Doctoral Thesis

Research, Development and Innovation of Hydrosustainable products based on pomegranate

Marina Cano Lamadrid

Supervisors:

Prof. Ángel Antonio Carbonell Barrachina

Prof. Aneta Wojdyło

2020

This doctoral thesis has been carried out thanks to an international double PhD title between WUELS (Poland) and UMH (Spain). This doctoral thesis has been partially funded by Fondo Europeo de Desarrollo Regional (FEDER), Ministerio de Ciencia e Innovación (MCI) y Agencia Estatal de Investigación (AEI) (e-SOS-water, AGL2013-482-45922-C2-2-R). Marina Cano Lamadrid was funded by a FPU grant (Reference number: FPU15/02158) from the Spanish Ministry of Education and by project PROM Programme – International scholarship exchange of PhD and young academic staff (Polish National Agency for Academic Exchange, NAWA).

A mi padre,

presente en mi mente durante todo este tiempo

AGRADEMICIMIENTOS

Quiero agradecer a mis directores de Tesis, el Dr. Ángel A. Carbonell Barrachina y a la Dra. Aneta Wojdyło por su confianza y apoyo, y por enseñarme a luchar por la ciencia, la investigación y la docencia. Muchísimas gracias por todo.

Gracias también al Dr. Francisco Burló, a la Dra. Francisca Hernández, a la Dra. Esther Sendra, al Dr. Ángel Calín y al Dr. David López ya que han sido para mí personas de referencia con personalidades, formas de trabajar y puntos de vista diferentes, siendo su experiencia y apoyo muy enriquecedor para mí tanto a nivel personal como profesional.

Es indiscutible el apoyo que he tenido por parte del Departamento de Tecnología Agroalimentaria, tanto a nivel personal, como investigador y docente. Gracias a todos de corazón, especialmente al Dr. José Ramón Díaz, Dr. Pedro Zapata, Dra. Raquel Muelas, Dra. Estrella Sayas, Dr. Manuel Viuda, y Dr. Juan Miguel Valverde.

Gracias a " Wrocław University of Environmental and Life Sciences" por abrirme las puertas y hacerme sentir parte de ella, especialmente quería agradecer el apoyo por parte de la Dra. Małgorzata Korzeniowska, a la Dra. Anna Michalska, al Dr. Adam Figiel, al Dr. Krzysztof Lech, a la Dra. Agnieszka Kita y a la Dra. Paulina Nowicka. "Serdecznie dziękuję"

Risas, lágrimas, esfuerzo, alegría, frustración, horas en el laboratorio, montaña rusa de emociones, viajes, congresos, jornadas… es lo que he compartido con determinadas personas durante estos años que nunca voy a olvidar y tengo que agradecerles todo: Lucía, Tina, Emma, Paula, Hussein, Camila, Chan, Raquel, María José, Igor, Karolina, Samiha, Jacinta, Estefanía, las nuevas incorporaciones de CSA, José copistería, MUDIC, Maricen, Salva, Fabián, Xose…

Muchas gracias a toda mi familia, en especial a mi madre y a mi pareja por estar orgullosos de mis logros y animarme. Sin vuestro apoyo y con "mi cabezonería", no hubiese conseguido todas las metas propuestas y alcanzadas durante todos estos a

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:** where the principal objectives and more relevant results obtained have been presented.
- **Aim and objectives:** The aim and specific objectives have been established in this section.
- **Results & Discussion: 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

  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

  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

  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

  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

  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

  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

- **Conclusions:** The main conclusions obtained after the achievements obtained and the future research lines opened by this work have been listed.

ABSTRACT / *RESUMEN* / *STRESZCZENIE*

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 hydroSOStainable 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 hydroSOStainable 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 ($DPPH^{\bullet}$, $ABTS^{\bullet+}$ 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 ºC; 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 (hydroSOStainable) 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 hydroSOStainable 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 hidroSOStenibles 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 hidroSOSsostenibles.
- *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 de 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 ($DPPH^{\bullet}$, $ABTS^{\bullet+}$ 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 4ºC.

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 hidroSOStenible 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 hidroSOStenible).

Streszczenie

Głównym celem rozprawy było opracowanie i scharakteryzowanie nowych produktów sporządzonych na bazie owoców granatowca pochodzących z produkcji hydroSOS, które powinny charakteryzować się niepowtarzalnymi cechami sensorycznymi i funkcjonalnymi w tym charakteryzować się podwyższonymi walorami prozdrowotnymi, przez co będą atrakcyjne i w pełni dostosowane do potrzeb i wymagań europejskich konsumentów.

Aby osiągnąć główny cel, praca została zrealizowana w 4 częściach badawczych:
- część I: uprawa hydroSOS owoców granatu.
- część II: przegląd komercyjnych produktów z granatów.
- część III: opracowanie produktu suszonego z owoców granatu w oparciu o „arils".
- część IV: opracowanie produktu smoothies na bazie granatów.

Część I

Zmiany klimatyczne ostatnich lat potwierdzają, że produkcja żywności zależy bardziej od dostępności wody niż od jakichkolwiek innych zasobów środowiska. Szczególne znaczenie ma to w przypadku rolnictwa jakie spotyka się w Hiszpanii na terenach śródziemnomorskich co sprawia iż niedobór wody stanowi dla jego funkcjonowania istotny problem.

Mając powyższe na uwadze konieczna jest zmiana polityki zrównoważonego zarządzania tym podstawowym zasobem jakim jest woda, a strategie irygacji deficytu wody (SDI) mogą być interesującym i pożądanym działaniem. Chociaż drzewa granatowca są odporne na suszę, oczekuje się, że hodowla granatów

w systemie SDI wywoła stres wodny drzew, które odpowiedzą na niego wzrostem zawartości związków bioaktywnych i atrakcyjniejszym profilem sensorycznym, przy jednoczesnej poprawie efektywności zużycia wody w uprawie.

Szczegółowym celem tej części było zebranie wiedzy na temat równoczesnego wpływu SDI (podczas wzrostu i dojrzewania owoców) i obciążenia plonu (przerzedzenie) na plon i jakość owoców granatowca odmiany Mollar de Elche. Ocenę jakości owoców przeprowadzono pod względem *(i)* fizycznym *(ii)* właściwości chemicznych oraz

(iii) atrybutów sensorycznych. Stwierdzono, że zabieg przerzedzenia zawiązków owoców skutecznie zwiększyć ich rozmiar i wagę, natomiast nie potwierdzono wpływu nawadniania na istotne zmiany w zawartość związków polifenolowych.

Natomiast stwierdzono, że owoce granatowca pod wpływem stresu wodnego i przerzedzania charakteryzowały się wyższą zawartością cukrów (glukozy i fruktozy), większymi i cięższymi owocami oraz wyższą zawartością alkoholi i monoterpenoidów oraz atrakcyjniejszymi notami w ocenie kluczowych cech sensorycznych (tj. kolor, owocowości, świeżości owoców granatu).

Część II

W ostatnim czasie prowadzone są szeroko zakrojone badania przez naukowców i przemysł spożywczy nad opracowaniem nowatorskich produktów opartych na owocach i warzywach uwzględniających preferencje konsumentów tj. produktów zrównoważonych, prozdrowotnych i gotowych do spożycia. Wiąże się to z faktem, iż w ostatnich latach wzrosła świadomość konsumentów na temat wpływu żywności na ich zdrowie i dobre samopoczucie. Promocja wielu produktów opracowanych na bazie owoców granatowca oparta jest na rozpowszechnionym zdrowym wizerunku tego owocu, podczas gdy rzeczywista zawartość bioaktywnych fitozwiązków pozostaje na niskim poziomie.

Komercyjne produkty z owoców granatowca, w tym kapsułki i suplementy, a także soki i nektary zostały poddane analizom poprzez porównanie informacji na etykiecie z rzeczywistą zawartością związków bioaktywnych (punikalaginy, kwasu elagowego i całkowitej zawartości związków polifenolowych) oraz związaną z nimi aktywnością przeciwutleniającą (DPPH$^{\bullet}$, ABTS$^{\bullet+}$ i FRAP). Wyniki przeprowadzonych badań wskazały na dużą zmienność w zawartości związków bioaktywnych oraz potrzebę dalszej weryfikacji głównych założeń technologicznych związanych z optymalizacją procesów i warunków przechowywania, a także etykietowania i standaryzacji, która zawiera tylko dane z rzeczywistej analizy, a nie „teoretycznie" oczekiwaną zawartość.

Część III

Ponieważ dzienne spożycie owoców i warzyw wciąż jest niższe niż zalecane (RDI), stąd pożądane jest zwiększenie podaży owoców i warzyw, co można upatrywać poprzez spożycie suszonych owoców i płynnych przekąsek owocowych. Dlatego znaczące urozmaicenie portfolio w produkty opracowane na bazie owoców granatowca jest jednym z ważnych celów niniejszej pracy: *(i)* odwodnione „arils" i *(ii)* smoothies z na bazie owoców granatowca.

Celem trzeciej części nieniejszej pracy była ocena kinetyki suszenia, parametrów jakościowych (zawartość antocyjanów, pojemność przeciwutleniająca, kolor, stopień rehydracji), właściwości sensorycznych i stopień akceptacji sensorycznej przez konsumentów suszonych „arils" otrzymanych z odmiany Mollar de Elche (ME) przygotowanych z wykorzystaniem procesu odwodnienia osmotycznego (OD) z wybranymi koncentratami soków owocowych (jabłko, granatu odmiany Wonderful i / lub aronia) i suszonych techniką łączoną [suszenie konwekcyjne (CPD) i dosuszanie próżniowo- mikrofalowe (VMFD)].

Zastosowanie OD zapewniło suszonym „arils" charakterystyczną słodycz aromatu ME, poprawiło kolor i złożoność sensoryczną charakterystyczną dla owoców granatowca odmiany Wonderful. Wszystkie próbki przygotowane przy użyciu proponowanych nowych technik suszenia były w większym stopniu akceptowane niż testowane próbki komercyjne. Najkorzystniejsze cechy uzyskano dla próbki suszonej przy użyciu odwodnienia osmotycznego w koncentracie soku z granatów odmiany Wonderful, a następnie utrwalone poprzez połączenie techniki suszenia (CD + VMFD).

Część IV

Jeśli chodzi o ostatnią część, jej celem była ocena efektu połączenia przecierów z owoców figi, głożyny oraz pigwy z sokiem z granatów (odmiany Wonderful i Mollar de Elche) celem przygotowania smoothies (przecier:sok - 40:60 i 60:40). Otrzymane produkty poddano analizie fizyko-chemicznej w tym oznaczono zawartość związków biologicznie aktywnych i odżywczych oraz aktywność przeciwutleniającą. Ponadto produkty zostały poddane przechowywaniu. Czynnikiem mającym największy wpływ na skład smoothies był rodzaj przecieru owocowego, gdyż smoothies figowe były bogate w antocyjany, z głożyny we flawonole i witaminę C, a pigwowe w kwasy fenolowe. Najwyższe zawartości antocyjanów, flawanoli, flawan-3-oli, polimerycznych procyjanidyn i kwasów fenolowych po 6 miesiącach przechowywania w 4°C zmierzono w przypadku połączenia przecieru pigwy z sokiem z granatowca w ilości 40:60.

Podsumowując, wykazano, że nowe produkty opracowane na bazie owoców granatowca z upraw hydroSOS powinny zwrócić szczególną uwagę zarówno przemysłu spożywczego, jak i konsumentów, gdyż ich wysoka jakość wynika z następujących faktów: *(i)* pochodzą one z upraw przyjaznych dla środowiska, *(ii)* są produktami o wysokiej akceptacji konsumenckiej co wzmaga fakt, iż pochodzą z regionu *(iii)* są smaczne i bogate w związki bioaktywne wytworzone w wyniku stresu wywołanego przez uprawę metodą hydroSOS.

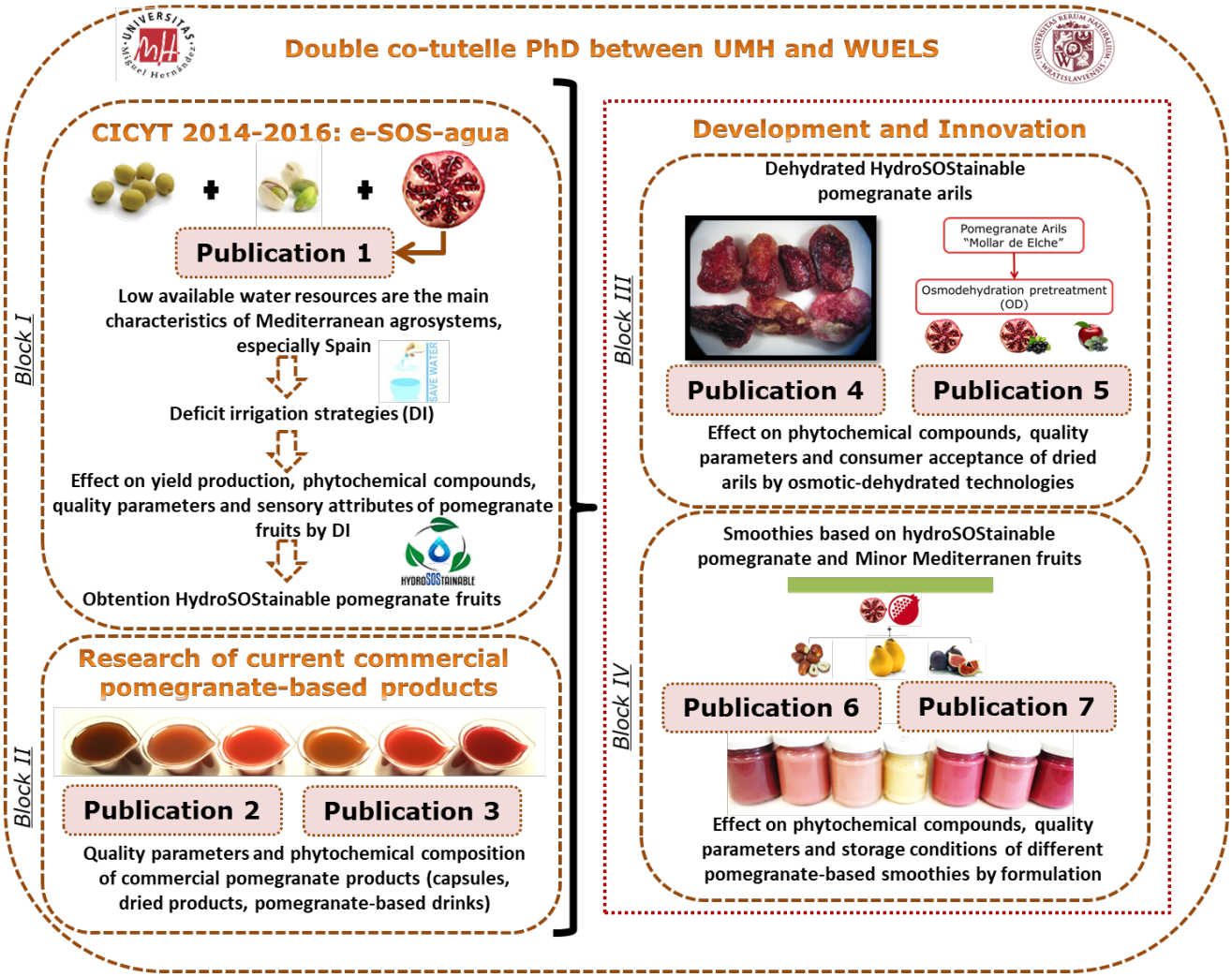
AIM and OBJECTIVES

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 ºC) 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.



CONCLUSIONS

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 produced 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) Nº 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 (hydroSOStainability) 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 (hydroSOStainable farming) raw materials .

Future work

1. A certification index of the hydroSOStainable 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 us 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.

PUBLICATIONS

Publication 1

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., Hernández F.

Journal of the Science of Food and Agriculture, 98: 3098-3108 (2018)

DOI:10.1002/jsfa.8810

Research Article

Received: 17 October 2017 Revised: 30 November 2017 Accepted article published: 6 December 2017 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8810

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

Marina Cano-Lamadrid,[a†] Alejandro Galindo,[b†] Jacinta Collado-González,[a†] Pedro Rodríguez,[c‡] Zulma N Cruz,[d‡] Pilar Legua,[e‡] Francisco Burló,[a‡] Donaldo Morales,[d‡] Ángel A Carbonell-Barrachina[a‡] and Francisca Hernández[e*‡]

Abstract

BACKGROUND: The working hypothesis of the present study was that, by proper simultaneous control of irrigation (hydroSOStainable 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; hydroSOStainable fruits; *Punica granatum* L.; water deficit

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).[1] 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.[2] Moreover, pomegranate tree exhibits high adaptability to water deficit in arid and semiarid areas because it possesses drought tolerance characteristics.[3] 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[4]; 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

* *Correspondence to: F Hernández, UMH, EPSO, Department of Plant Sciences and Microbiology, Plant Production and Technology Research Group, Orihuela, Alicante, Spain. E-mail: francisca.hernandez@umh.es*

† *These authors contributed equally to this work.*

‡ *These authors contributed equally to this work.*

a *Department of Agrofood Technology, Food Quality and Safety (CSA) Research Group, Escuela Politécnica Superior de Orihuela (EPSO), Universidad Miguel Hernández de Elche (UMH), Orihuela, Alicante, Spain*

b *Departamento of Water Engineering & Management, Faculty of Engineering Technology, University of Twente, AE, Enschede, The Netherlands*

c *Centro de Investigación Obonuco. Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Pasto, Nariño, Colombia*

d *Instituto Nacional de Ciencias Agrícolas (INCA), San José de Las Lajas, Mayabeque, Cuba*

e *UMH, EPSO, Department of Plant Sciences and Microbiology, Plant Production and Technology Research Group, Orihuela, Alicante, Spain*

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.[5] 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.[6] However, pomegranate juice obtained from SDI trees, under severe water stress, was of lower quality and less healthy than that that from fully irrigated trees.[7] Recently, however, other studies[8] 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.[9]

On the other hand, previous studies with fruits such as pistachios[10,11] and table olives,[12,13] grown under deficit irrigation strategies were shown to have a proper and specific identity and were termed 'hydroSOStainable' 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-calcicorthid 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 (ETo), as calculated using the Penman–Monteith equation (FAO method),[14] and a crop factor based on the time of the year[15] and also the percentage of ground area shaded by the tree canopy.[16] 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^{-1}) 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^{-1}, maturity index (MI) [TSS (°Brix)/TA (g 100 mL^{-1})] 21.5 ± 6.3 and (ii) *ME*: 12.7 °Brix, 0.140 g 100 mL^{-1}, 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 (Ψ_{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).[5]

Morphological, physical and chemical parameters

The moisture (*M*) percentage of arils was determined in accordance with Alcaraz-Mármol *et al.*[17] 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.[18] 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.[19] 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 α and β isomers) in the pomegranate juices were analyzed using the method proposed by Cano-Lamadrid *et al.*[20]

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.[17] 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 μL of 1000 mg L^{-1}, 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 μm 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,[21] 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 μm.

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.[22] 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. Ψ_{stem} and g_{leaf} values for each replicate were averaged before the mean ± 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).

