable fruits and based products.
7. It is worth to continue the research in this area to know the effect of farming, types of commercial products, drying techniques, formulation of novel pomegranate products, blend of fruits, heat treatments but going further by studying the effects on human health, for instance starting by biological activities such as anti-diabetic, anti-obesity and anti-cholinesterase action.

## Conclusiones

### **Visión general de las principales contribuciones**

#### **Bloque I: Cultivo de la granada hidroSOStenible (Objetivo 1)**

1. La aplicación de la estrategia de riego deficitario sostenido estudiada (T1) causó estrés hídrico en los árboles de granada. Aunque se observó una disminución del rendimiento comercial después del riego deficitario (T1) y el aclareo (A1), los frutos que quedaban en el árbol fueron de mayor peso y tamaño, lo cual es un hecho positivo y resulta en un precio más alto de los frutos comerciales.
2. El aclareo tuvo un efecto negativo sobre el contenido de fenoles totales (TPC) de los frutos *Wonderful* y *Mollar de Elche*. Además, ni el TPC ni el contenido de punicalagina se vieron afectados por los tratamientos de riego aplicado.
3. Los frutos *Wonderful* fueron más sensibles que los frutos *Mollar de Elche* a los cambios en el perfil de azúcares, siendo los valores de glucosa y fructosa mayores tras la aplicación de las estrategias de riego deficitario (T1), lo cual es importante debido a la fuerte acidez característica de estas frutas. Además, T1 causó una reducción del contenido de aldehídos totales (principalmente hexanal) y terpenoides en ambos cultivares, perdiendo notas vegetales en su sabor.
4. **El mejor tratamiento para los cultivares de granada *Wonderful* y *Mollar de Elche* fue T1A1, que consistió en la aplicación simultánea de riego deficitario sostenido y suave durante el crecimiento y maduración del fruto (T1) y la aplicación de aclareo (A1).**

#### **Bloque II: Visión general de los productos comerciales de granada (Objetivo 2)**

5. Se observó una gran variabilidad en el contenido de compuestos bioactivos entre los productos a base de granada disponibles en el mercado español y "desafortunadamente" el hecho de que la granada esté presente en la lista de

ingredientes de un producto no garantiza la presencia del "esperado contenido de compuestos bioactivos"

6. Existe la necesidad de estandarizar el etiquetado de los productos comerciales de granada debido a la amplia variabilidad de los compuestos clave (punicalaginas, ácido elálgico y los compuestos polifenólicos totales) entre los productos de granada similares "teóricamente".
7. **Los contenidos "reales y analizados" de los compuestos clave deberían declararse en las etiquetas de los productos, como indicadores de los posibles beneficios para la salud, y deben reemplazar las descripciones funcionales subjetivas o esperadas.** Esta falta de correlación permite la confusión de los potenciales consumidores.
8. Los productores de zumo y néctar de granada deberían evaluar el contenido real de punicalaginas, ácido elálgico y fenoles totales en sus productos para optimizar la formulación, el tratamiento térmico y las condiciones de almacenamiento para garantizar altos niveles de compuestos bioactivos durante la vida útil y capaz de etiquetar sus productos en el futuro con los contenidos esperados.

**Bloque III:** Preparación de arilos de granada deshidratados (Objetivo 3, 4 & 5)

9. La deshidratación osmótica utilizando zumos concentrados de granada y aronia mejoró la calidad de los arilos de granada *Mollar de Elche* deshidratados en términos de tasa de rehidratación, capacidad antioxidante, color y perfil sensorial.
10. Una mejora del perfil volátil y la calidad sensorial de los arilos de granada deshidratados desarrollados fue claramente notable en comparación con los de los productos comerciales disponibles.
11. **Los principales impulsores de aceptación de los arilos deshidratados desarrollados fueron el alto contenido de ésteres, el**

**bajo contenido de furanos, altos valores de las notas frutales, el sabor dulce y la baja dureza de las semillas.**

**Bloque IV:** Preparación de *smoothies* a base de granada (Objetivo 3, 6 & 7)

12. Se observó un efecto positivo de la adición de higo, jínjol y membrillo sobre la calidad nutricional y la funcionalidad de los *smoothies* de granada.
13. La adición de jínjol contribuyó a un enriquecimiento en vitamina C, flavonoles y ácidos orgánicos, mientras que se observó un aumento del contenido de pectina y antocianinas en los *smoothies* a base de higo y de membrillo.
14. **La mejor formulación y condiciones de almacenamiento fue la adición de puré de membrillo al zumo de granada *Wonderful* en una proporción 40:60 puré:zumo, almacenado a 4 ° C.** Las razones principales fueron: i) el parámetro de calidad más valorado de los productos de granada (color rojo) se mejoró significativamente y se mantuvo durante el almacenamiento; y ii) se encontró un mayor contenido de polifenoles totales [TPC: suma de antocianinas, flavanoles, flavan-3-oles, procianidinas poliméricas y ácido fenólicos] tras la preparación de los *smoothies* y se mantuvo durante 6 meses de almacenamiento.