Ψ_{stem} and g_{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 Ψ_{stem} and g_{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,[23] stomatal conductance,[24] leaf photosynthesis[25] and tree water use.[26] However, studies also reported a reduction in water uptake as a result of a high crop load.[27] Finally, further studies concluded that the effect of crop load on tree water status is not obvious[28] or is apparent only under severe deficit irrigation conditions.[28] In this sense, the results from the current experiment indicate that Ψ_{stem} and g_{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

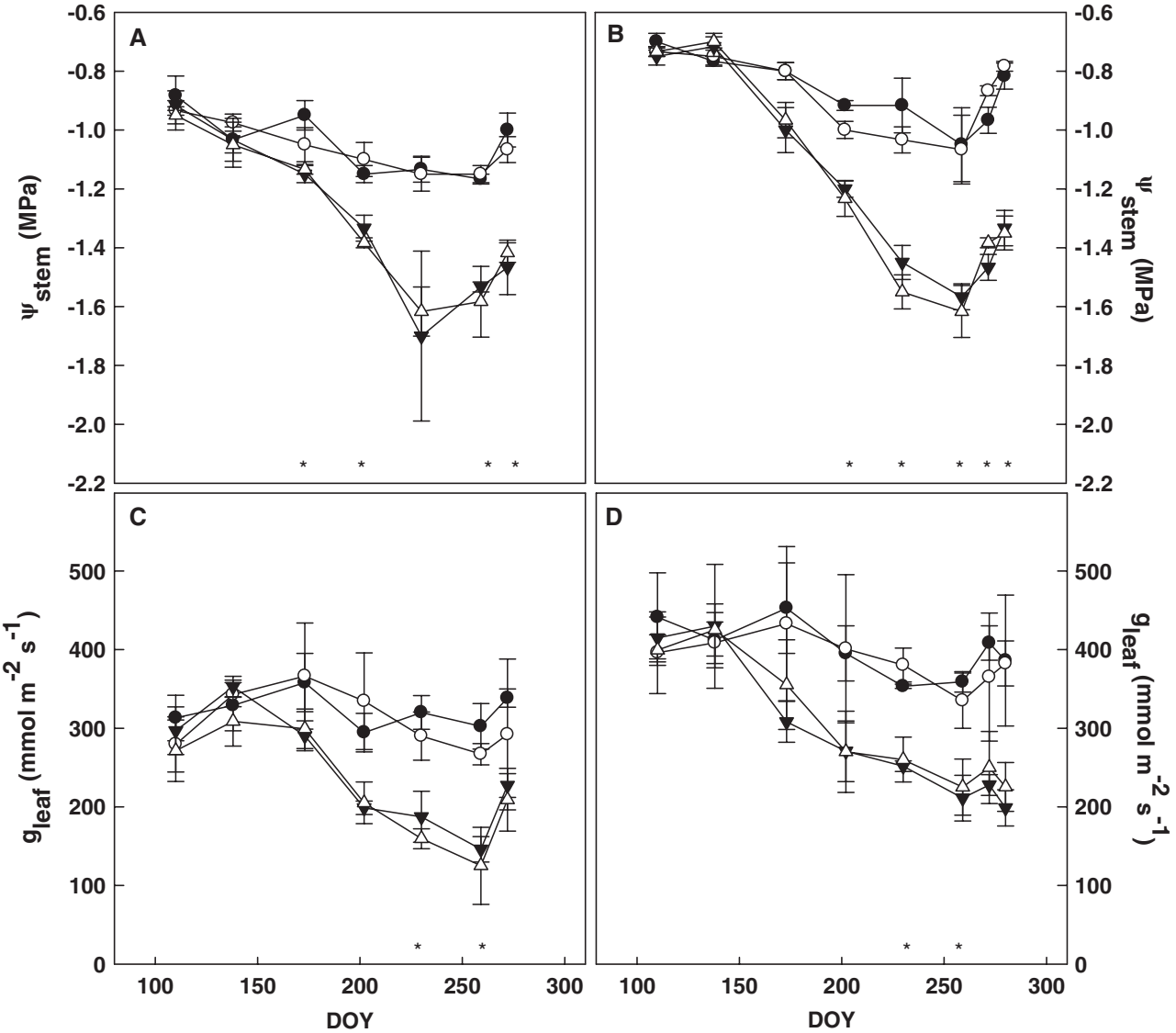


Figure 1. Midday stem water potential (Ψ_{stem}) and leaf conductance (g_{leaf}) values in control (T0, circles) and deficit irrigated (T1, triangles) *Wond* (A, C) and *ME* (B, D) pomegranate trees, which were hand-thinned (A1, open symbols) or non-thinned (A0, closed symbols), during the experimental 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.[5,6] However, water deficit did not affect fruit weight and size. In this respect, and taking into consideration the results shown previously,[5] 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-phloroglucinol and monomeric ACNs,[29] 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.[30] 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%.[18] 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^{-1} FW), and, punicalagin (Pn, mg mL^{-1} 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^{-1} Trolox FW)	Pn (mg mL^{-1} FW)
				WONDERFUL			
				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
				MOLLAR DE ELCHE			
				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 α and β punicalagin isomers.
FW, fresh weight.

of different cultivars, and probably also because pomegranate juices were stored at −18 °C during 2 weeks and then defrosted at 4 °C. Previous studies indicated that a prolonged storage at 4 °C, or even at −18 °C, produced the polymerization of ACNs with other compounds, mainly condensed tannins.[29,31] 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*).[17] 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.[32] 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[30]; 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,[20] implying that Pn (isomers α and β) 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

	Sugars		Organic acids	
	Glucose	Fructose	Citric acid	Ascorbic acid
Treatment	(g L^{-1} FW)			
	WONDERFUL			
	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 × 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
	MOLLAR DE ELCHE			
	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 × 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,[17,32] two sugars (glucose and fructose) and two organic acids (citric and ascorbic) were identified in the *Wond* and *ME* pomegranate juices (Table 2).

In terms of sugar profile, the glucose/fructose ratios were 0.84 and 0.82 in *Wond* and *ME*, respectively; similar results were reported previously.[17,32] An increase of glucose and fructose in *Wond* 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.[33] The interaction on SDI and thinning (T1A1) gave rise to the highest increase in glucose and fructose in the *Wond* 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 *Wond* ones.[9]

The content of citric acid was higher in the *Wond* fruits than in the *ME* fruits, which agreed with a previous study stating that citric acid predominated in sour varieties, such as *Wond*.[17] 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.[34] 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 *Wond* 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.[35] 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.[36,37]

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): β-pinene (V10), *p*-cymene (V11) and α-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):

- *Wond*: ALCs (mean of all treatments 67.0%) >> MTOs (11.5%) > MTEs (10.1%) ≈ 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 *Wond* fruits (Table 4); these results agreed with those reported previously.[38] 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.[39]

Changes on volatile composition as affected by irrigation or thinning treatments have been found in different fruits, such as jujube,[40] table olive[13] and pistachio,[10] 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 *Wond* 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	β-Pinene	12.33	992	998	Woody
V11	*p*-Cymene	14.63	1043	1051	Citrus
V12	α-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 (β-pinene), was observed; however, in the *ME* cultivar, ALDs also decreased (hexanal and nonanal), although MTEs decreased as well (β-pinene and α-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.[41] 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.[42] 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.[43]

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 *Wond* 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 *Wond* 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 *Wond* (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 *Wond* fruits (Fig. 2A), T0A0 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 *Wond* fruits, all other three treatments (T0A1, 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 (T0A0, T0A1 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. *Wond* 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 Elche* pomegranates as affected by deficit irrigation and thinning treatments

	Aldehydes				Esters				Aliphatic alcohols				Monoterpenes				Monoterpenoids		
Compounds	V1±	V2	V3	∑ALD	V4	V5	V6	∑EST	V7	V8	V9	∑ALC	V10	V11	V12	∑MTE	V13	V14	∑MTO
	Relative abundance (%)																		
WONDERFUL																			
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	*	*	*	*	*
Tukey's multiple range test																			
Irrigation																			
T0	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
T1	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																			
A0	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
A1	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 × Thinning																			
T0A0	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
T0A1	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
T1A0	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
T1A1	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
MOLLAR DE ELCHE																			
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																			
T0	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
T1	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																			
A0	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
A1	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 × Thinning																			
T0A0	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
T0A1	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
T1A0	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
T1A1	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.
V1, hexanal; V2, octanal; V3, nonanal; ∑ALD, total aldehydes; V4, benzyl acetate; V5, ethyl hexanoate; V6, ethyl octanoate; ∑EST, total esters; V7, 2-ethyl-1-hexanol; V8, 3- hexen-1-ol; V9, 1-hexanol; ∑ALC, total aliphatic alcohols; V10, β-pinene; V11, *p*-cymene; V12, α-terpineol; ∑MTE, total monoterpenes; V13, linalool; V14, limonene; ∑MTO, total monoterpenoids.

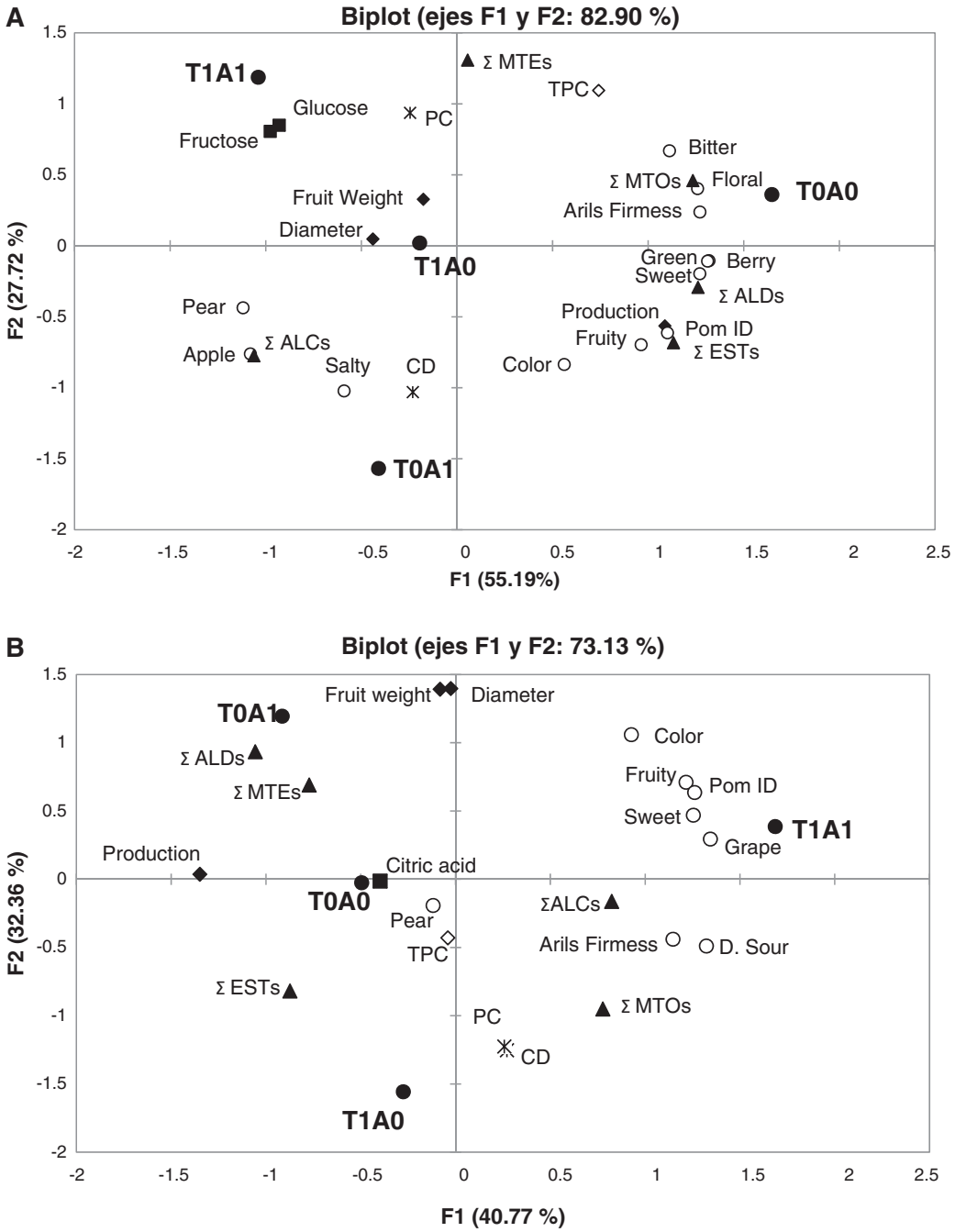


Figure 2. PCA scores plot showing the relationship among production, morphological parameters, total polyphenolic content, polymeric color, organic acids and sugars contents, volatile composition, and descriptive sensory analysis and treatments (T0A0, T0A1, T1A0 and T1A1) in cultivars *Wond* (A) and *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 (∑ALDs), total esters (∑ESTs), total aliphatic alcohols (∑ALCs), total monoterpenes (∑MTEs) and total monoterpenoids (∑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, T0A0, 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, T0A0, 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	Arils Firmesss
							WONDERFUL							
							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 × 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
							MOLLAR DE ELCHE							
							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 × 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).

ACKNOWLEDGEMENTS

We are grateful to the *Ministerio de Economía y Competitividad* (MINECO) for funding this research through the projects (CICYT/FEDER AGL2013-45922-C2-1-R and AGL2013-45922-C2-2-R). MC-L was funded by a FPU grant (FPU15/02158) from the Spanish Government. AG and JC-G acknowledge the postdoctoral financial support received from the Ramón Areces Fondation and Juan de la Cierva program, respectively. This work is the result of the internships of PR and DM (19925/IV/15 and 20127/IV/17) funded by the Seneca Foundation-Agency for Science and Technology in the Region of Murcia under the Jiménez de la Espada Program for Mobility, Cooperation and Internationalization.

REFERENCES

1 Gómez-Caravaca AM, Verardo V, Toselli M, Segura-Carretero A, Fernández-Gutiérrez A and Caboni MF, Determination of the major phenolic compounds in pomegranate juices by HPLC–DAD–ESI-MS. *J Agric Food Chem* **61**:5328–5337 (2013).
2 Viuda-Martos M, Fernández-López J and Pérez-Álvarez JA, Pomegranate and its many functional components as related to human health: a review. *Compr Rev Food Sci F* **9**:635–654 (2010).
3 Rodríguez P, Mellisho CD, Conejero W, Cruz ZN, Ortuño MF, Galindo A *et al.*, Plant water relations of leaves of pomegranate trees under different irrigation conditions. *Environ Expl Bot* **77**:19–24 (2012).
4 MAPAMA, Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, *Anuario de Estadística* [Online]. Available: http://www.mapama.gob.es/estadistica/pags/anuario/2016-Avance/avance/AvAE16.pdf [8 November 2017].
5 Galindo A, Calín-Sánchez Á, Collado-González J, Ondoño S, Hernández F, Torrecillas A *et al.*, Phytochemical and quality attributes of pomegranate fruits for juice consumption as affected by ripening stage and deficit irrigation. *J Sci Food Agr* **94**:2259–2265 (2014).
6 Mellisho CD, Egea I, Galindo A, Rodríguez P, Rodríguez J, Conejero W *et al.*, Pomegranate (*Punica granatum* L.) fruit response to different deficit irrigation conditions. *Agr Water Manage* **114**:30–36 (2012).

7 Mena P, Galindo A, Collado-González J, Ondoño S, García-Viguera C, Ferreres F *et al.*, Sustained deficit irrigation affects the colour and phytochemical characteristics of pomegranate juice. *J Sci Food Agr* **93**:1922–1927 (2013).
8 Peña-Estévez ME, Gómez PA, Artés F, Aguayo E, Martínez-Hernández GB, Otón M *et al.*, Quality changes of fresh-cut pomegranate arils during shelf life as affected by deficit irrigation and postharvest vapour treatments. *J Sci Food Agr* **95**:2325–2336 (2015).
9 Laribi AI, Palou L, Intrigliolo DS, Nortes PA, Rojas-Argudo C, Taberner V *et al.*, Effect of sustained and regulated deficit irrigation on fruit quality of pomegranate cv. 'Mollar de Elche' at harvest and during cold storage. *Agr Water Manage* **125**:61–70 (2013).
10 Carbonell-Barrachina ÁA, Memmi H, Noguera-Artiaga L, Gijón-López MdC, Ciapa R and Pérez-López D, Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *J Sci Food Agr* **95**:2866–2873 (2015).
11 Noguera-Artiaga L, Lipan L, Vázquez-Araújo L, Barber X, Pérez-López D and Carbonell-Barrachina ÁA, Opinion of Spanish Consumers on Hydrosustainable Pistachios. *J Food Sci* **81**:2559–2565 (2016).
12 Cano-Lamadrid M, Girón IF, Pleite R, Burló F, Corell M, Moriana A *et al.*, Quality attributes of table olives as affected by regulated deficit irrigation. *LWT – Food Sci and Technol* **62**:19–26 (2015).
13 Cano-Lamadrid M, Hernandez F, Corell M, Burló F, Legua P, Moriana A *et al.*, Antioxidant capacity, fatty acids profile, and descriptive sensory analysis of table olives as affected by deficit irrigation. *J Sci Food Agric* **97**:444–451 (2017).
14 Allen RG, Pereira LS, Raes D and Smit M, *Crop Evapotranspiration – Guidelines for Computing Crop Water Requirements*, Vol. 56. FAO – Food and Agriculture Organization of the United Nations, Rome (1998).
15 Bhantana P and Lazarovitch N, Evapotranspiration, crop coefficient and growth of two young pomegranate (*Punica granatum* L.) varieties under salt stress. *Agr Water Manage* **97**:715–722 (2010).
16 Fereres E and Goldhamer D, *Deciduous Fruit and Nut Trees*, Vol. 30). ASA, CSSA, SSSA, Madison, WI (1990).
17 Alcaraz-Mármol F, Nuncio-Jáuregui N, García-Sánchez F, Martínez-Nicolás JJ and Hernández F, Characterization of twenty pomegranate (*Punica granatum* L.) cultivars grown in Spain: Aptitudes for fresh consumption and processing. *Sci Hortic-Amsterdam* **219**:152–160 (2017).
18 Giusti MM and Wrolstad RE, Characterization and measurement of anthocyanins by UV-visible spectroscopy, in *Current Protocols in Food Analytical Chemistry*, ed. by Wrolstad RE. John Wiley & Sons, New York, NY (2001).
19 Singleton VL, Orthofer R and Lamuela-Raventós RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol* **299**:152–178 (1999).
20 Cano-Lamadrid M, Marhuenda-Egea FC, Hernández F, Rosas-Burgos EC, Burgos-Hernández A and Carbonell-Barrachina AA, Biological activity of conventional and organic pomegranate juices: antioxidant and antimutagenic potential. *Plant Food Hum Nutr* **71**:375–380 (2016).
21 El-Zaeddi H, Martínez-Tomé J, Calín-Sánchez Á, Burló F and Carbonell-Barrachina ÁA, Irrigation dose and plant density affect the volatile composition and sensory quality of dill (*Anethum graveolens* L.). *J Sci Food Agr* **97**:427–433 (2017).
22 Vázquez-Araújo L, Koppel K, Chambers IV, Adhikari K and Carbonell-Barrachina AA, Instrumental and sensory aroma profile of pomegranate juices from the USA: differences between fresh and commercial juice. *Flavour Frag J* **26**:129–138 (2011).
23 Moriana A and Fereres E, *Establishing Reference Values of Trunk Diameter Fluctuations and Stem Water Potential for Irrigation Scheduling of Olive Trees*. International Society for Horticultural Science (ISHS), Leuven, pp. 407–412 (2004).
24 Dejong TM, Effects of reproductive and vegetative sink activity on leaf conductance and water potential in *Prunus persica* L. Batsch. *Sci Hortic-Amsterdam* **29**:131–137 (1986).
25 Gucci R, Corelli-Grappadelli L, Tusti S and Ravaglia G, The effect of defruiting at different stages of fruit development on leaf photosynthesis of 'Golden Delicious' apple. *Tree Physiol* **15**:35–40 (1995).
26 Mpelasoka B, Behboudian M and Green S, Water use, yield and fruit quality of lysimeter-grown apple trees: responses to deficit irrigation and to crop load. *Irrigation Sci* **20**:107–113 (2001).
27 Williamson J and Coston D, The relationship among root growth, shoot growth and fruit growth of peach. *J Am Soc Hortic Sci* **114**:180–183 (1989).
28 Naor A, The interactions of soil- and stem-water potentials with crop level, fruit size and stomatal conductance of field-grown 'Black Amber' Japanese plum. *J Hortic Sci Biotechnol* **79**:273–280 (2004).
29 Türkyılmaz M and Özkan M, Effects of condensed tannins on anthocyanins and colour of authentic pomegranate (*Punica granatum* L.) juices. *Food Chem* **164**:324–331 (2014).
30 Mena P, Martí N and García-Viguera C, Varietal blends as a way of optimizing and preserving the anthocyanin content of pomegranate (*Punica granatum* L.) juices. *J Agric Food Chem* **62**:6936–6943 (2014).
31 Turfan Ö, *Changes in Anthocyanins of Pomegranate Juice Concentrate During Production and Storage*. Ankara University, Ankara (2008).
32 Mena P, García-Viguera C, Navarro-Rico J, Moreno DA, Bartual J, Saura D *et al.*, Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *J Sci Food Agr* **91**:1893–1906 (2011).
33 Kulkarni AP and Aradhya SM, Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chem* **93**:319–324 (2005).
34 Lopez G, Hossein Behboundian M, Girona J and Marsal J, Drought in deciduous fruit trees: implications for yield and fruit quality, in *Plant Responses to Dought Stress*, ed. by Aroca R. Springer, Berlin, Heidelberg, pp. 441–459 (2012).
35 Calín-Sánchez Á, Martínez JJ, Vázquez-Araújo L, Burló F, Melgarejo P and Carbonell-Barrachina ÁA, Volatile composition and sensory quality of Spanish pomegranates (*Punica granatum* L.). *J Sci Food Agric* **91**:586–592 (2011).
36 SAFC, *Sigma-Aldrich Flavors & Fragrances* [Online]. Available: http://www.sigmaaldrich.com/industries/flavors-and-fragrances.html [10 June 2017].
37 El-Sayed AM, *The Pherobase. Database of Pheromones and Semiochemicals* [Online]. Available: http://www.pherobase.com/ [10 June 2017].
38 Mayuoni-kirshinbaum L, Tietel Z, Porat R and Ulrich D, Identification of aroma-active compounds in 'Wonderful' pomegranate fruit using solvent-assisted flavour evaporation and headspace solid-phase micro-extraction methods. *Eur Food Res Technol* **235**:277–283 (2012).
39 Melgarejo P, Calín-Sánchez Á, Vázquez-Araújo L, Hernández F, Martínez JJ, Legua P *et al.*, Volatile composition of pomegranates from 9 Spanish cultivars using headspace solid phase microextraction. *J Food Sci* **76**:S114–S120 (2011).
40 Galindo A, Noguera-Artiaga L, Cruz ZN, Burló F, Hernández F, Torrecillas A *et al.*, Sensory and physico-chemical quality attributes of jujube fruits as affected by crop load. *LWT – Food Sci and Technol* **63**:899–905 (2015).
41 Klee HJ, Improving the flavor of fresh fruits: genomics, biochemistry, and biotechnology. *New Phytol* **187**:44–56 (2010).
42 Osorio S, Muñoz C and Valpuesta V, Physiology and biochemistry of fruit flavors, in *Handbook of Fruit and Vegetable Flavors*, ed. by Hui YH. John Wiley & Sons, Inc., Hoboken, NJ, pp. 25–43 (2010).
43 Song J, Shellie KC, Wang H and Qian MC, Influence of deficit irrigation and kaolin particle film on grape composition and volatile compounds in Merlot grape (*Vitis vinifera* L.). *Food Chem* **134**:841–850 (2012).

Publication 2

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., Carbonell-Barrachina Á.A.

***Journal of Food Science*,** 82: 1820-1826 (2017)

DOI: 10.1111/1750-3841.13788

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

Marina Cano-Lamadrid, Leontina Lipan, Ángel Calín-Sánchez, Francisca Hernández, and Ángel A. Carbonell-Barrachina

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$^{\bullet}$, ABTS$^{\bullet+}$, 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$^{\bullet}$ 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 (α-PC and β-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-Jáuregui 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 the 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

JFDS-2017-0289 Submitted 2/16/2017, Accepted 5/20/2017. *Authors Cano-Lamadrid, Lipan, Calín-Sánchez, and Carbonell-Barrachina are with Dept. of Agro-Food Technology, Food Quality and Safety Group, Univ. Miguel Hernández de Elche, Escuela Politécnica Superior de Orihuela, Carretera de Beniel, km 3.2, 03312, Orihuela, Alicante, Spain. Author Hernández is with Dept. of Vegetal Production, Univ. Miguel Hernández de Elche, Escuela Politécnica Superior de Orihuela, Carretera de Beniel, km 3.2, 03312, Orihuela, Alicante, Spain. Direct inquiries to author Carbonell-Barrachina (E mail: angel.carbonell@umh.es).*

doi: 10.1111/1750-3841.13788

Journal of Food Science A Publication of the Institute of Food Technologists

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-Jáuregui 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 [α and β], and EA) and AOC (DPPH$^{\bullet}$, FRAP, and ABTS$^{\bullet+}$) 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 (α and β 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 μm 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 × 4 mm, particle size, 5 μm; Merck, Darmstadt, Germany), and a precolumn C18 (LiChrospher 100 RP-18, 5 μm; 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/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 (α-PC, β-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$^{\bullet}$, ABTS$^{\bullet+}$, 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$^{\bullet}$ radical method, as described by previous research (Brand-Williams and others 1995), allowing a reaction time of 15 min. The ABTS$^{\bullet+}$ 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 ($R^2 \geq 0.998$). 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, Mercers 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 (α and β 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 measureable 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, α-PC ranged from nonmeasurable contents (1B, 1E, 1I, and 1K) up to 176 mg/g (1A), whereas β-PC registered also from nonmeasurable values (1B, 1E, and 1K) to 132 mg/g (1A). In the 2nd group, the values of α-PC and β-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 Group 1: CAPSULES AND SUPPLEMENTS	Commercial brand	Country	Recommended dose Capsules	Amount (mL)	 (g)	 (capsules)
1A	Pomegranate extract 833 mg. Bulking agent silicone dioxide 10 mg, magnesium stearate 25 mg	Granatum Plus	Spain	1	–	–	30
1B	Pomegranate extract 250 mg. Rice flour, gelatin. Contains <2% of: silica, vegetable cellulose, vegetable magnesium stearate	Puritan´s Pride Premium	USA	1–2	–	–	120
1C	Pomegranate extract 200 mg. Capsule: gelatin, rice flour, bulking agent: magnesium stearate and silica	Solarray	USA	1	–	–	60
1D	Pomegranate extract (77%). Gelatin, ascorbate, bulking agent (corn starch), anti-adherent agent (magnesium stearate)	Snact Bernhard	Germany	2	–	–	90
1E	Powdered pomegranate extract from peel	ClubVitis	UK	2	–	–	30
1F	70% concentrate powder of pomegranate juice (pomegranate juice, cornstarch), 10% pomegranate extract, granulated sugar and palm oil	RAAB Vitalfood	Germany	9	–	–	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	Germany	2-4	–	–	100
1H	350 mg pomegranate dry extract. Bulking agent (microcrystalline cellulose), anti-adherent agent (magnesium stearate and silicone dioxide)	Naturlider	European Union, EU	2	–	–	60
1I	100% pomegranate kernel flour	RAAB Vitalfood	USA	–	–	240	–
1J	Pomegranate juice (30 mL) from concentrated	SuperDiet	France	1–2 ampoules	300	–	–
1K	Pomegranate powder made from the fleshy seeds of ripe pomegranate	Biotona	Belgium	10 g	–	200	–

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

Table 2–Contents of punicalagin (α-PC and β-PC) and ellagic acid (EA) in the studied pomegranate products, expressed in mg/g.

Sample	α-PC	β-PC	Σ PC	EA
	(mg/g)			
	ANOVA[a]			
Group 1	***	***	***	***
1A	176 a[b]	132 a	308 a	13.1 a
1B	nd[c]	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[a]			
Group 2	**	**	**	*
2A	0.89 c[b]	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[c]	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

[a] *, **, and ***, significant at $P < 0.05$, 0.01, and 0.001, respectively;
[b] *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;*
[c] 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$^{\bullet}$	ABTS$^{\bullet+}$	FRAP
	(mg GAE/g)	(mmol TroloX/kg)		
	ANOVA[a]			
Group 1	***	***	***	***
1A	353 a[b]	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[a]			
Group 2	**	**	**	**
2A	17.5 a[b]	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

[a] ** and ***, significant at $P < 0.01$ and 0.001, respectively;
[b] *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;*
[c] nd = below limit of quantification (LOQ).

no detectable values of α-PC but remarkable β-PC content (85 mg/g). This statement agreed quite well with previous studies, where a higher concentration of β-PC than α-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$^{\bullet+}$, FRAP, and DPPH$^{\bullet}$) 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•+, and FRAP).

	TPC	PC	EA	DPPH•	ABTS•+	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•+	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•+	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•+	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•+ 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-Jáuregui 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•+, 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 IJ, with 24.8 mmol Trolox/kg. It can be noticed that sample 1A showed the highest ability to inhibit both DPPH• and ABTS•+ 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•+ 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[a]	Daily TPC (mg GAE)
Group 1	***	***	***
1A	256 ab	10.9 a	294 a
1B	nd[c]	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
		ANOVA[a]	
Group 2	***	***	***
2A	56.7 d [b]	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.*
[b] *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.*
[c] 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$^{\bullet}$ and TPC values. TPC was highly correlated ($R = 0.80$; $P < 0.001$) with DPPH$^{\bullet}$ assay. FRAP and ABTS$^{\bullet+}$ values were correlated ($P < 0.05$) with TPC, PC, and DPPH$^{\bullet}$. 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.

Acknowledgments

The authors are grateful to the project AGL2013-45922-C2-2-R (Ministerio de Economía y Competitividad, Spain). Author Marina Cano-Lamadrid was funded by a FPU grant from the Spanish Ministry of Education (FPU15/02158).

References

Al-Muammar MN, Khan F. 2012. Obesity: The preventive role of the pomegranate (*Punica granatum*). Nutrition 28(6):595–604.
Alaei, B, Amiri Chayjan R. 2015. Drying characteristics of pomegranate arils under near infrared-vacuum conditions. Journal of Food Processing and Preservation 39(5):469–79.
Aloqbi A, Omar U, Marwa Y, Grace M, Lila MA, Howell N. 2016. Antioxidant activity of pomegranate juice and punicalagin. Natural Science 8:235–46.
Bchir B, Besbes S, Karoui R, Attia H, Paquot M, Blecker C. 2012. Effect of air-drying conditions on physico-chemical properties of osmotically pre-treated pomegranate seeds. Food and Bioprocess Technology 5(5):1840–52.
Benzie I, Strain J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry 239(1):70–6.
Benzie I, Szeto Y. 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry 47(2):633–36.
Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28(1):25–30.

Calín-Sánchez Á, Figiel A, Hernández F, Melgarejo P, Lech K, Carbonell-Barrachina ÁA. 2013. Chemical composition, antioxidant capacity, and sensory quality of pomegranate (*Punica granatum* L.) arils and rind affected by drying method. Food and Bioprocess Technology 6(7):1644–54.
Cano-Lamadrid M, Hernandez F, Corell M, Burlo F, Legua P, Moriana A, Carbonell-Barrachina AA. 2016. Antioxidant capacity, fatty acids profile, and descriptive sensory analysis of table olives as affected by deficit irrigation. J Sci Food Agric 97:444–51.
Chun OK, Chung SK, Song WO. 2007. Estimated Dietary Flavonoid Intake and Major Food Sources of U.S. Adults. J Nutr 137(5):1244–52.
Filannino P, Azzi L, Cavoski I, Vincentini O, Rizzello CG, Gobbetti M, Di Cagno R. 2013. Exploitation of the health-promoting and sensory properties of organic pomegranate (Punica granatum L.) juice through lactic acid fermentation. International Journal of Food Microbiology 163(2–3):184–92.
Fischer UA, Dettmann JS, Carle R, Kammerer DR. 2011. Impact of processing and storage on the phenolic profiles and contents of pomegranate (Punica granatum L.) juices. Eur Food Res Technol 233:797–816.
Grosso G, Stepaniak U, Topor-Madry R, Szafraniec K, Pajak A. 2014. Estimated dietary intake and major food sources of polyphenols in the Polish arm of the HAPIEE study. Nutrition 30(11-12):1398–403.
MFA (Ministry of Foreign Affaris). 2015. CBI Product Factsheet: Fresh Pomegranates in Europe. CBI. Market Intelligence.
Nuncio-Jáuregui N, Calín-Sánchez A, Hernández F, Carbonell-Barrachina AA. 2014. Pomegranate juice adulteration by addition of grape or peach juices. Journal of the Science of Food and Agriculture 94:646–65.
Nuncio-Jáuregui N, Munera-Picazo S, Calin-Sanchez A, Wojdyło A, Hernandez F, Carbonell-Barrachina AA. 2015. Bioactive compound composition of pomegranate fruits removed during thinning. Journal of Food Composition and Analysis 37:11–9.
Nuncio-Jauregui N, Nowicka P, Munera-Picazo S, Hernandez F, Carbonell-Barrachina AA, Wojdyło A. 2015. Identification and quantification of major derivatives of ellagic acid and antioxidant properties of thinning and ripe Spanish pomegranates. Journal of Functional Foods 12:354–64.
Ovaskainen ML, Torronen R, Koponen JM, Sinkko H, Hellstrom J, Reinivuo H, Mattila P. 2008. Dietary intake and major food sources of polyphenols in Finnish adults. Journal of Nutrition 138(3):562–6.
Perez-Jimenez J, Fezeu L, Touvier M, Arnault N, Manach C, Hercberg S, Scalbert A. 2011. Dietary intake of 337 polyphenols in French adults. American Journal of Clinical Nutrition 93(6):1220–28.
Qu W, Breksa AP, Pan Z, Ma H. 2012. Quantitative determination of major polyphenol constituents in pomegranate products. Food Chemistry 132(3):1585–91.
Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26(9–10):1231–37.
Rinaldi M, Caligiani A, Borgese R, Palla G, Barbanti D, Massini R. 2013. The effect of fruit processing and enzymatic treatments on pomegranate juice composition, antioxidant activity and polyphenols content. LWT - Food Science and Technology 53(1):355–9.
Rummun N, Somanah J, Ramsaha S, Bahorun T, Neergheen-Bhujun VS. 2013. Bioactivity of nonedible parts of Punica granatum L.: a potential source of functional ingredients. Vol. 2013: Article ID 602312.
Scalbert A, Williamson G. 2000. Dietary intake and bioavailability of polyphenols. J Nutr 130(8S Suppl):2073–85
Seeram NP, Adams LS, Henning SM, Niu YT, Zhang YJ, Nair MG, Heber D. 2005. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. Journal of Nutritional Biochemistry 16(6):360–7.
Szychowski PJ, Frutos MJ, Burlo F, Perez-Lopez AJ, Carbonell-Barrachina AA, Hernandez F. 2015. Instrumental and sensory texture attributes of pomegranate arils and seeds as affected by cultivar. LWT-Food Science and Technology 60(2):656–63.
Talcott ST, Lee JH. 2002. Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. Journal of Agricultural and Food Chemistry 50(11), 3186–92.
Tresserra-Rimbau A, Medina-Remon A, Perez-Jimenez J, Martinez-Gonzalez MA, Covas MI, Corella D, Lamuela-Raventos RM. 2013. Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: The PREDIMED study. Nutrition Metabolism and Cardiovascular Diseases 23(10):953–9.
Turrini E, Ferruzzi L, Fimognari C. 2015. Potential effects of pomegranate polyphenols in cancer prevention and therapy. Oxidative Medicine and Cellular Longevity 2015:1–19.
Vázquez-Araújo L, Chambers E, Carbonell-Barrachina ÁA. 2015. Effects of albedo addition on pomegranate juice physicochemical, volatile and chemical markers. Beverages 1(1):17–33.
Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Sendra E, Sayas-Barbera E, Perez-Alvarez JA. 2011. Antioxidant properties of pomegranate (*Punica granatum* L.) bagasses obtained as co-product in the juice extraction. Food Research International 44(5):1217–23.
Zamora-Ros R, Knaze V, Rothwell JA, Hemon B, Moskal A, Overvad K, Scalbert A. 2016. Dietary polyphenol intake in Europe: the european prospective investigation into cancer and nutrition (EPIC) study. European Journal of Nutrition 55(4):1359–75.
Zhang Y, Wang D, Lee R, HenNing SM, Heber D. 2009. Absence of pomegranate ellagitannins in the majority of commercial pomegranate extracts: implications for standardization and quality control. Journal of Agricultural and Food Chemistry 2009 57(16):7395–740.

Publication 3

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., Carbonell-Barrachina A.A.

***Journal of Food Science*,** 84: 886-894 (2019)

DOI: 10.1111/1750-3841.14497

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

Marina Cano-Lamadrid, Igor Piotr Turkiewicz, Karolina Tkacz, Lucía Sánchez-Rodríguez, David López-Lluch, Aneta Wojdyło, Esther Sendra, and Angel A. Carbonell-Barrachina

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 α, β], 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 (ellagitannis), 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, açaí juice, or blueberry juice.

JFDS-2018-1639 Submitted 10/24/2018, Accepted 2/12/2019. *Authors Cano-Lamadrid, Sánchez-Rodríguez, and Carbonell-Barrachina are with Dept. Tecnología Agroalimentaria, Grupo Calidad y Seguridad Alimentaria, Escuela Politécnica Superior de Orihuela (EPSO), Univ. Miguel Hernández de Elche (UMH), Carretera de Beniel, km 3.2, 03312 Orihuela, Alicante, Spain. Authors Turkiewicz, Tkacz, and Wojdyło are with Dept. of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław Univ. of Environmental and Life Sciences, 37 Chełmońskiego Street, 51–630 Wrocław, Poland. Author Sendra is with Dept. Tecnología Agroalimentaria, Grupo Industrialización de Productos de Origen Animal, Escuela Politécnica Superior de Orihuela (EPSO), Univ. Miguel Hernández de Elche (UMH), Carretera de Beniel, km 3.2, 03312 Orihuela, Alicante, Spain. Direct inquiries to author Carbonell-Barrachina (E-mail: angel.carbonell@umh.es).*

Authors M. Cano-Lamadrid, I.P. Turkiewicz, and K. Tkacz contributed equally to this work.

doi: 10.1111/1750-3841.14497

Journal of Food Science A Publication of the Institute of Food Technologists

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 α, β, 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) + 1 % 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 α and β isomers) and EA contents were determined using the method proposed by Cano-Lamadrid, Lipan, Calín-Sánchez, 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
Group 1: 100% pomegranate juice				
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
Group 2: Pomegranate juice based drinks				
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® (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®	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-phloroglucinol and monomeric ACNs ($R = 0.866$) (Türkyılmaz & 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 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 2–Titratable acidity (TA), color density (CD) and polymeric color (PC) of pomegranate juice and pomegranate juice based drinks.

Sample	Titratable acidity, TA (g citric acid L^{-1})	Color density, CD	Polymeric color, PC (%)
	ANOVA *(LSD)*[a]		
Group 1	*** *(0.10)*	*** *(0.08)*	*** *(2.1)*
1A	1.02 d[b]	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)*[a]		
Group 2	*** *(0.11)*	*** *(0.09)*	*** *(2.1)*
2A	1.27 c[b]	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

[a]*, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively, and LSD means lower significant difference.
[b]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.

Table 3–Contents of punicalagin (α-, β-Pn, and sum), ellagic acid (EA), and total polyphenolic content (TPC) in the studied pomegranate juices and pomegranate juice based drinks.

Sample	α-Pn	β-Pn	Σ Pn	EA	TPC
	(mg/mL)				(mmol gallic acid/L)
	ANOVA (LSD)[a]				
	***	**	***	*	***
Group 1	*(0.04)*	*(0.01)*	*(0.05)*	*(0.01)*	*(0.80)*
1A	nd[c]	nd	nd	nd	12.5cd
1B	3.08 a[b]	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)*[a]				
	***	*	***	*	***
Group 2	*(0.05)*	*(0.02)*	*(0.07)*	*(0.01)*	*(0.80)*
2A	0.66 cd[b]	nd[c]	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

[a]*, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively, and LSD means lower significant difference.
[b]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.
[c]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 [α, β isomers, and total ($\Sigma = \alpha + \beta$)] 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 α- and β-Pn, the α-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 β-Pn in commercial 100% Pom juices, with values being 0.1 to 0.24 mg α-Pn/mL and 0.12 to 0.28 β-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 α-Pn and β-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-Jáuregui, 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	**1.000**							
Pom-ID	0.854***	**1.000**						
TA	0.075	0.082	**1.000**					
CD	0.208	0.256	0.187	**1.000**				
PC	0.241	0.321	−0.220	−0.246	**1.000**			
TPC	0.354	0.228	0.124	0.286	0.446**	**1.000**		
Pn	0.709***	0.706***	0.086	0.153	0.344	0.216	**1.000**	
EA	0.364**	0.425**	−0.021	0.353	−0.226	0.427**	0.270	**1.000**

[a]**, 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 β-Pn isomer only reached measurable levels in two samples (2B and 2I), while the isomer α-PC was the predominant one in all nectar samples. Recently, Borges et al. (2010) reported in that the α-Pn and β-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 galllic 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.