**Implicaciones en la agricultura y en la Industria Alimentaria**

1. La agricultura bajo condiciones de riego deficitario (hidroSOStenible) puede ayudar a mitigar el cambio climático global al reducir el consumo incontrolado de los recursos hídricos estableciendo una gestión de riego optimizada. Esta estrategia de cultivo producirá frutos con una identidad específica que puede usarse para producir productos novedosos con valor añadido a partir de granadas hidrosostenibles y con la mayor calidad posible.
2. La preferencia del consumidor es esencial para mejorar la calidad de los productos alimenticios procesados, pero las pequeñas empresas a veces



carecen de conocimientos o herramientas para realizar estudios de consumidores. Las organizaciones de productores pueden beneficiarse de la armonización del etiquetado de los productos de granada, por lo que pueden cumplir con las expectativas de los consumidores y estar preparados en caso de que EFSA finalmente autorice las declaraciones de propiedades saludables para estos productos. Los autores sugieren una posible mejora del Reglamento (UE) N° 432/2012, revisando las acciones no reguladas para mejorar el etiquetado y evitar la confusión de los consumidores, especialmente en el caso específico de su aplicación en productos de granada.

3. Mejorar la calidad de los arilos deshidratados y los *smoothies* a base de granada y otros cultivos mediterráneos con menor producción (higo, jínjol, membrillo) debe aumentar la popularidad de estas frutas (algunas de ellas altamente infrautilizadas incluso en regiones productoras), especialmente en grupos con un consumo reducido de frutas, como adolescentes y niños, lo que lleva a una mayor aceptación del consumidor, una mayor demanda de productos y, finalmente, mayores beneficios para los agricultores y para la industria.
4. Esta tesis doctoral también proporciona información útil para comprender las preferencias de los consumidores con respecto a los productos de granada, como los arilos de granada deshidratados. Además, el vínculo de las herramientas fisicoquímicas y sensoriales se describe claramente y ayuda a seleccionar los indicadores de calidad sensorial adecuados.
5. Los nuevos productos a base de granada preparados a partir de frutos producidos bajo prácticas respetuosas con el medio ambiente (hidroSOSTenibles) deberían alentar tanto a la industria alimentaria como a los consumidores a producir, comprar y consumir estos productos de alta calidad porque son seguros, sabrosos y socialmente aceptables ya que usan materias primas locales (DOP, cultivares tradicionales) y respetuosas con el

medio ambiente (hidroSOSostenible).

### **Futuras líneas de investigación**

1. Se debe desarrollar un índice de certificación hidroSOSostenible (incluyendo marcadores agronómicos, químicos, funcionales y sensoriales) para los frutos de granada y sus productos derivados.
2. Se necesita más investigación para la optimización completa de los tratamientos combinados de secado ya que la muestra liofilizada todavía tuvo un mayor contenido de antocianinas y mejores parámetros de color instrumental. Además, la mejora debe llevarse a cabo evitando compuestos indeseables generados por la reacción de Maillard. Puede valer la pena estudiar otros tratamientos de deshidratación osmótica aumentando los compuestos bioactivos, como el uso de zumos concentrados fortificados con compuestos bioactivos propios a partir del co-producto piel de granada.
3. El consumo de energía y el coste del tratamiento de secado deben evaluarse en futuros estudios.
4. Después de conocer la información nutricional y funcional de los *smoothies*, vale la pena continuar la investigación en esta área para dilucidar sus perfiles sensoriales completos y la aceptación del consumidor.
5. La composición volátil de otras formulaciones de *smoothies* y otros tipos de tratamientos de pasteurización debe usarse para evitar compuestos y aromas indeseables; y posteriormente, sería interesante conocer sus compuestos odoríferos activos. Además, se deben realizar estudios de consumidores para conocer los impulsores de la aceptación de los nuevos *smoothies*.
6. Existe la necesidad de evaluar la influencia de la información hidroSOSostenible (por ejemplo, el logotipo) en la aceptación y preferencia de granadas hidroSOSostenibles y sus productos por parte de los consumidores.

7. Vale la pena continuar la investigación en esta área para conocer el efecto de las prácticas agrícolas en los productos, los diferentes tipos de productos comerciales, las técnicas de secado, la formulación de nuevos productos de granada, la mezcla de frutas, los tratamientos térmicos, pero yendo más allá al estudiar los efectos sobre la salud humana, por ejemplo comenzando por actividades biológicas como la acción antidiabética (inhibición de la alfa-amilasa), la acción antiobesidad (inhibición de la lipasa pancreática) y la acción anticolinesterasa (inhibición de la acetilcolinesterasa).

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