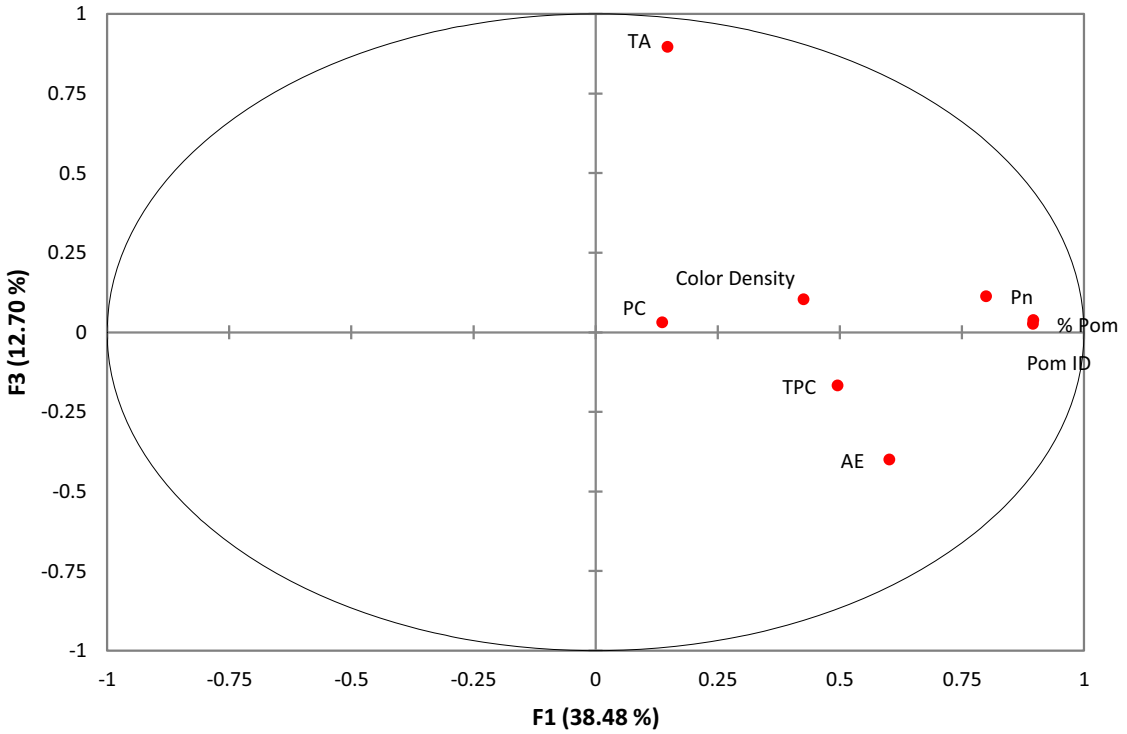
The TPC of organic juices ranged from 9.0 to 16.5 mmol gallic acid/L, while the TPC levels in conventional juices were higher, from 11.4 to 70.9 mmol gallic acid/L (which was even fourfold more for sample 1K). On the contrary, Mena et al. (2011) reported lower values of TPC in conventional Pom juices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



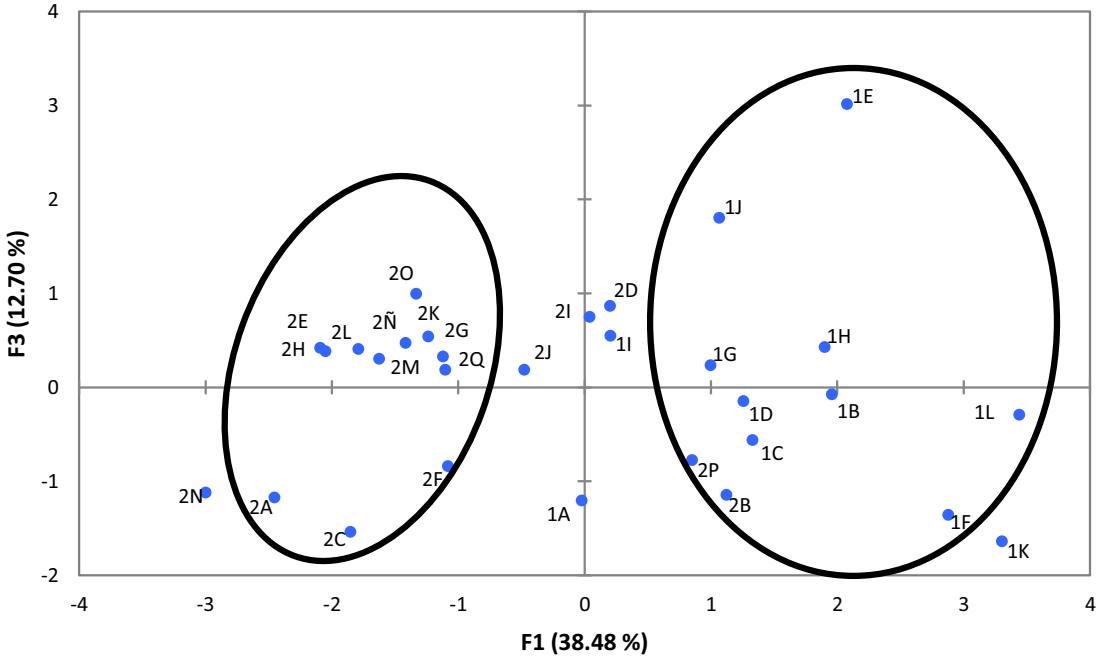


Figure 1–PCA map showing the relationships among chemical content (TPC, punicalagin [PC], and ellagic acid [EA]) and percentage of pomegranate juice (% Pom), sensory analysis (Pom ID), titratable acidity (TA), color density (CD), and polymeric color (PC) of pomegranate juice based drinks.

(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 2Ñ) 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-Jáuregui et al., 2015) indicated that the effect of thermal treatment must take into account due to the fact that a temperature raise will increased Pn because of degradation of other elagitannins. Abountiolas 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 Abountiolas 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 Türkyılmaz, 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% (F1 = 38.48% and F2 = 12.70%, respectively) of the total variation of the experimental data. Considering F1 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 F1 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 heath claims under European Regulations. The knowlegde of phytochemical content is essential to guarantee the quality of procesed 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 jeopardize 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).

Acknowledgments

Author Marina Cano-Lamadrid was funded by a FPU grant from the Spanish Ministry of Education (FPU15/02158). This work has been carried out, thanks to a double co-tutelle PhD between WUELS (Poland) and UMH (Spain).

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.

References

Aboelsoud, N. H. (2010). Herbal medicine in ancient Egypt. *Journal of Medicinal Plants Research*, *4*(2), 082–086.

Abountiolas, M., & Nascimento Nunes, C. (2018). Polyphenols, ascorbic acid and antioxidant capacity of commercial nutritional drinks, fruit juices, smoothies and teas. *International Journal of Food Science & Technology*, *53*(1), 188–198. https://doi.org/10.1111/ijfs.13573

Akhavan, H., Barzegar, M., Weidlich, H., & Zimmermann, B. F. (2015). Phenolic compounds and antioxidant activity of juices from ten Iranian pomegranate cultivars depend on extraction. *Journal of Chemistry*, *2015*, article ID 907101.

Alper, N., Bahçeci, K. S., & Acar, J. (2005). Influence of processing and pasteurization on color values and total phenolic compounds of pomegranate juice. *Journal of Food Processing and Preservation*, *29*(5-6), 357–368.

Bhandari, P. R. (2012). Pomegranate (Punica granatum L). Ancient seeds for modern cure? Review of potential therapeutic applications. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, *2*(3), 171.

Borges, G., Mullen, W., & Crozier, A. (2010). Comparison of the polyphenolic composition and antioxidant activity of European commercial fruit juices. *Food & Function*, *1*(1), 73–83.

Bunea, C.-I., Pop, N., Babeş, A. C., Matea, C., Dulf, F. V., & Bunea, A. (2012). Carotenoids, total polyphenols and antioxidant activity of grapes (Vitis vinifera) cultivated in organic and conventional systems. *Chemistry Central Journal*, *6*(1), 66.

Cano-Lamadrid, M., Lipan, L., Calín-Sánchez, Á., Hernández, F., & Carbonell-Barrachina, Á. A. (2017a). A Comparative Study Between Labeling and Reality: The Case of Phytochemical Composition of Commercial Pomegranate-Based Products. *Journal of Food Science*, *82*(8), 1820–1826.

Cano-Lamadrid, M., Lech, K., Michalska, A., Wasilewska, M., Figiel, A., Wojdyło, A., & Carbonell-Barrachina, Á. A. (2017b). 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.

Cano-Lamadrid, M., Marhuenda-Egea, F. C., Hernández, F., Rosas-Burgos, E.-C., Burgos-Hernández, A., & Carbonell-Barrachina, A. (2016). Biological activity of conventional and organic pomegranate juices: Antioxidant and antimutagenic potential. *Plant Foods for Human Nutrition*, *71*(4), 375–380.

Cano-Lamadrid, M., Vázquez-Araújo, L., Sánchez-Rodríguez, L., Wodyło, A., & Carbonell-Barrachina, Á. A. (2018). Consumers' Opinion on Dried Pomegranate Arils to Determine the Best Processing Conditions. *Journal of Food Science*, *83*, 3085–3091.

Cao, X., Zhang, Y., Zhang, F., Wang, Y., Yi, J., & Liao, X. (2011). Effects of high hydrostatic pressure on enzymes, phenolic compounds, anthocyanins, polymeric color and color of strawberry pulps. *Journal of the Science of Food and Agriculture*, *91*(5), 877–885.

Costa, Y., & Melgarejo, P. (2000). A study of the production costs of two pomegranate varieties grown in poor quality soils. In P. Melgarejo, J. J. Martínez-Nicolás, & J. Martínez-Tomé (Eds.), *Production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology* (pp. 49–53). Zaragoza, Spain: CIHEAM.

Crecente-Campo, J., Nunes-Damaceno, M., Romero-Rodríguez, M., & Vázquez-Odériz, M. (2012). Color, anthocyanin pigment, ascorbic acid and total phenolic compound determination in organic versus conventional strawberries (Fragaria× ananassa Duch, cv Selva). *Journal of Food Composition and Analysis*, *28*(1), 23–30.

EFSA. (2018). Nutritionl and health claim. Retrieved from http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=register.home

Fawole, O. A., Makunga, N. P., & Opara, U. L. (2012). Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. *BMC Complementary and Alternative Medicine*, *12*(1), 200.

FDA. (2008). Food. Retrieved from https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm063064.htm

Fernandes, L., Pereira, J. A., Lopéz-Cortés, I., Salazar, D. M., González-Álvarez, J., & Ramalhosa, E. (2017). Physicochemical composition and antioxidant activity of several pomegranate (Punica granatum L.) cultivars grown in Spain. *European Food Research and Technology*, *243*(10), 1799–1814.

Ferrari, G., Maresca, P., & Ciccarone, R. (2010). The application of high hydrostatic pressure for the stabilization of functional foods: Pomegranate juice. *Journal of Food Engineering*, *100*(2), 245–253.

Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. In *Current Protocols in Food Analytical Chemistry* (pp. F1.2.1–F1.2.13). Hoboken, NJ, USA: John Wiley & Sons, Inc.

González-Díaz, C., Gil-González, D., & Álvarez-Dardet, C. (2018). Scientific evidence on functional food and its commercial communication: A review of legislation in Europe and the USA. *Journal of Food Science*, *83*, 2710–2717

Kaur, R., & Malik, C. (2016). Punica granatum: Cultivation and medicinal importances. *The Journal of Plant Science Research*, *32*(2), 149.

Les, F., Prieto, J. M., Arbonés-Mainar, J. M., Valero, M. S., & López, V. (2015). Bioactive properties of commercialised pomegranate (Punica granatum) juice: Antioxidant, antiproliferative and enzyme inhibiting activities. *Food & Function*, *6*(6), 2049–2057.

Li, J., Li, G., Zhao, Y., & Yu, C. (2009). Composition of pomegranate peel polyphenols and its antioxidant activities. *Scientia Agricultura Sinica*, *42*(11), 4035–4041.

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D., & Martí, N. (2011). Phytochemical characterisation for industrial use of pomegranate (Punica granatum L.) cultivars grown in Spain. *Journal of the Science of Food and Agriculture*, *91*(10), 1893–1906.

Mena, P., Martí, N., Saura, D., Valero, M., & García-Viguera, C. (2013). Combinatory effect of thermal treatment and blending on the quality of pomegranate juices. *Food and Bioprocess Technology*, *6*(11), 3186–3199.

Nuncio-Jáuregui, N., Cano-Lamadrid, M., Hernández, F., Carbonell-Barrachina, Á. A., & Calín-Sánchez, Á (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.

Palou, L., Rosales, R., Montesinos-Herrero, C., & Taberner, V. (2016). Short-term exposure to high CO2 and O2 atmospheres to inhibit postharvest gray mold of pomegranate fruit. *Plant Disease*, *100*(2), 424–430.

Qu, W., Breksa, III, A. P., Pan, Z., & Ma, H. (2012). Quantitative determination of major polyphenol constituents in pomegranate products. *Food Chemistry*, *132*(3), 1585–1591.

Rein, M. J., Ollilainen, V., Vahermo, M., Yli-Kauhaluoma, J., & Heinonen, M. (2005). Identification of novel pyranoanthocyanins in berry juices. *European Food Research and Technology*, *220*(3–4), 239–244.

Saha, P., San Yeoh, B., Singh, R., Chandrasekar, B., Vemula, P. K., Haribabu, B., . . . Jala, V. R. (2016). Gut microbiota conversion of dietary ellagic acid into bioactive phytoceutical urolithin a inhibits heme peroxidases. *PLoS One*, *11*(6), e0156811.

Sánchez-Salcedo, E. M., Mena, P., García-Viguera, C., Martínez, J. J., & Hernández, F. (2015). Phytochemical evaluation of white (Morus alba L.) and black (Morus nigra L.) mulberry fruits, a starting point for the assessment of their beneficial properties. *Journal of Functional Foods*, *12*, 399–408.

Seeram, N. P., Adams, L. S., Henning, S. M., Niu, Y., Zhang, Y., Nair, M. G., & Heber, D. (2005). In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *The Journal of Nutritional Biochemistry*, *16*(6), 360–367.

Seeram, N. P., Aviram, M., Zhang, Y., Henning, S. M., Feng, L., Dreher, M., & Heber, D. (2008). Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *Journal of Agricultural and Food Chemistry*, *56*(4), 1415–1422.

Sidhu, J. S., & Zafar, T. A. (2012). *Superfruits: Pomegranate, wolfberry, aronia (chokeberry), acai, noni, and amla. Handbook of Fruits and Fruit Processing, Second Edition*, 653–679. Ames, Iowa, USA: Wiley-Blackwell.

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2018). Phenolic compounds as beneficial phytochemicals in pomegranate (Punica granatum L.) peel: A review. *Food Chemistry*, *261*, 75–86.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent *Methods in Enzymology*, *299*, 152–178.

Sun, Y.-Q., Xin, T., Men, X.-M., Xu, Z.-W., & Tian, W. (2017). In vitro and in vivo antioxidant activities of three major polyphenolic compounds in pomegranate peel: Ellagic acid, punicalin, and punicalagin. *Journal of Integrative Agriculture*, *16*(8), 1808–1818.

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B., & Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, *115*(3), 873–877.

Todaro, A., Cavallaro, R., La Malfa, S., Continella, A., Gentile, A., Fischer, U., . . . Spagna, G. (2016). Anthocyanin profile and antioxidant activity of freshly squeezed pomegranate (Punica granatum l.) juices of sicilian and spanish provenances. *Italian Journal of Food Science*, *28*(3), 464–479.

Türkyılmaz, M., & Özkan, M. (2014). Effects of condensed tannins on anthocyanins and colour of authentic pomegranate (Punica granatum L.) juices. *Food Chemistry*, *164*, 324–331.

Türkyılmaz, M., Tağı, Ş., Dereli, U., & Özkan, M. (2013). Effects of various pressing programs and yields on the antioxidant activity, antimicrobial activity, phenolic content and colour of pomegranate juices. *Food Chemistry*, *138*(2–3), 1810–1818.

Tzulker, R., Glazer, I., Bar-Ilan, I., Holland, D., Aviram, M., & Amir, R. (2007). Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *Journal of Agricultural and Food Chemistry*, *55*(23), 9559–9570.

Vázquez-Araújo, L., Chambers, I., Adhikari, K., & Carbonell-Barrachina, Á. A. (2010). Sensory and physicochemical characterization of juices made with pomegranate and blueberries, blackberries, or raspberries. *Journal of Food Science*, *75*(7), S398–404.

Vázquez-Araújo, L., Koppel, K., Chambers Iv, E., Adhikari, K., & Carbonell-Barrachina, A. (2011). Instrumental and sensory aroma profile of pomegranate juices from the USA: Differences between fresh and commercial juice. *Flavour and Fragrance Journal*, *26*(2), 129–138.

Zou, H., Lin, T., Bi, X., Zhao, L., Wang, Y., & Liao, X. (2016). Comparison of high hydrostatic pressure, high-pressure carbon dioxide and high-temperature short-time processing on quality of mulberry juice. *Food and Bioprocess Technology*, *9*(2), 217–231.

Publication 4

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

Cano-Lamadrid M., Lech K., Michalska A., Wasilewska,M., Figiel A., Wojdyło A., Carbonell-Barrachina A.A.

***Food Chemistry*,** 232: 306-315 (2017)

DOI: 10.1016/j.foodchem.2017.04.033

Food Chemistry 232 (2017) 306–315

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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

Marina Cano-Lamadrid [a], Krzysztof Lech [b], Anna Michalska [d], Malwina Wasilewska [b], Adam Figiel [b], Aneta Wojdyło [c], Ángel A. Carbonell-Barrachina [a,*]

[a] Universidad Miguel Hernández de Elche (UMH), Escuela Politécnica Superior de Orihuela, Department of Agro-Food Technology, Ctra. Beniel, km 3.2, 03312 Orihuela, Alicante, Spain
[b] Institute of Agricultural Engineering, Wrocław University of Environmental and Life Sciences, 37a Chełmońskiego Street, 51-630 Wrocław, Poland
[c] Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chełmońskiego Street, 51-630 Wrocław, Poland
[d] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, Olsztyn, Poland

ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form 28 March 2017
Accepted 4 April 2017
Available online 6 April 2017

Keywords:
Convective drying
Vacuum-microwave drying
Punica granatum L.
Sensory analysis
Antioxidant capacity
Anthocyanins

ABSTRACT

"*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^{-1}), red colour (a^* coordinate 15.6), and antioxidant capacity (e.g. ABTS mean of 5.7 mmol Trolox 100 g^{-1}). 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-Özgüven, 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).

* Corresponding author.
E-mail address: angel.carbonell@umh.es (Á.A. Carbonell-Barrachina).

http://dx.doi.org/10.1016/j.foodchem.2017.04.033
0308-8146/

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^{-1}, 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^{-1} 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^3. 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^{-1}, 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^{-1}, 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 led 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 decreas-

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

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

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 μm, 2.1 × 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^{-1} ($R^2 \leq 0.9998$) 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$^+$ 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 (1500*g*, 10 min, 4 °C). The antioxidant capacity of the extracts was examined using the Trolox Equivalent Antioxidant Capacity test (TEAC ABTS$^+$) 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^{-1} dry weight, dw (±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 (ΔE) was calculated by Eq. (3):

$$\Delta E = [(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2]^{0.5} \quad (3)$$

The ΔE 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 ΔE 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, Wojdylo, 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).

$$RR = m_1 m_0^{-1} \quad (4)$$

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 μS cm^{-1}) 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 ± 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 ± 0.7% down to an average value of 72.1 ± 0.4% (Table 1). The *WL* was not affected

by the nature of the osmotic solution; it reached a mean of 30.2 ± 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 ± 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 4.2×10^{-3} and 7.0×10^{-3} for CPD and 5.0×10^{-4} and 1.9×10^{-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 ($\sum$) of pomegranate arils before and after osmotic dehydration (OD) in different fruit juices.

Samples[†]	Before OD	After OD			Rehydration	
	MC	*MC*	*WL*[±]	*SG*[±±]	*RR*	$\sum$
	(g *per* 100 g fw)					(μS cm^{-1})
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

[†] 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[†]	Drying kinetics $MR = A \cdot e^{-k \cdot \tau^n}$					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	323[¶] 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

[†] 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.
[‡] 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.
[¶] The OD step lasted 90 min for all treatments.

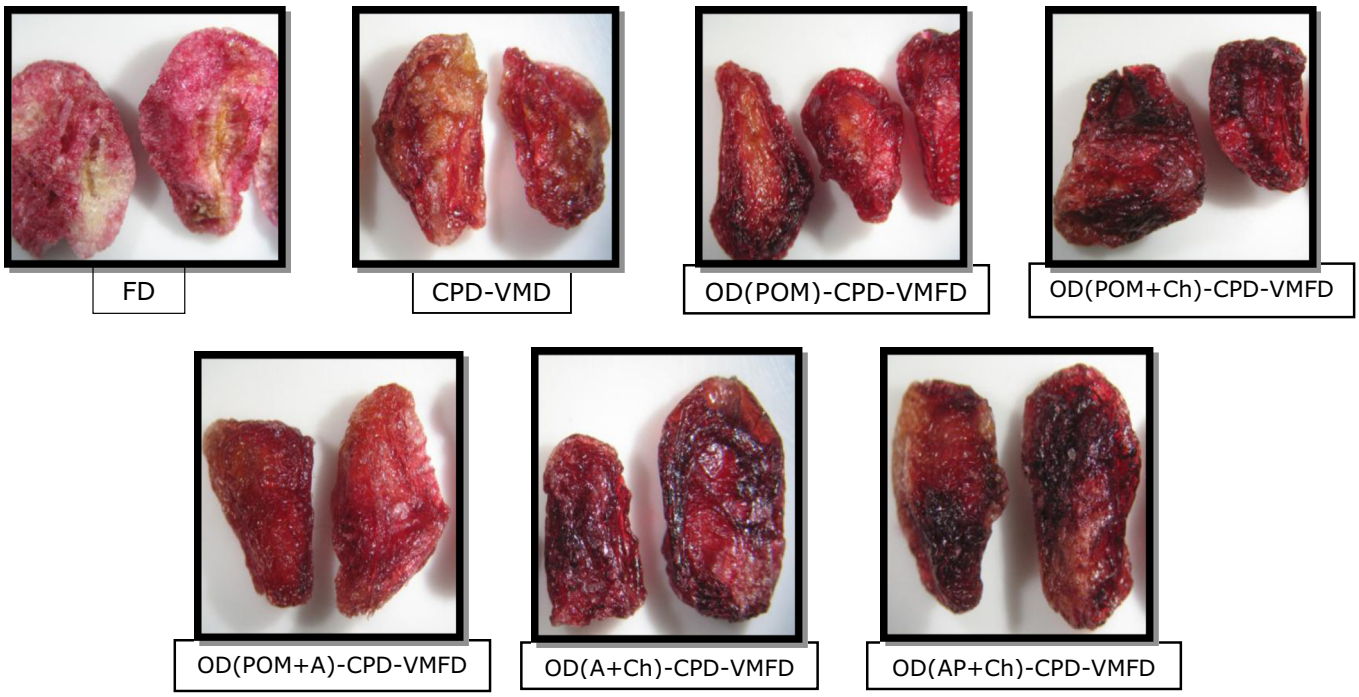


Fig. 1. "*Mollar de Elche*" pomegranate arils dried by freeze drying (FD), combined drying (CPD-VMFD), and osmotic dehydration (OD) before combined drying method (CPD-VMFD).

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 extend 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.

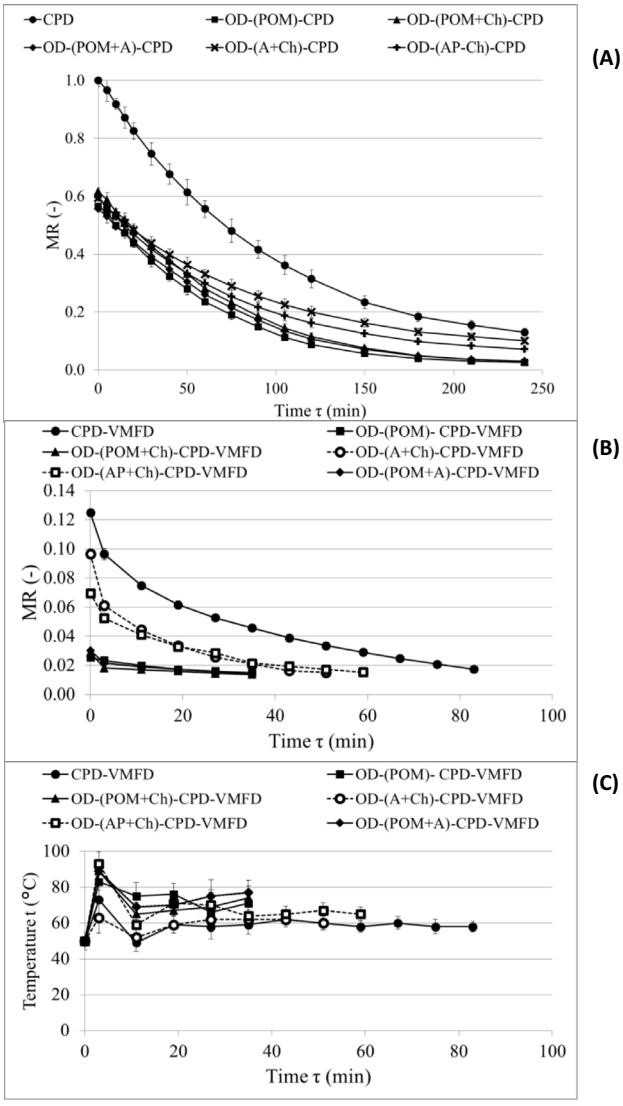
Finally, the electrical conductivity ($\sum$) is a measure of the soluble solids and electrolytes in the medium, which was indicative of the release of intercellular ions from sample tissue, due to an induced damage or creation of a porous structure in the material during drying. The $\sum$ values ranged between 200 ± 1 and 284 ± 2 $\mu S\ cm^{-1}$ (FD sample, with porous structure), and were significantly higher than that of the water used for rehydration, 6 $\mu S\ cm^{-1}$ (Table 1). The OD pre-treatment increased the $\sum$ 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 mL^{-1} of juice, respectively (total of 694 mg 100 mL^{-1}).
- Chokeberry juice: A4 and A7 with contents being 28.2 and 1032 mg 100 mL^{-1}, respectively.
- Apple juice: A4 and A7 with 10.4 and 76.9 mg 100 mL^{-1}, respectively.

A total of 6 anthocyanins typical of pomegranate products were identified: delpinidin-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 mg kg^{-1} - dm) in dried arils and pomegranate juice (295 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[†]	Anthocyanins[‡]								Antioxidant capacity		Colour			
	A1	A2	A3	A4	A5	A6	A7	Total A	$ABTS^+$	FRAP	L^*	a^*	b^*	ΔE
	(mg kg^{-1} dm)								(mmol Trolox 100 g^{-1})					
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

[†] 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.
[‡] 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.
[¶] 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 $ABTS^+$ and FRAP)

The $ABTS^+$ antioxidant capacity was affected by the osmotic dehydration pre-treatments as well as the drying methods (Table 3). The highest $ABTS^+$ 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 $ABTS^+$ 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 $ABTS^+$ or FRAP values; however, a positive and significant ($p < 0.001$) correlation was found between $ABTS^+$ 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

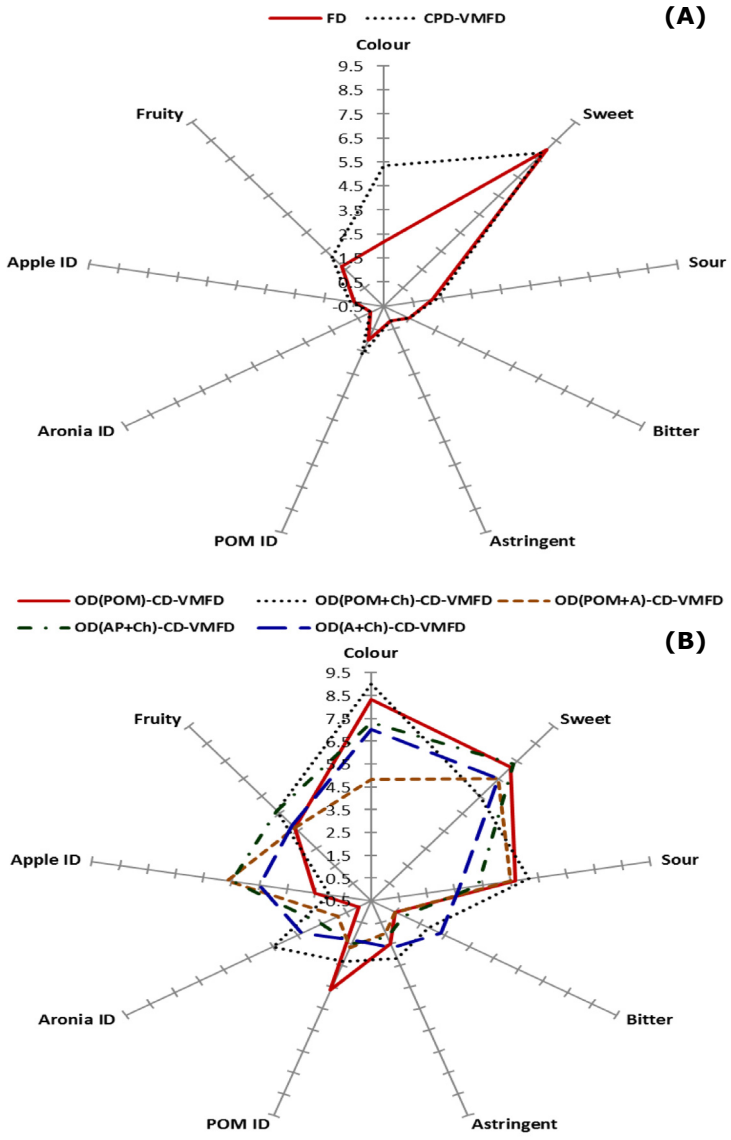
The dried pomegranate arils available in the market now are usually brownish and not attractive to consumers, due to degradation of arils anthocyanins (Maskan, Kaya, & Maskan, 2002).

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.

control (A) and osmotic-dehydrated (B) samples.



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 (ΔE) of samples ranged from 2.5 to 24.3 (Table 3). The lowest ΔE 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, 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 significantly 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 exists.

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).

Acknowledgments

The authors are grateful to the projects AGL2013-45922-C2-1-R y AGL2013-45922-C2-2-R (*Ministerio de Economía y Competitividad*, Spain). Author Marina Cano-Lamadrid was funded by a FPU grant from the Spanish Ministry of Education. This study was supported by a grant of the KNOW Consortium "Healthy Animal – Safe Food", MS&HE Decision No. 05-1/KNOW2/2015.

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.

References

Alaei, B., & Amiri Chayjan, R. (2015). Drying characteristics of pomegranate arils under near infrared-vacuum conditions. *Journal of Food Processing and Preservation, 39*(5), 469–479.

Bchir, B., Besbes, S., Karoui, R., Attia, H., Paquot, M., & Blecker, C. (2012). Effect of air-drying conditions on physico-chemical properties of osmotically pre-treated pomegranate seeds. *Food and Bioprocess Technology, 5*(5), 1840–1852.

Bchir, B., Besbes, S., Karoui, R., Paquot, M., Attia, H., & Blecker, C. (2012). Osmotic dehydration kinetics of pomegranate seeds using date juice as an immersion solution base. *Food and Bioprocess Technology, 5*(3), 999–1009.

Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in enzymology* (Vol. 299, pp. 15–27). Academic Press.

Calín-Sánchez, Á., Figiel, A., Hernández, F., Melgarejo, P., Lech, K., & Carbonell-Barrachina, Á. A. (2013). Chemical composition, antioxidant capacity, and sensory quality of pomegranate (*Punica granatum* L.) arils and rind as affected by drying method. *Food and Bioprocess Technology, 6*(7), 1644–1654.

Calín-Sánchez, Á., Figiel, A., Szarycz, M., Lech, K., Nuncio-Jáuregui, N., & Carbonell-Barrachina, Á. A. (2014). Drying kinetics and energy consumption in the dehydration of pomegranate (*Punica granatum* L.) arils and rind. *Food and Bioprocess Technology, 7*, 2071–2083.

Calín-Sánchez, Á., Kharaghani, A., Lech, K., Figiel, A., Carbonell-Barrachina, Á. A., & Tsotsas, E. (2015). Drying kinetics and microstructural and sensory properties of black chokeberry (*Aronia melanocarpa*) as affected by drying method. *Food and Bioprocess Technology, 8*(1), 63–74.

Dak, M., Sagar, V. R., & Jha, S. K. (2014). Shelf-life and kinetics of quality change of dried pomegranate arils in flexible packaging. *Food Packaging and Shelf Life, 2*(1), 1–6.

Elyatem, S. M., & Kader, A. A. (1984). Post-harvest physiology and storage behaviour of pomegranate fruits. *Scientia Horticulturae, 24*, 287–298.

EUFIC, E. F. I. C. (2012). *Fruit and vegetable consumption in Europe-do Europeans get enough?* (vol September 2016). European Food Information Council. http://www.eufic.org/article/en/expid/Fruit-vegetable-consumption-Europe/.

Figiel, A. (2010). Drying kinetics and quality of beetroots dehydrated by combination of convective and vacuum-microwave methods. *Journal of Food Engineering, 98*, 461–470.

Galindo, A., Calín-Sánchez, A., Collado-González, J., Ondoño, S., Hernández, F., Torrecillas, A., & Carbonell-Barrachina, Á. A. (2014). Phytochemical and quality attributes of pomegranate fruits for juice consumption as affected by ripening stage and deficit irrigation. *Journal of the Science of Food and Agriculture, 94*, 2259–2265.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry, 48*(10), 4581–4589.

Haghighian, M. K., Rafraf, M., Moghaddam, A., Hemmati, S., Jafarabadi, M. A., & Gargari, B. P. (2016). Pomegranate (*Punica granatum* L.) peel hydro alcoholic extract ameliorates cardiovascular risk factors in obese women with dyslipidemia: A double blind, randomized, placebo controlled pilot study. *European Journal of Integrative Medicine, 8*, 676–682.

Horuz, E., & Maskan, M. (2015). Hot air and microwave drying of pomegranate (*Punica granatum* L.) arils. *Journal of Food Science and Technology, 52*(1), 285–293.

Jaiswal, V., DerMarderosian, A., & Porter, J. R. (2010). Anthocyanins and polyphenol oxidase from dried arils of pomegranate (Punica granatum L.). *Food Chemistry, 118*(1), 11–16.

Juszczak, L., Witczak, M., & Galkowska, D. (2009). Flow behaviour of black chokeberry (Aronia melanocarpa) juice. *International Journal of Food Engineering, 5*(1), 1556–3758.

Kingsly, A. R. P., & Singh, D. B. (2007). Drying kinetics of pomegranate arils. *Journal of Food Engineering, 79*(2), 741–744.

Kingsly, A. R. P., Singh, D. B., Manikantan, M. R., & Jain, R. K. (2006). Moisture dependent physical properties of dried pomegranate seeds (anardana). *Journal of Food Engineering, 75*(4), 492–496.

Kulling, S. E., & Rawel, H. M. (2008). Chokeberry *(Aronia melanocarpa)* – A review on the characteristic components and potential health effects. *Planta Medica, 74*, 1625–1634.

Lech, K., Figiel, A., Wojdyło, A., Korzeniowska, M., Serowik, M., & Szarycz, M. (2015). Drying kinetics and bioactivity of beetroot slices pretreated in concentrated chokeberry juice and dried with vacuum microwaves. *Drying Technology, 33*(13), 1644–1653.

Maskan, A., Kaya, S., & Maskan, M. (2002). Effect of concentration and drying processes on color change of grape juice and leather (pestil). *Journal of Food Engineering, 54*(1), 75–80.

Megías-Pérez, R., Gamboa-Santos, J., Soria, A. C., Villamiel, M., & Montilla, A. (2014). Survey of quality indicators in commercial dehydrated fruits. *Food Chemistry, 150*, 41–48.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). *Sensory evaluation techniques* (4th ed.). Boca Raton, FL: CRC Press, Taylor & Francis Group (Chapter 10).

Mena, P., Martí, N., & García-Viguera, C. (2014). Varietal blends as a way of optimizing and preserving the anthocyanin content of pomegranate (Punica granatum L.) juices. *Journal of Agricultural and Food Chemistry, 62*(29), 6936–6943.

Mundada, M., Hathan, B. S., & Maske, S. (2011). Mass transfer kinetics during osmotic dehydration of pomegranate arils. *Journal of Food Science, 76*(1), E31–E39.

Mundada, M., Singh, B., & Maske, S. (2010). Optimisation of processing variables affecting the osmotic dehydration of pomegranate arils. *International Journal of Food Science and Technology, 45*, 1732–1738.

Nowicka, P., Wojdylo, A., Lech, K., & Figiel, A. (2015a). Influence of osmodehydration pretreatment and combined drying method on the bioactive potential of sour cherry fruits. *Food and Bioprocess Technology, 8*, 824–836.

Nowicka, P., Wojdyło, A., Lech, K., & Figiel, A. (2015b). Chemical composition, antioxidant capacity, and sensory quality of dried sour cherry fruits pre-dehydrated in fruit concentrates. *Food and Bioprocess Technology, 8*(10), 2076–2095.

Orgil, O., Spector, L., Holland, D., Mahajna, J., & Amir, R. (2016). The anti-proliferative and anti-androgenic activity of different pomegranate accessions. *Journal of Functional Foods, 26*, 517–528.

Oszmiański, J., & Lachowicz, S. (2016). Effect of the production of dried fruits and juice from chokeberry (*Aronia melanocarpa* L.) on the content and antioxidative activity of bioactive compounds. *Molecules, 21*(8), 1098.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay – Electron-transfer reactions with organic compounds in solutions containing nitrite or nitrate. *Free Radical Biology and Medicine, 26*, 1231–1237.

Szychowski, P. J., Frutos, M. J., Burló, F., Pérez-López, A. J., Carbonell-Barrachina, A. A., & Hernández, F. (2015). Instrumental and sensory texture attributes of pomegranate arils and seeds as affected by cultivar. *LWT-Food Science and Technology, 60*, 656–663.

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B., & Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry, 115*(3), 873–877.

Trigueros, L., Wojdyło, A., & Sendra, E. (2014). Antioxidant activity and protein–polyphenol interactions in a pomegranate (*Punica granatum* L.) yogurt. *Journal of Agricultural and Food Chemistry, 62*(27), 6417–6425.

Vázquez-Araújo, L., Nuncio-Jáuregui, P. N., Cherdchu, P., Hernández, F., Chambers, E., & Carbonell-Barrachina, Á. A. (2014). Physicochemical and descriptive sensory characterization of Spanish pomegranates: Aptitudes for processing and fresh consumption. *International Journal of Food Science & Technology, 49*(7), 1663–1672.

Wojdyło, A., Nowicka, P., Carbonell-Barrachina, Á. A., & Hernández, F. (2016). Phenolic compounds, antioxidant and antidiabetic activity of different cultivars of Ficus carica L. fruits. *Journal of Functional Foods, 25*, 421–432.

Yilmaz, Y., & Toledo, R. (2005). Antioxidant activity of water-soluble Maillard reaction products. *Food Chemistry, 93*, 273–278.

Publication 5

Consumers' opinion on dried pomegranate arils to determine the best processing conditions

Cano-Lamadrid M., Vázquez-Araújo L., Sánchez-Rodríguez L., Wojdyło A., Carbonell-Barrachina Á.A.

Journal of Food Science, 83: 3085-3091 (2018)

DOI: 10.1111/1750-3841.14390

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

Marina Cano-Lamadrid, Laura Vázquez-Araújo, Lucía Sánchez-Rodríguez, Aneta Wodyło, and Ángel A. Carbonell-Barrachina

Abstract: Consumers' preference is essential to improve processed food products quality, but small companies sometimes lacks 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

JFDS-2018-0807 Submitted 5/25/2018, Accepted 10/11/2018. *Authors Cano-Lamadrid, Sánchez-Rodríguez, and Carbonell-Barrachina are with Dept. of Agro-Food Technology, Research Group "Food Quality and Safety", Escuela Politécnica Superior de Orihuela (EPSO), Univ. Miguel Hernández de Elche, Carretera de Beniel, km 3.2, 03312, Orihuela, Alicante, Spain. Author Vázquez-Araújo is with BCCInnovation, Technological Center in Gastronomy, Juan Avelino Barriola 101, 20009, Donostia-San Sebastián, Gipuzkoa, Spain. Author Vázquez-Araújo is also with Basque Culinary Center, Mondragon Unibersitatea, Juan Avelino Barriola 101, 20009, Donostia-San Sebastián, Gipuzkoa, Spain. Author Wodyło is with Department of Fruit and Vegetable Processing, Wroclaw Univ. of Environmental and Life Science, 37 Chełmońskiego Street, 51–630, Wroclaw, Poland. Direct inquiries to author Vázquez-Araújo (E-mail: lvazquez@bculinary.com).*

Journal of Food Science A Publication of the Institute of Food Technologists

doi: 10.1111/1750-3841.14390

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 led 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 × 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 distillated 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[γ]	RT (min)[†]	Retention Indexes[¥]					Descriptors[¶]
				Exp. A	Exp. B	Exp. C	Exp. D	UMH	
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	α-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

[γ] A, commercial; B, CD-VMD; C, OD(POM)-CD-VMD; and D, OD(POM-Ch)-CD-VMD;
[†] RT, retention time;
[¥] 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 matrixes. All 19 compounds found in the dehydrated pomegranate arils have been identified by using Sigma-Aldrich (Merk KGaA, Darmstadt, Germany) standards;
[¶] 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 trough 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 α-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 (α- thujene), and monoterpenoids (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 led 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†	Content (%)ʸ			
			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	α-Thujene	***	0.91 c	24.8 a	0.08 c	15.4 b
V8	2-Acetylfuran	***	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-(Mydroxymethyl)-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

†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;
ʸA, 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†	Aʸ	B	C	D
			Appearance		
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
			Basic taste		
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
			Flavor		
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
			Texture		
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

†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;
ʸA, 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[†]	A[ɣ]	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

[†]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;
[ɣ]A, commercial; B, CD-VMD; C, OD(POM)-CD-VMD; and D, OD(POM-Ch)-CD-VMD.

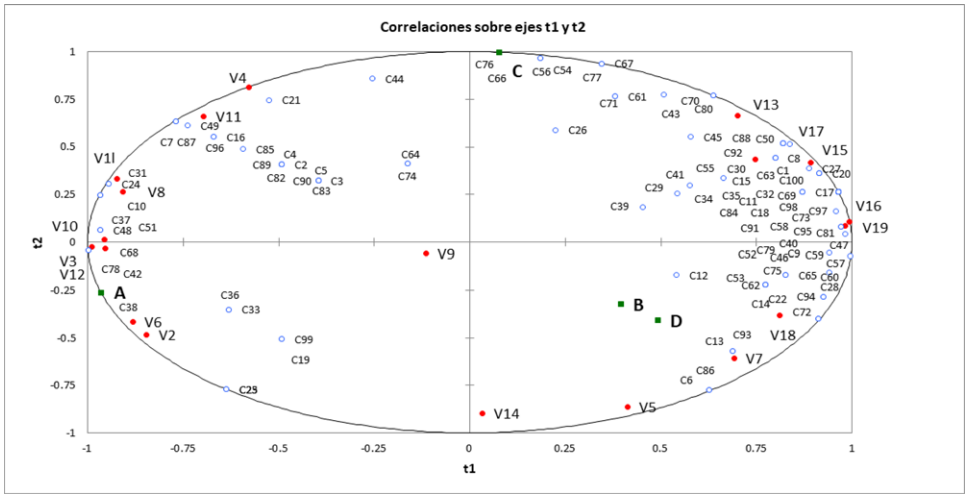


Figure 1–Partial least squares regression (PLS) of the volatile compounds (x) and consumers' overall liking (Y) of the samples (square, samples; unfilled circle unfilled, consumers; filled circle, volatile compounds¥). ¥V1, hexanal; V2, 2,3-butanediol; V3, furfural; V4, 1-hexanol; V5, furfuryl alcohol; V6, 5-methyl-2(3H)-furanone; V7, α-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 linking 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 fist 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

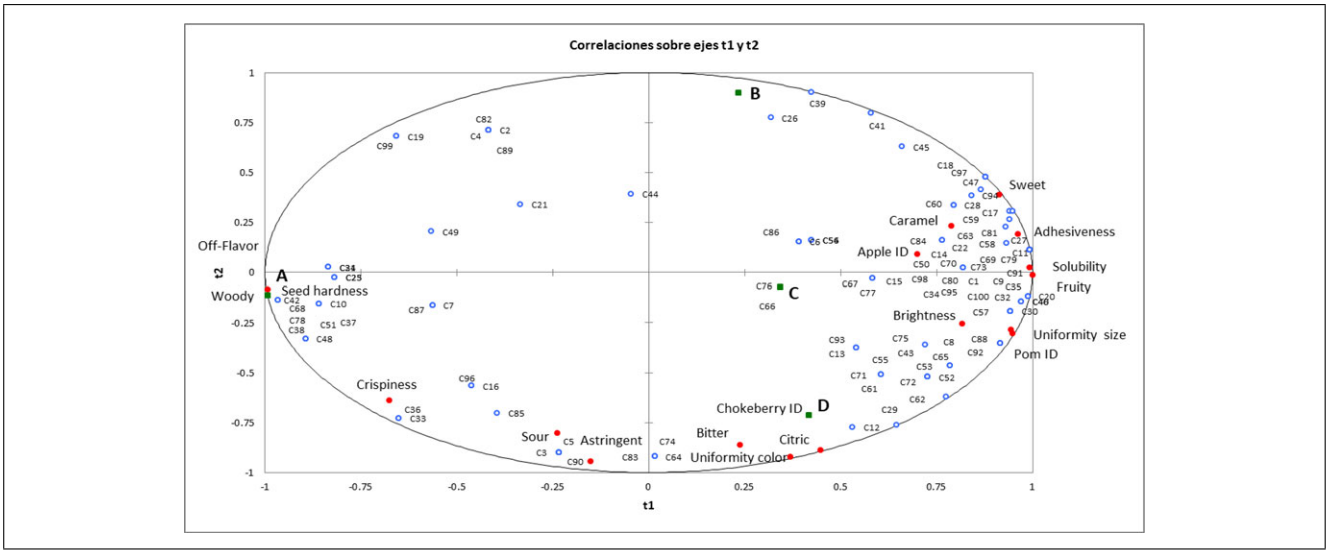


Figure 2–Partial least squares regression (PLS) of the descriptive sensory profile (X) and consumers' overall liking (Y) of the samples (squares, samples; unfilled circle, consumers; filled circle, descriptive attributes).

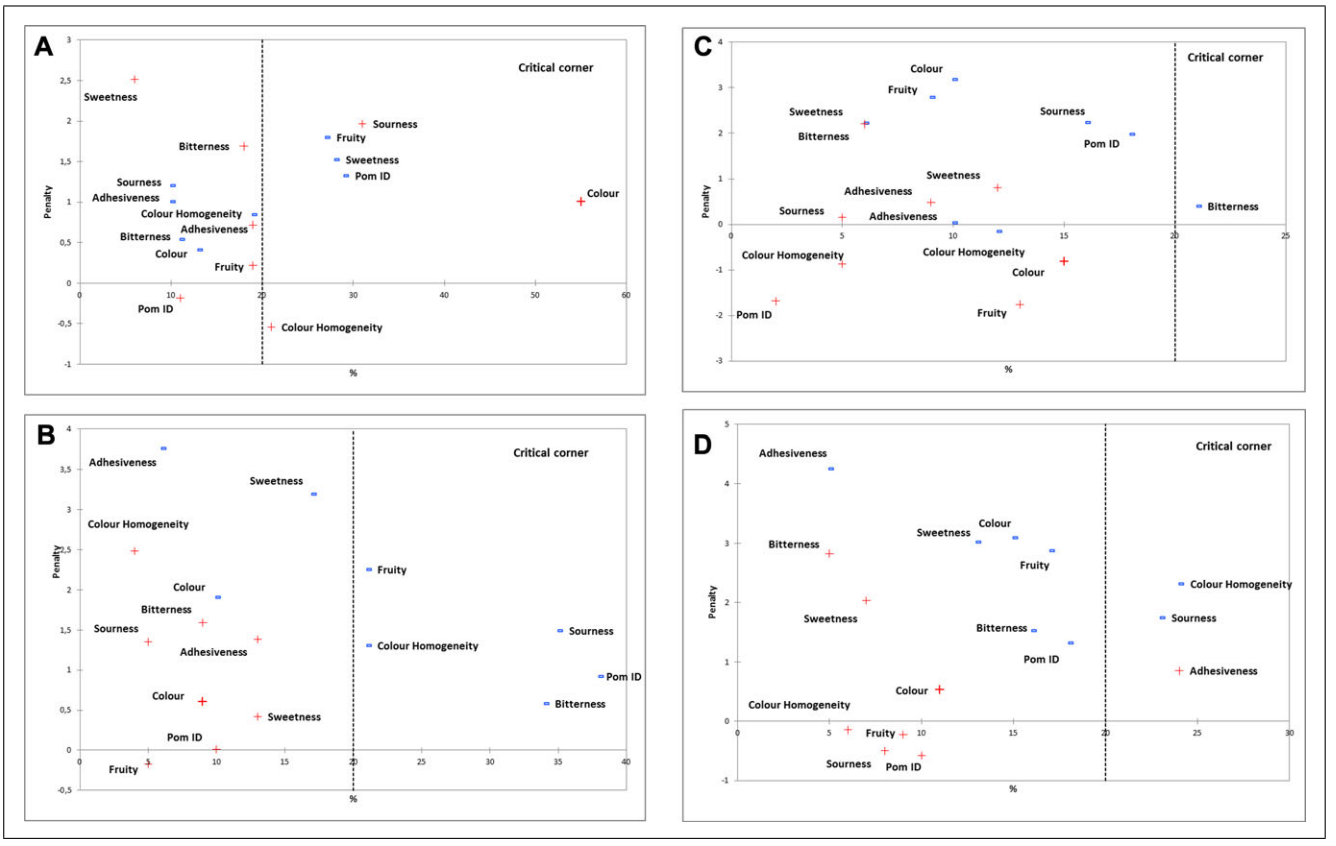


Figure 3–Penalty analysis of samples (sample code indicated on the top left of each figure; "too low intensity" indicated with "-", and "too high intensity" indicated with "+").

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 number 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 linking 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-favor, 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 osmodehydration (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

Author Cano-Lamadrid was funded by an FPU grant (reference number: FPU15/02158) from the Spanish Ministry of Education. The authors are grateful to the project AGL2013-482-45922-C2-2-R (Ministerio de Economía y Competitividad, Spain). This work has been carried out thanks to a double co-tutelle PhD between WUELS (Poland) and UMH (Spain).

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.

References

Alcaraz-Mármol, F., Calín-Sánchez, Á., Nuncio-Jáuregui, N., Carbonell-Barrachina, Á. A., Hernández, F., & Martínez, J. J. (2015). Classification of pomegranate cultivars according to their seed hardness and wood perception. *Journal of Texture Studies*, *46*(6), 467–474.

Alcaraz-Mármol, F., Nuncio-Jáuregui, N., García-Sánchez, F., Martínez-Nicolás, J. J., & Hernández, F. (2017). Characterization of twenty pomegranate (Punica granatum L.) cultivars grown in Spain: Aptitudes for fresh consumption and processing. *Scientia Horticulturae*, *219*, 152–160.

Butler, E. (2018). Tasting off-flavors: Food science, sensory knowledge and the consumer sensorium. *The Senses and Society*, *13*(1), 75–88.

Calín-Sánchez, Á., Figiel, A., Hernández, F., Melgarejo, P., Lech, K., & Carbonell-Barrachina, Á. A. (2013). Chemical composition, antioxidant capacity, and sensory quality of pomegranate (*Punica granatum* L.) arils and rind as affected by drying method. *Food and Bioprocess Technology*, *6*(7), 1644–1654.

Calín-Sánchez, Á., Martínez, J. J., Vázquez-Araújo, L., Burló, F., Melgarejo, P., & Carbonell-Barrachina, A. A. (2011). Volatile composition and sensory quality of Spanish pomegranates (*Punica granatum* L.). *Journal of the Science of Food and Agriculture*, *91*(3), 586–592.

Cano-Lamadrid, M., Lech, K., Michalska, A., Wasilewska, M., Figiel, A., Wojdyło, A., & 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.

Cano-Lamadrid, M., Galindo, A., Collado-González, J., Rodríguez, P., Cruz, Z. N., Legua, P., . . . Hernández, F. (2018). Influence of deficit irrigation and crop load on the yield and fruit quality in Wonderful and Mollar de Elche pomegranates.*Journal of the Science of Food and Agriculture*, *98*(8), 3098–3108.

Dak, M., Sagar, V. R., & Jha, S. K. (2014). Shelf-life and kinetics of quality change of dried pomegranate arils in flexible packaging. *Food Packaging and Shelf Life*, *2*(1), 1–6.

Fromberg, A., Mariotti, M. S., Pedreschi, F., Fagt, S., & Granby, K. (2014). Furan and alkylated furans in heat processed food, including home cooked products. *Czech Journal of Food Sciences*, *32*(5), 443–448.

Kalaycıoğlu, Z., & Erim, F. B. (2017). Total phenolic contents, antioxidant activities, and bioactive ingredients of juices from pomegranate cultivars worldwide. *Food Chemistry*, *221*, 496–507.

Karimi, M., Sadeghi, R., & Kokini, J. (2017). Pomegranate as a promising opportunity in medicine and nanotechnology. *Trends in Food Science & Technology*, *69*, 59–73.

Koppel, K., & Chambers, E, IV. (2010). Development and application of a lexicon to describe the flavor of pomegranate juice. *Journal of Sensory Studies*, *25*(6), 819–837.

Mayuoni-Kirshinbaum, L., & Porat, R. (2014). The flavor of pomegranate fruit: A review. *Journal of the Science of Food and Agriculture*, *94*(1), 21–27.

Merck KGaA (2018). Flavor & Fragrance Ingredients: 2018 New Product Supplement. Retrieved from https://secure.sigmaaldrich.com/productsupplement.

Narayanan, P., Chinnasamy, B., Jin, L., & Clark, S. (2014). Use of just-about-right scales and penalty analysis to determine appropriate concentrations of stevia sweeteners for vanilla yogurt. *Journal of Dairy Science*, *97*(6), 3262–3272.

Noguera-Artiaga, L., Lipan, L., Vázquez-Araújo, L., Barber, X., Pérez-López, D., & Carbonell-Barrachina, Á. A. (2016). Opinion of Spanish consumers on hydrosustainable pistachios. *Journal of Food Science*, *81*(10), S2559–S2565.

Szychowski, P. J., Frutos, M. J., Burló, F., Pérez-López, A. J., Carbonell-Barrachina, Á. A., & Hernández, F. (2015). Instrumental and sensory texture attributes of pomegranate arils and seeds as affected by cultivar. *LWT - Food Science and Technology*, *60*(2, Part 1), 656–663.

van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances*, *24*(2), 230–233.

Vardin, H., & Yilmaz, F. M. (2018). The effect of blanching pretreatment on the drying kinetics, thermal degradation of phenolic compounds and hydroxymethyl furfural formation in pomegranate arils. *Italian Journal of Food Science*, *30*(1), 156–169.

Vázquez-Araújo, L., Nuncio-Jáuregui, P. N., Cherdchu, P., Hernández, F., Chambers, E., & Carbonell-Barrachina, Á. A. (2014). Physicochemical and descriptive sensory characterization of Spanish pomegranates: Aptitudes for processing and fresh consumption. *International Journal of Food Science & Technology*, *49*(7), 1663–1672.

Wojdyło A., Figiel A., Lech K., Nowicka P., & Oszmiański Jan. (2014). Effect of convective and vacuum–microwave drying on the bioactive compounds, color, and antioxidant capacity of sour cherries. *Food Bioprocess Technology*, 7, 829–841.

Publication 6

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., Wojdyło, A.

***Journal of the Science of Food and Agriculture*,** 98: 5731-5741 (2018)

DOI: 10.1002/jsfa.9120

Research Article

Received: 21 March 2018 Revised: 30 April 2018 Accepted article published: 8 May 2018 Published online in Wiley Online Library: 27 June 2018

(wileyonlinelibrary.com) DOI 10.1002/jsfa.9120

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

Marina Cano-Lamadrid,[a] Paulina Nowicka,[b] Francisca Hernández,[c] Angel A Carbonell-Barrachina[a] and Aneta Wojdyło[b]*

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^{-1} fresh weight (FW), followed by jujube and figs, with 374 and 320 mg 100 g^{-1} 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).[1] 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.[2] 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,[3] broccoli, tomato,[4] sour cherry,[5] apple[6] and *Prunus* fruits.[7] 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.[8]

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

* *Correspondence to: A Wojdyło, Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chełmońskiego Street, 51-630, Wrocław, Poland. E-mail: aneta.wojdylo@upwr.edu.pl*

a *Research Group 'Food Quality and Safety', Department of Agro-Food Technology, Escuela Politécnica Superior de Orihuela (EPSO), Universidad Miguel Hernández de Elche (UMH), Alicante, Spain*

b *Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland*

c *Research Group 'Plant Production and Technology', Department of Plant Sciences and Microbiology, EPSO, UMH, Alicante, Spain*

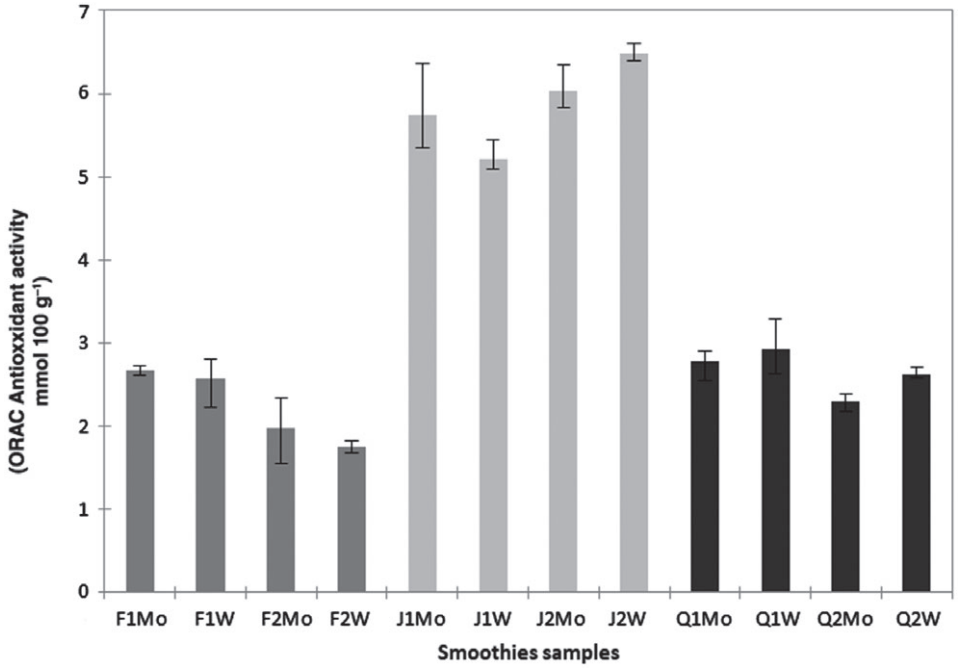


Figure 1. Antioxidant activity (ORAC method) as affected by smoothie formulation; see Table 1 for specific formulation.

with huge potential because of their healthy properties, such as figs,[9] jujubes[10] and quinces;[11,12] 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.[13] 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).[14–17] Pomegranate cvs 'Mollar de Elche' and 'Wonderful', figs (*Ficus carica*, cv. 'Colar'), jujubes (*Ziziphus jujube*, cv. 'Grandes de Albatera') 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

Titratable acidity (TA) was determined by titrating sample aliquots using 0.1 mol L^{-1} 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_3 and 2 mL

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

No.	Code[a]	Formulation[b]
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

[a] Mo, 'Mollar de Elche' pomegranate juice; W, 'Wonderful' pomegranate juice; F, fig purée; J, jujube purée; Q, quince purée.
[b] The percentage of each component was expressed as weight (w/w).

H_2O_2 (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.[7] Vitamin C was analyzed using high-performance liquid chromatography (HPLC), as described by Wojdyło *et al.*[12]

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.*[7] 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^{-1} 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.*[7] 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^{-1} ($R^2 \geq 0.9998$).

Phytochemical composition and antioxidant capacity

Polyphenols and procyanidins

The extraction of polyphenols was conducted using the conditions reported by Wojdyło *et al.*[12] 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 Wojdyło *et al.*[18] The analysis of polymeric procyanidins by the phloroglucinol method was performed according to the protocol described previously by Kennedy and Jones.[19]

ORAC assay

Extraction of compounds with antioxidant activity was conducted according to a previous study.[9] The ORAC assay was prepared as described by Ou *et al.*[20] 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 significantly 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.[21–23] pH and TA ranges were 3.31–4.52 and 0.28–1.77 g malic acid 100 g^{-1}, 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^{-1}) as compared to 'Mollar de Elche' fruits (4.09 and 0.53 g malic acid 100 g^{-1}, respectively). These experimental findings agreed with previous data for fresh pomegranate fruits.[24]

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[25–27] 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^{-1} 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^{-1}) than jujubes and quinces (21 and 11 mg 100 g^{-1}, respectively).[28] Regarding Mg and

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

Sample	TSS (°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
ANOVA test[a]						
Purée:juice ratio	NS	NS	NS	NS	*	*
Pomegranate cultivar	NS	**	**	NS	**	**
Purée fruit	**	**	NS	***	**	**
Tukey's multiple range test[b]						
Purée:juice ratio (%, w/w)						
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
Pomegranate cultivar						
'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
Purée fruit						
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

[a] NS, not significant at $P < 0.05$; asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.
[b] 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.[29]

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.[30] Pectin content increased when using the highest content of fruit purée, as previously concluded by Nowicka *et al.*[7] 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}).[31] This experimental finding is based on the high vitamin C content of jujubes (387–555 mg 100 g^{-1} FW).[32]

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.[24] The glucose:fructose ratio indicated previously for pomegranate juice (0.80–0.95) was maintained in the pomegranate-based smoothies (mean value: 0.85).[13]

According to previous studies,[11] 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

	Minerals				Pectin	Vitamin C
	Ca	Mg	K	Zn		
Sample	(mg 100 g^{-1} FW)				(g 100 g^{-1} FW)	(mg 100 g^{-1} 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
ANOVA test[a]						
Purée:juice ratio	NS	NS	*	NS	*	**
Pom. cultivar	NS	NS	***	NS	NS	NS
Purée fruit	***	**	*	***	**	***
Tukey's multiple range test[b]						
Ratio purée: juice (%:%, w:w)						
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
Pomegranate cultivar						
'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
Purée fruit						
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

[a] NS, not significant at $P < 0.05$; asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.
[b] 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).[33] 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.[34]

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.[35]

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^{2+} and Mg^{2+}.[36] Moreover, oxalic acid, which inhibits the mineral bioavailability and forms calcium oxalates in urinary stones,[37] was found in all samples but in low quantities (0.06–0.18 g 100 g^{-1} 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; $[M-H]^-$ 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)[38] and based on *Prunus* fruits, such as peach, apricot and plum (representing 0–26% of total polyphenolic content).[7] In the smoothies under

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

	Sugars				Organic acids							
	Fructose	Sorbitol	Glucose	Sucrose	Oxalic	Malic	Citric	Tartaric	Quinic	Shikimic	Succinic	Formic
Sample	(g 100 g^{-1} 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
ANOVA test[a]												
Purée:juice ratio	**	*	**	*	NS	NS	NS	NS	NS	NS	NS	NS
Pom. cultivar	*	NS	*	NS	NS	NS	NS	***	NS	NS	NS	***
Purée fruit	***	***	**	**	***	***	***	*	***	***	***	***
Tukey's multiple range test[b]												
Purée:juice ratio (%, w/w)												
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
Pomegranate cultivar												
'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
Purée fruit												
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

[a] NS, not significant at $P < 0.05$; asterisks indicate significance at $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.
[b] 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^{-1} FW in the smoothie sample Q1Mo to 103 mg 100 g^{-1} 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,[9] 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.[38] 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,[35] 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.[39–41] It is worth mentioning, however, that a recent review[42] 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,[26] which indicated that the pH in a food matrix is the main factor controlling the changes in ACN color.[43]

Flavonols (Fvs)

Table 5 shows the identification and quantification of Fvs (11 compounds): quercetin-3-*O*-galactoside (Fv1; $[M-H]^-$ at $m/z = 601.06$; MS/MS fragment at $m/z = 463.16$, and $R_t = 6.30$ min); quercetin-3-*O*-rutinose (Fv2; 609.14; 300.03; $R_t = 6.60$ min); quercetin-3-*O*-glusoside (Fv3; 433.17; 301.03; 6.84 min); kaempferol-3-*O*-galactoside (Fv4; 447; 284; 8.04 min); kaempferol-3-*O*-rutinoside (Fv5; 593.15; 285.05; 7.32 min); quercetin-3-*O*-rutinoside-7-*O*-hexoside (Fv6; 771; 301.02; 4.41 min); quercetin-3-*O*-rutinoside-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^{-1} FW) in the smoothies under analysis

	Anthocyanins[a]					Flavonols[b]										
	A1	A2	A3	A4	A5	Fv1	Fv2	Fv3	Fv4	Fv5	Fv6	Fv7	Fv8	Fv9	Fv10	Fv11
Sample	(mg 100 g^{-1} FW)					(mg 100 g^{-1} 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[c]					**ANOVA test[c]**										
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[d]					**Tukey's multiple range test[d]**										
Purée:juice ratio (%, w:w)																
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
Pomegranate cultivar																
'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
Purée fruit																
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

[a] 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.
[b] Fv1, quercetin-3-*O*-galactoside; Fv2, quercetin-3-*O*-rutinose; Fv3, quercetin-3-*O*-glusoside; Fv4, kaempferol-3-*O*-galactoside; Fv5, kaempferol-3-*O*-rutinoside; 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.
[c] Asterisks indicate significance at *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.
[d] 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^{-1}).[7] The total Fv content of the current smoothies ranged from 33.4 mg 100 g^{-1} in sample Q1W to 67.4 mg 100 g^{-1} 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^{-1}), followed by the jujube and fig samples, with values of 9.08 and 2.12 mg 100 g^{-1}, 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).[9,12,32] 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.[44]

Flavan-3-ols (F3os)

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

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

	Flavan-3-ols[a]							Hydroxycinnamic acids[b]					
	C	PB1	E	PB2	P3	P4	PP	NCh	4op	Ch	4cq	cq	Dcq
Sample	(mg 100 g^{-1} FW)						(g 100 g^{-1} FW)	(mg 100 g^{-1} 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[c]								**ANOVA test[c]**					
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[d]								**Tukey's multiple range test[d]**					
Purée:juice ratio (%, w/w)													
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
Pomegranate cultivar													
'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
Purée fruit													
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

[a] C, (+) catechin; PB1, procyanidin B1; E, (−)-epicatechin; PB2, procyanidin B2; 3, procyanidin trimer; P4, procyanidin tetramer; PP, polymeric procyanidins.
[b] 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**.**
[c] Asterisks indicate significance at *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.
[d] 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 $R_t = 3.41$ 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).[45]

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^{-1}. 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^{-1}, respectively. Previously, (+)-catechin and (−)-epicatechin were the predominant F3os in smoothies based on peach, apricot and plum.[7] 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).[9,12,32]

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,[12,46] jujubes,[32,47] and some fig cultivars.[9] 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; $[M-H]^-$ at

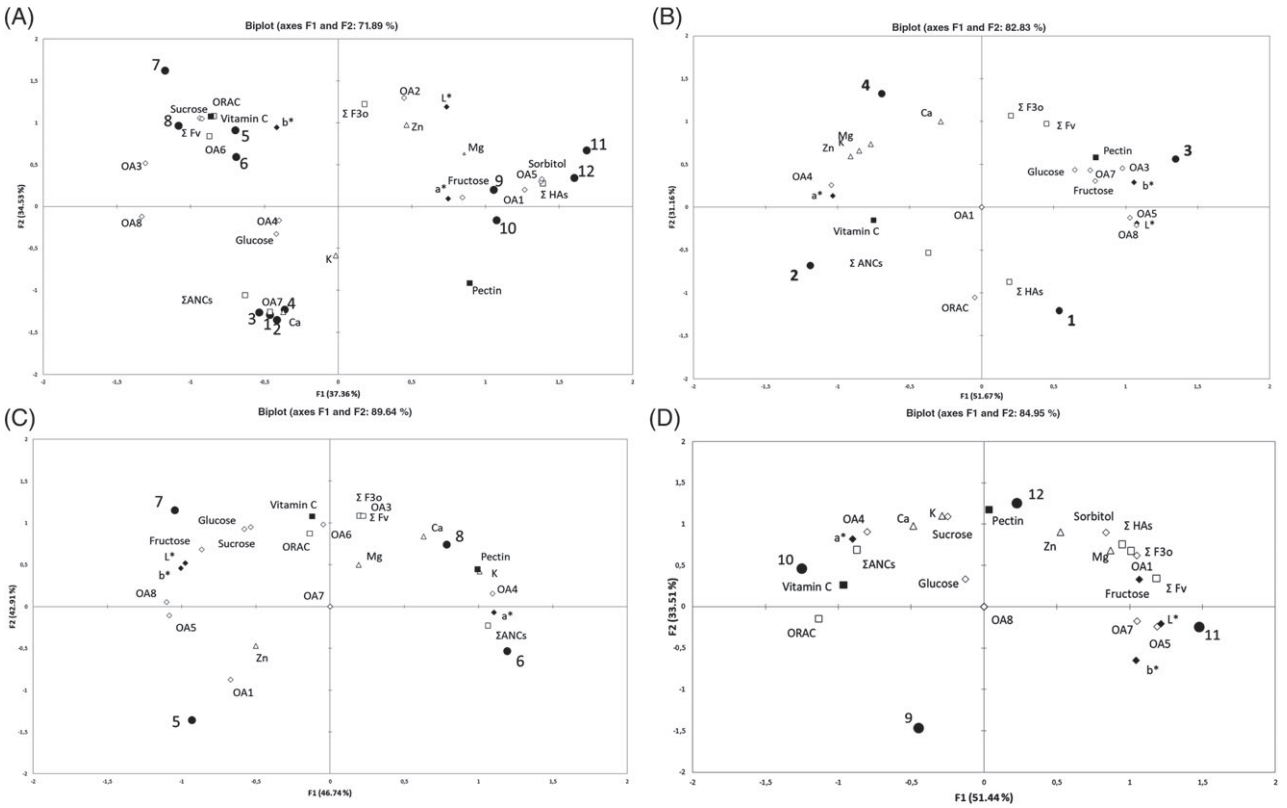


Figure 2. PCA scores plot showing the relationships among color coordinates, chemical composition, antioxidant capacity (ORAC) and phenolic profile of the smoothies under study: (A) all smoothies; (B) fig smoothies; (C) jujube smoothies; and (D) quince smoothies. ♦: color coordinates (L^*, a^*, b^*); ◇: 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 (Σ ACNs, anthocyanins; Σ Fv, flavonols; Σ F3o, flavan-3-ols; Σ 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;[32] these profiles are supported by the profiles of fresh quinces and figs.[9,12] Although the absorption of HAs when passing through the human digestive tract depends on the esterase activity on intestine mucosa and the microflora,[48] several healthy properties were shown such as improvements in diabetes, cardiovascular disease, obesity and cancer.[49]

Antioxidant capacity (AC)

The AC determined by the ORAC assay ranged from 1.75 to 6.48 mmol Trolox 100 g^{-1}, with quince purée leading to the highest values of AC (5.78 mmol Trolox 100 g^{-1}) as compared to quince and fig purées, at 2.61 and 2.34 mmol Trolox 100 g^{-1}, 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 HAcs. 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.[50,51] Contrarily, these authors concluded that vitamin C did not present ORAC activity.[50] 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.

ACKNOWLEDGEMENTS

Author MCL was funded by an FPU grant (reference number: FPU15/02158) from the Spanish Ministry of Education, Culture and Sport. The authors are grateful to the project AGL2013-482-45922-C2-2-R (Ministerio de economía y Competitividad, Spain). This work has been carried out thanks to a double co-tutelle PhD between WUELS (Poland) and UMH (Spain).

REFERENCES

1 Di Cagno R, Minervini G, Rizzello CG, De Angelis M and Gobbetti M, Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. *Food Microbiol* **28**:1062–1071 (2011).
2 FAO/WHO, *Promoting Fruit and Vegetable Consumption around the World*. [Online]. Ed. by FAO/WHO (2016). Available: http://www.who.int/dietphysicalactivity/fruit/en/ [9 June 2018].
3 Formica-Oliveira AC, Martínez-Hernández GB, Aguayo E, Gómez PA, Artés F and Artés-Hernández F, A functional smoothie from carrots with induced enhanced phenolic content. *Food Bioprocess Technol* **10**:491–502 (2017).
4 Castillejo N, Martínez-Hernández GB, Gómez PA, Artés F and Artés-Hernández F, Red fresh vegetables smoothies with extended shelf life as an innovative source of health-promoting compounds. *J Food Sci Technol* **53**:1475–1486 (2016).
5 Nowicka P, Wojdyło A, Teleszko M and Samoticha J, Sensory attributes and changes of physicochemical properties during storage of smoothies prepared from selected fruit. *LWT – Food Sci Technol* **71**:102–109 (2016).
6 Markowski J, Celejewska K, Rosłonek A and Kosmala M, Impact of different thermal preservation technologies on the quality of apple-based smoothies. *LWT – Food Sci Technol* **85**:470–473 (2017).
7 Nowicka P, Wojdyło A and Samoticha J, Evaluation of phytochemicals, antioxidant capacity, and antidiabetic activity of novel smoothies from selected Prunus fruits. *J Funct Foods* **25**:397–407 (2016).
8 Smith V, Green-Petersen D, Møgelvang-Hansen P, Christensen RHB, Qvistgaard F and Hyldig G, What's (in) a real smoothie: a division of linguistic labour in consumers' acceptance of name–product combinations? *Appetite* **63**:129–140 (2013).
9 Wojdyło A, Nowicka P, Carbonell-Barrachina ÁA and Hernández F, Phenolic compounds, antioxidant and antidiabetic activity of different cultivars of *Ficus carica* L. fruits. *J Funct Foods* **25**:421–432 (2016).
10 Hernández F, Noguera-Artiaga L, Burló F, Wojdyło A, Carbonell-Barrachina ÁA and Legua P, Physico-chemical, nutritional, and volatile composition and sensory profile of Spanish jujube (*Ziziphus jujuba* Mill.) fruits. *J Sci Food Agric* **96**:2682–2691 (2016).
11 Szychowski PJ, Munera-Picazo S, Szumny A, Carbonell-Barrachina ÁA and Hernández F, Quality parameters, bio-compounds, antioxidant activity and sensory attributes of Spanish quinces (*Cydonia oblonga* Miller). *Sci Hortic* **165**:163–170 (2014).
12 Wojdyło A, Oszmiański J and Bielicki P, Polyphenolic composition, antioxidant activity, and polyphenol oxidase (PPO) activity of quince (*Cydonia oblonga* Miller) varieties. *J Agric Food Chem* **61**:2762–2772 (2013).
13 Kalaycıoğlu Z and Bedia Erim F, Total phenolic contents, antioxidant activities, and bioactive ingredients of juices from pomegranate cultivars worldwide. *Food Chem* **221**:496–507 (2017).
14 Cano-Lamadrid M, Girón IF, Pleite R, Burló F, Corell M, Moriana A *et al.*, Quality attributes of table olives as affected by regulated deficit irrigation. *LWT – Food Sci Technol* **62**:19–26 (2015).
15 Cano-Lamadrid M, Hernández F, Corell M, Burló F, Legua P, Moriana A *et al.*, Antioxidant capacity, fatty acids profile, and descriptive sensory analysis of table olives as affected by deficit irrigation. *J Sci Food Agric* **97**:444–451 (2016).
16 Carbonell-Barrachina ÁA, Memmi H, Noguera-Artiaga L, Gijón-López MC, Ciapa R and Pérez-López D, Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. *J Sci Food Agric* **95**:2866–2873 (2015).
17 Noguera-Artiaga L, Lipan L, Vázquez-Araújo L, Barber X, Pérez-López D and Carbonell-Barrachina ÁA, Opinion of Spanish consumers on hydrosustainable pistachios. *J Food Sci* **81**:S2559–S2565 (2016).
18 Wojdyło A, Nowicka P, Laskowski P and Oszmiański J, Evaluation of sour cherry (*Prunus cerasus* L.) fruits for their polyphenol content, antioxidant properties, and nutritional components. *J Agric Food Chem* **62**:12332–12345 (2014).
19 Kennedy JA and Jones GP, Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J Agric Food Chem* **49**:1740–1746 (2001).

20 Ou B, Huang D, Hampsch-Woodill M, Flanagan JA and Deemer EK, Analysis of antioxidant activities of common vegetables employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) assays: a comparative study. *J Agric Food Chem* **50**:3122–3128 (2002).
21 Legua P, Serrano M, Melgarejo P, Valero D, Martínez JJ, Martínez R *et al.*, Quality parameters, biocompounds and antioxidant activity in fruits of nine quince (*Cydonia oblonga* Miller) accessions. *Sci Hortic* **154**:61–65 (2013).
22 Pereira C, Serradilla MJ, Martín A, Villalobos MC, Pérez-Gragera F and López-Corrales M, Agronomic behaviour and quality of six fig cultivars for fresh consumption. *Sci Hortic* **185**:121–128 (2015).
23 Galindo A, Noguera-Artiaga L, Cruz ZN, Burló F, Hernández F, Torrecillas A *et al.*, Sensory and physico-chemical quality attributes of jujube fruits as affected by crop load. *LWT – Food Sci Technol* **63**:899–905 (2015).
24 Alcaraz-Mármol F, Nuncio-Jáuregui N, García-Sánchez F, Martínez-Nicolás JJ and Hernández F, Characterization of twenty pomegranate (*Punica granatum* L.) cultivars grown in Spain: aptitudes for fresh consumption and processing. *Sci Hortic* **219**:152–160 (2017).
25 Bursać Kovačević D, Putnik P, Dragović-Uzelac V, Pedisić S, Režek Jambrak A and Herceg Z, Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chem* **190**:317–323 (2016).
26 Cano-Lamadrid M, Trigueros L, Wojdyło A, Carbonell-Barrachina ÁA and Sendra E, Anthocyanins decay in pomegranate enriched fermented milks as a function of bacterial strain and processing conditions. *LWT – Food Sci Technol* **80**:193–199 (2017).
27 Berenguer M, Vegara S, Barrajón E, Saura D, Valero M and Martí N, Physicochemical characterization of pomegranate wines fermented with three different *Saccharomyces cerevisiae* yeast strains. *Food Chem* **190**:848–855 (2016).
28 USDA, *National Nutrient Database for Standard Reference Release*. Food Composition Databases. Available: https://ndb.nal.usda.gov/ndb/ [5 July 2017].
29 Shad AA, Ahmad S, Ullah R, AbdEl-Salam NM, Fouad H, Rehman NU *et al.*, Phytochemical and biological activities of four wild medicinal plants. *Sci World J* **2014**:7 (2014). Available: https://doi.org/10.1155/2014/857363.
30 Naqash F, Masoodi FA, Rather SA, Wani SM and Gani A, Emerging concepts in the nutraceutical and functional properties of pectin: a review. *Carbohydr Polym* **168**:227–239 (2017).
31 CCE, Etiquetado propiedades nutritivas de los productos alimenticios, Consejo Dd. *Diario Oficial de las Comunidades Europeas*:40–44 (1990).
32 Wojdyło A, Carbonell-Barrachina ÁA, Legua P and Hernández F, Phenolic composition, ascorbic acid content, and antioxidant capacity of Spanish jujube (*Ziziphus jujube* Mill.) fruits. *Food Chem* **201**:307–314 (2016).
33 Montalto M, Gallo A, Ojetti V and Gasbarrini A, Fructose, trehalose and sorbitol malabsorption. *Eur Rev Med Pharmacol Sci* **17**:26–29 (2013).
34 FAO/WHO, *Fruit and Vegetable Processing*. FAO Agricultural Services Bulletin. Rome (1995).
35 Mena P, García-Viguera C, Navarro-Rico J, Moreno DA, Bartual J, Saura D *et al.*, Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *J Sci Food Agric* **91**:1893–1906 (2011).
36 Urbano G, López-Jurado M, Aranda P, Vidal-Valverde C, Tenorio E and Porres J, The role of phytic acid in legumes: antinutrient or beneficial function? *J Physiol Biochem* **56**:283–294 (2000).
37 Kayashima T and Katayama T, Oxalic acid is available as a natural antioxidant in some systems. *Biochim Biophys Acta* **1573**:1–3 (2000).
38 Teleszko M and Wojdyło A, Bioactive compounds vs. organoleptic assessment of 'smoothies'-type products prepared from selected fruit species. *Int J Food Sci Technol* **49**:98–106 (2014).
39 Kong J-M, Chia L-S, Goh N-K, Chia T-F and Brouillard R, Analysis and biological activities of anthocyanins. *Phytochemistry* **64**:923–933 (2003).
40 Ma T, Hu N, Ding C, Zhang Q, Li W, Suo Y *et al.*, In vitro and in vivo biological activities of anthocyanins from *Nitraria tangutorun* Bobr. fruits. *Food Chem* **194**:296–303 (2016).
41 Trikas ED, Melidou M, Papi RM, Zachariadis GA and Kyriakidis DA, Extraction, separation and identification of anthocyanins from red wine by-product and their biological activities. *J Funct Foods* **25**:548–558 (2016).
42 Braga ARC, Murador DC, de Souza Mesquita LM and de Rosso VV, Bioavailability of anthocyanins: gaps in knowledge, challenges and future research. *J Food Compos Anal* **68**:31–40 (2018).
43 Cheynier V, Phenolic compounds: from plants to foods. *Phytochem Rev* **11**:153–177 (2012).
44 Rauf A, Imran M, Patel S, Muzaffar R and Bawazeer SS, Rutin: exploitation of the flavonol for health and homeostasis. *Biomed Pharmacother* **96**:1559–1561 (2017).
45 Grzesik M, Naparło K, Bartosz G and Sadowska-Bartosz I, Antioxidant properties of catechins: comparison with other antioxidants. *Food Chem* **241**:480–492 (2018).
46 Hamauzu Y, Yasui H, Inno T, Kume C and Omanyuda M, Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *J Agric Food Chem* **53**:928–934 (2005).
47 Collado-González J, Cruz ZN, Rodríguez P, Galindo A, Díaz-Baños FG, García de la Torre J *et al.*, Effect of water deficit and domestic storage on the procyanidin profile, size, and aggregation process in pear-jujube (*Z. jujuba*) fruits. *J Agric Food Chem* **61**:6187–6197 (2013).
48 Liang N and Kitts DD, Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. *Nutrients* **8**:16 (2016).
49 Tajik N, Tajik M, Mack I and Enck P, The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. *Eur J Nutr* **56**:2215–2244 (2017).
50 Prior RL, Sintara M and Chang T, Multi-radical (ORACMR5) antioxidant capacity of selected berries and effects of food processing. *J Berry Res* **6**:159–173 (2016).
51 Yilmaz Y and Toledo RT, Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. *J Agric Food Chem* **52**:255–260 (2004).

Publication 7

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

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

LWT- Food Science and Technology, 96, 322-328 (2018)

DOI: 10.1016/j.lwt.2018.05.047

LWT - Food Science and Technology 96 (2018) 322–328

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

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

M. Cano-Lamadrid[a], F. Hernández[b], P. Nowicka[c], A.A. Carbonell-Barrachina[a], A. Wojdyło[c,*]

[a] Universidad Miguel Hernández de Elche (UMH), Escuela Politécnica Superior de Orihuela (EPSO), Department of Agro-Food Technology, Research Group "Food Quality and Safety", Ctra. Beniel, km 3.2, 03312, Orihuela, Alicante, Spain

[b] UMH, EPSO, Department of Plant Sciences and Microbiology, Research Group "Plant Production and Technology", Ctra. Beniel, km 3.2, 03312, Orihuela, Alicante, Spain

[c] Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chełmońskiego Street, 51-630, Wrocław, Poland

ARTICLE INFO

Keywords:
Punica granatum L.
Mollar de Elche
Wonderful
Anthocyanins
Polymeric procyanidins
FRAP
ABTS

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.

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

* Corresponding author.
E-mail address: aneta.wojdylo@upwr.edu.pl (A. Wojdyło).

https://doi.org/10.1016/j.lwt.2018.05.047
Received 26 March 2018; Received in revised form 22 April 2018; Accepted 20 May 2018
Available online 21 May 2018

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 Albatera*) 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.

Nº	Code[a]	Formulation[b]
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

[a] Mo, Mollar de Elche pomegranate juice; W, Wonderful pomegranate juice; F, fig purée; J, jujube purée; Q, quince purée.

[b] 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* ¼ 93.92; a* ¼ 1.03; b* ¼ 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 ($ABTS^+$ 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 $ABTS^+$ 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^{-1} 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

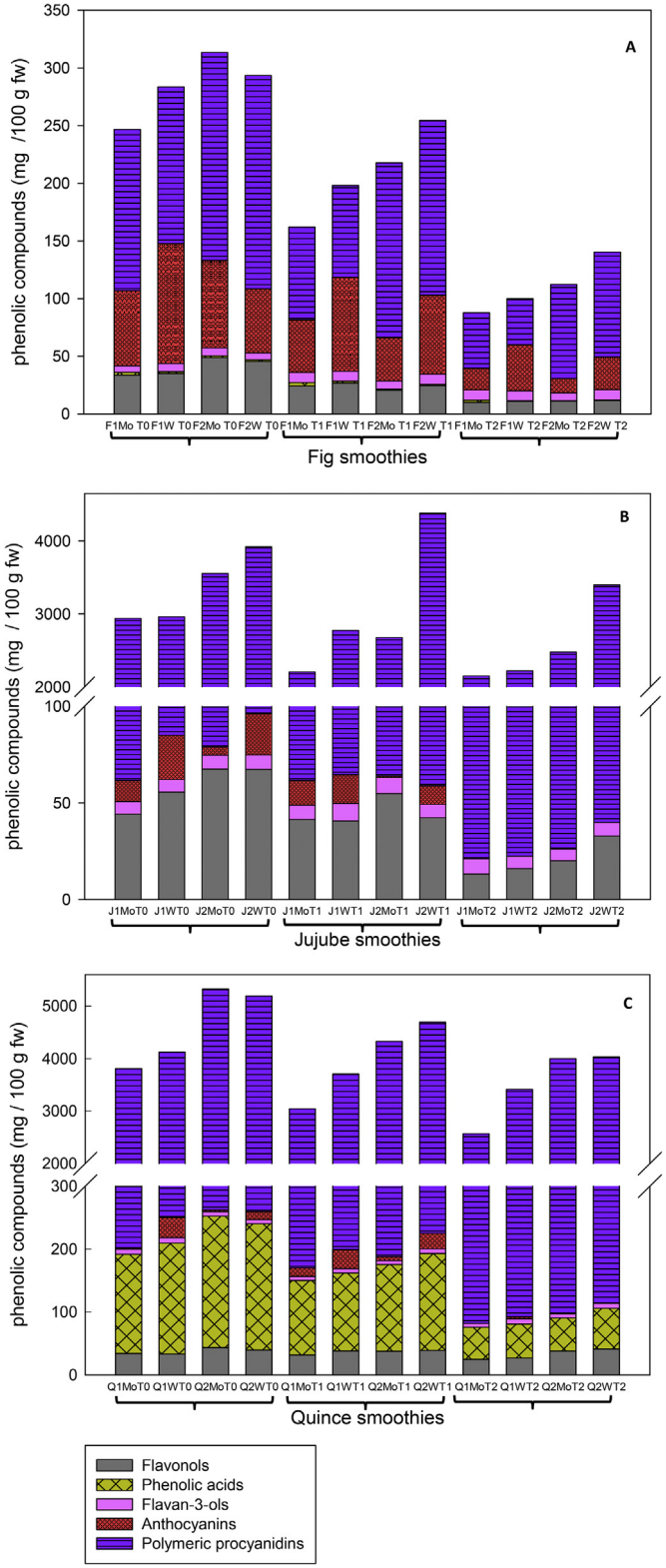


Fig. 1. Content of flavonols, phenolic acids, flavan-3-ols (as monomeric, dimeric and polymeric procyanidins), and anthocyanins, in mg/100 g fw (fresh weight) of fig (A), jujube (B), and quince (C) smoothies before (T0) and after 6 months of storage time at 4 °C (T1) and 20 °C (T2).

Table 2
Flavonols, phenolic acid, flavan-3-ols and antioxidant capacity (ABTS^{+} 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[†]	Smoothies[‡]											
		F1Mo	F1W	F2Mo	F2W	J1Mo	J1W	J2Mo	J2W	Q1Mo	Q1W	Q2Mo	Q2W
Flavonols[¥]	***	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 [¥]	***	2.32c	1.15d	0.84d	0.96d	nd	nd	nd	nd	109b	118b	133a	140a
Flavan-3-ols[¥]	***	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[¥]	***	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[†]	Ratio purée:juice		ANOVA[†]	Pomegranate cultivar		ANOVA	Fruit purée			ANOVA	Storage temperature		
		40:60	60:40		Mo	W		Fig	Jujube	Quince		T0	T1	T2
Flavonols[¥]	**	30.0b	38.2a	NS	33.3	34.9	***	25.3c	41.3a	35.7b	***	45.7a	35.2b	21.4c
Phenolic ac. [¥]	**	38.4b	45.8a	NS	40.9	43.3	***	1.32b	nd	125a	***	62.5a	35.2b	18.7c
Flavan-3-ols[¥]	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[¥]	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

[¥]expressed in followed units: mg/100 g fw smoothie.
[±] expressed in followed units: mmol Trolox/100 g fw smoothie.
[†]NS = not significant F ratio ($p > 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
[‡]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.
[α]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 (Wojdyło 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 than 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 ΣANCs 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 delphidin-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 that 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 (Wojdyło, Nowicka, Carbonell-Barrachina, & Hernández, 2016). Moreover, as expected fig smoothies presented 6.5 and 5.0 higher times of ACNs than jujube and quinces smoothies, respectively due to the presence of ACNs 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†	Smoothies‡											
		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	9.31bc	0.00d	6.32bc	3.25bc	14.0abc	1.46c	7.68bc
A3	NS	3.28a	3.55a	0.74a	1.48	1.17	0.75	0.23	0.35	0.82	1.32	0.59	1.15
A4	***	13.9a	12.5a	11.8ab	15.0ab	1.21a	0.88b	1.39b	1.67b	0.73b	1.93b	0.89b	1.08b
A5	***	10.2b	17.4a	18.6a	4.37c	0.00d	0.00d	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†	Ratio purée:juice		ANOVA†	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

± 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.
† NS = not significant F ratio ($p < 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
¥ 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.
αT0: 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 ACNs 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 ACNs 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 ACNs 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 ACNs 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
CIE$L^*a^*b^*$ 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[†]	Smoothies[‡]											
		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[†]	Ratio purée:juice		ANOVA[†]	Pomegranate cultivar		ANOVA	Fruit purée			ANOVA	Storage temperature [α]		
		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

[†] NS = not significant F ratio ($p > 0.05$); *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.
[¥] 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.
[α]T0: freshly made; T1: storage 6 months 4 °C; and, T2: storage 6 months 20 °C.

the $ABTS^+$ 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 $ABTS^+$ ($R = 0.44$; $p < 0.05$) data. Besides, anthocyanin content was significantly but negatively correlated with FRAP ($R = -0.47$; $p < 0.05$) and $ABTS^+$ ($R = -0.40$; $p < 0.05$) data. No significant ($p < 0.05$) correlation was observed among flavonols and flavan-3-ols with FRAP and $ABTS^+$ 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.

Acknowledgments

Author M.C-L. was funded by a FPU grant (Reference number: FPU15/02158) from the Spanish Ministry of Education. The authors are grateful to the project AGL2013-482-45922-C2-2-R (Ministerio de Economía y Competitividad, Spain). This work has been carried out thanks to a double co-tutelle PhD between WUELS (Poland) and UMH (Spain).

References

Aloqbi, A., Omar, U., Yousr, M., Grace, M., Lila, M. A., & Howell, N. (2016). Antioxidant activity of pomegranate juice and punicalagin. *Natural Science, 8*(6), 235–246.
Andrés, V., Villanueva, M. J., & Tenorio, M. D. (2016). The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry, 192*, 328–335. https://doi.org/10.1016/j.foodchem.2015.07.031.
Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant Power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292.
Cano-Lamadrid, M., Galindo, A., Collado-González, J., Rodríguez, P., Cruz, Z. N., Legua, P., ... Hernández, F. (2018). Influence of deficit irrigation and crop load on the yield and fruit quality in Wonderful and Mollar de Elche pomegranates. *Journal of the Science of Food and Agriculture*. http://dx.doi.org/10.1002/jsfa.8810 n/a-n/a.
Cano-Lamadrid, M., Trigueros, L., Wojdyło, A., Carbonell-Barrachina, Á. A., & Sendra, E. (2017). Anthocyanins decay in pomegranate enriched fermented milks as a function of bacterial strain and processing conditions. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 80*, 193–199. https://doi.org/10.1016/j.lwt.2017.02.023.
Castillejo, N., Martínez-Hernández, G. B., Gómez, P. A., Artés, F., & Artés-Hernández, F. (2016). Red fresh vegetables smoothies with extended shelf life as an innovative source of health-promoting compounds. [journal article]. *Journal of Food Science & Technology, 53*(3), 1475–1486. http://dx.doi.org/10.1007/s13197-015-2143-2.
Di Cagno, R., Minervini, G., Rizzello, C. G., De Angelis, M., & Gobbetti, M. (2011). Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. *Food Microbiology, 28*(5), 1062–1071. https://doi.org/10.1016/j.fm.2011.02.011.
Fernandes, A., Brás, N. F., Mateus, N., & de Freitas, V. (2014). Understanding the molecular mechanism of anthocyanin binding to pectin. *Langmuir, 30*(28), 8516–8527. http://dx.doi.org/10.1021/la501879w.
Gossé, F., Guyot, S., Roussi, S., Lobstein, A., Fischer, B., Seiler, N., et al. (2005). Chemopreventive properties of apple procyanidins on human colon cancer-derived metastatic SW620 cells and in a rat model of colon carcinogenesis. *Carcinogenesis, 26*(7), 1291–1295. http://dx.doi.org/10.1093/carcin/bgi074.
Keenan, D. F., Brunton, N., Gormley, R., & Butler, F. (2011). Effects of thermal and high hydrostatic pressure processing and storage on the content of polyphenols and some quality attributes of fruit smoothies. *Journal of Agricultural and Food Chemistry, 59*(2), 601–607. http://dx.doi.org/10.1021/jf1035096.
Kennedy, J. A., & Jones, G. P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry, 49*, 1740–1746.
Lin, Z., Fischer, J., & Wicker, L. (2016). Intermolecular binding of blueberry pectin-rich fractions and anthocyanin. *Food Chemistry, 194*, 986–993. https://doi.org/10.1016/j.foodchem.2015.08.113.
Li, L., & Sun, B. (2017). Grape and wine polymeric polyphenols: Their importance in

enology. *Critical Reviews in Food Science and Nutrition,* 1–17. http://dx.doi.org/10.1080/10408398.2017.1381071.

Mena, P., Martí, N., & García-Viguera, C. (2014). Varietal blends as a way of optimizing and preserving the anthocyanin content of pomegranate (punica granatum L.) juices. *Journal of Agricultural and Food Chemistry, 62*(29), 6936–6943. http://dx.doi.org/10.1021/jf405129q.

Miller, V., Mente, A., Dehghan, M., Rangarajan, S., Zhang, X., Swaminathan, S., ... Yusuf, S. (2017). Fruit, vegetable, and legume intake, and cardiovascular disease and deaths in 18 countries (PURE): A prospective cohort study. *The Lancet, 390*(10107), 2037–2049. https://doi.org/10.1016/S0140-6736(17)32253-5.

Nowicka, P., Wojdyło, A., Teleszko, M., & Samoticha, J. (2016). Sensory attributes and changes of physicochemical properties during storage of smoothies prepared from selected fruit. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 71*, 102–109. https://doi.org/10.1016/j.lwt.2016.03.021.

Nunes, M. A., Costa, A. S. G., Barreira, J. C. M., Vinha, A. F., Alves, R. C., Rocha, A., et al. (2016). How functional foods endure throughout the shelf storage? Effects of packing materials and formulation on the quality parameters and bioactivity of smoothies. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 65*, 70–78. https://doi.org/10.1016/j.lwt.2015.07.061.

Oszmiański, J., & Wojdyło, A. (2008). Polyphenol content and antioxidative activity in apple purées with rhubarb juice supplement. *International Journal of Food Science and Technology, 43*(3), 501–509.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

Sinela, A., Rawat, N., Mertz, C., Achir, N., Fulcrand, H., & Dornier, M. (2017). Anthocyanins degradation during storage of Hibiscus sabdariffa extract and evolution of its degradation products. *Food Chemistry, 214*, 234–241. https://doi.org/10.1016/j.foodchem.2016.07.071.

Szychowski, P. J., Munera-Picazo, S., Szumny, A., Carbonell-Barrachina, Á. A., & Hernández, F. (2014). Quality parameters, bio-compounds, antioxidant activity and sensory attributes of Spanish quinces (*Cydonia oblonga Miller*). *Scientia Horticulturae, 165*, 163–170.

Tiwari, U. (2018). *Chapter 14-production of fruit-based smoothies fruit juices.* San Diego: Academic Press261–278.

Walkling-Ribeiro, M., Noci, F., Cronin, D. A., Lyng, J. G., & Morgan, D. J. (2010). Shelf life and sensory attributes of a fruit smoothie-type beverage processed with moderate heat and pulsed electric fields. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 43*(7), 1067–1073. https://doi.org/10.1016/j.lwt.2010.02.010.

Wojdyło, A., Carbonell-Barrachina, Á. A., Legua, P., & Hernández, F. (2016). Phenolic composition, ascorbic acid content, and antioxidant capacity of Spanish jujube (*Ziziphus jujube* Mill.) fruits. *Food Chemistry, 201*, 307–314. https://doi.org/10.1016/j.foodchem.2016.01.090.

Wojdyło, A., Nowicka, P., Carbonell-Barrachina, Á. A., & Hernández, F. (2016). Phenolic compounds, antioxidant and antidiabetic activity of different cultivars of *Ficus carica* L. fruits. *Journal of Functional Foods, 25*, 421–432. https://doi.org/10.1016/j.jff.2016.06.015.

Wojdyło, A., Oszmiański, J., & Bielicki, P. (2013). Polyphenolic composition, antioxidant activity, and polyphenol oxidase (PPO) activity of quince (cydonia oblonga miller) varieties. *Journal of Agricultural and Food Chemistry, 61*(11), 2762–2772. http://dx.doi.org/10.1021/jf304969b.

Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry, 51*(3), 609–614.