Research Article

Received: 18 July 2017 Revised: 23 October 2017 Accepted article published: 14 December 2017 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8836

Evaluation of microfiltration and heat treatment on the microbiological characteristics, phenolic composition and volatile compound profile of pomegranate (*Punica granatum* L.) juice

Antonio Colantuono, Paola Vitaglione, Nadia Manzo,* Giuseppe Blaiotta, Immacolata Montefusco, Andrea Marrazzo, Fabiana Pizzolongo and Raffaele Romano

Abstract

BACKGROUND: Since processing technology and storage may influence the sensory and nutritional value as well as the shelf life of pomegranate juice (PJ), mild technologies based on microfiltration may be a promising alternative to heat treatments for fruit juice preservation. In this study, physicochemical and microbiological properties of raw (RPJ), microfiltered (MPJ) and cloudy pasteurized (PPJ) PJ were compared over a period of 4 weeks.

RESULTS: Data demonstrated that microfiltration was comparable to pasteurization in guaranteeing microbiological stability of the juice, avoiding spoilage of the final product. After treatment, PPJ showed the highest amounts of gallic acid (GA) and ellagic acid derivatives (EAs). During storage, the amount of ellagitannins, EAs and GA similarly decreased in all types of juice. Trends towards variations of monomeric anthocyanins in MPJ and variations of polymeric and copigmented anthocyanins in both MPJ and PPJ were found over storage.

CONCLUSION: The optimization of pretreatments and filtration parameters can lead to the industrial scale-up of microfiltration technology for the development of high-quality non-heat-treated PJ.

Keywords: antioxidant capacity; microfiltration; volatile organic compounds; anthocyanins; total polyphenols; ellagitannins

INTRODUCTION

In recent years, the market demand for pomegranate juice (PJ) has increased at an impressive pace, and this product has gained widespread popularity as a functional food and a source of nutraceuticals.[1] The growing interest is supported by the health benefits attributed to PJ consumption and associated with the content of several bioactive compounds such as ellagitannins (ETs), gallic acid (GA), ellagic acid (EA) and its glycosylated derivatives (EAs), or formed upon intestinal metabolism of ETs and EA, such as urolithins.[2,3] Recent clinical studies have demonstrated the efficacy of PJ consumption towards oxidative stress and in the prevention of several inflammation-related diseases such as some types of cancer, type 2 diabetes, metabolic syndrome and obesity.[4–14] In Italy, owing to the good prospects of the market, pomegranate cultivation has increased rapidly in the last 5 years, growing from 50 ha in 2012 to 620 ha in 2016, mainly in Sicily (346 ha) and Apulia (187 ha), with a current total production of 4634 tons year^{-1}.[15] PJ is the major food product obtained from pomegranate arils, which constitute about 50% of the fruit weight and contain ~78% juice and 22% seeds.[16,17] Conversely, pomegranate peels are the most abundant by-products resulting from pomegranate fruit industrial processing and can be extracted in order to obtain polyphenol-rich extracts for cosmetic and nutraceutical applications or use as functional ingredients in the formulation of new foods.[18–22]

The production process of PJ includes several steps such as peduncle removal, fruit washing, separation of arils and peels, pressing, clarification and pasteurization, which is the technology commonly used to extend the shelf life of PJ. However, thermal treatments and storage may affect the aroma profile of PJ and can result in color alteration.[23–25] Several chemical reactions can take place during thermal processes, affecting the color of fruit juices. These include the degradation of bioactive compounds such as carotenoids, anthocyanins and chlorophyll as well as browning

* *Correspondence to: N Manzo, Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy. E-mail: nadia.manzo@unina.it*

Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy

reactions such as Maillard reaction, enzymatic browning and oxidation of ascorbic acid.[23] Therefore preservation of the nutritional and sensory quality of PJ throughout processing and storage is a technological challenge of the PJ industry. Mild technologies avoiding heating processes are promising alternatives to preserve the nutritional quality of food products.[24,26–28] Ultrafiltration and microfiltration are the most commonly used membrane filtration techniques for the clarification and sterilization of beverages and fruit juices. In comparison with traditional heat treatments, membrane filtration requires lower energy and can satisfy the consumer demand of additive-free fruit juices possessing sensory attributes of fresh fruit. Additionally, the use of membrane filtration techniques for fruit juice clarification has a low environmental impact compared with the use of conventional fining agents such as gelatin, bentonite, silica sol, diatomaceous earth, etc. A technological disadvantage may be the fouling effect, i.e. the retention of some food components on the membrane surface or in membrane pores. This effect may negatively impact both microfiltration performance and system maintenance, because it decreases the flow rate, increases the processing time, causes partial losses of valuable components and results in time-costly membrane cleaning.[29,30]

The main objective of this study was to assess whether microfiltration could replace pasteurization as a microbiological stabilization technique of minimally processed fresh PJ. For this purpose, physicochemical and microbiological parameters of raw (RPJ), microfiltered (MPJ) and pasteurized (PPJ) PJ were compared during 4 weeks of storage under conditions simulating commercial ones, i.e. 5 °C for RPJ and MPJ and room temperature for PPJ.

MATERIALS AND METHODS

Chemicals

Water, methanol and acetonitrile of high-performance liquid chromatography (HPLC) grade were obtained from Merck (Darmstadt, Germany). All other reagents and analytical standards used in this study were purchased from Sigma-Aldrich (St Louis, MO, USA) unless stated otherwise.

RPJ production

RPJ was obtained from pomegranate fruit cultivar 'Wonderful' harvested in Sicily (Italy). Pomegranate arils were mechanically separated from pomegranate peels and pressed in a wine press to obtain RPJ, which was filtered through a nylon mesh and subsequently frozen (−20 °C) until analysis.

Pasteurization and microfiltration of PJ

To obtain PPJ, an aliquot of RPJ was bottled in a sterilized glass container and heated to 80 °C for 30 s in a laboratory-scale pasteurization system comprising two tanks, one containing water at 80 °C and the other with water at 5 °C for the cooling step. One bottle was used as reference sample to control the time/temperature profile with a thermocouple inserted in the headspace. To obtain MPJ, another aliquot of RPJ was enzymatically treated at 13 °C for 15 h with a 2 g hL^{-1} pectinolytic preparation (Everzym GPC, Ever, Venezia, Italy) and then centrifuged at 10 000 × *g* for 5 min. The supernatant was collected and microfiltered in aseptic conditions using a sterilized filter holder (Thermo Scientific, Rockford, IL, USA) equipped with 0.45 µm mixed cellulose ester membranes. A third aliquot of RPJ was used as control. All samples were stored for 28 days, avoiding light exposure. RPJ and MPJ were kept at 5 ± 1 °C, while PPJ was stored at room temperature. The temperatures for MPJ and PPJ simulated storage on the market of fresh and thermally treated juice respectively. RPJ was stored at the same temperature as MPJ to serve as control for the effect of microfiltration on microbiological stability.

Microbiological analysis

Microbiological analysis of RPJ, PPJ and MPJ was performed using the spread-plate technique. Specifically, 1 mL of each sample was serially diluted in physiological solution (8.5 g L^{-1} NaCl, 1 g L^{-1} peptone, 0.5 g L^{-1} Tween 80, 100 g L^{-1} sucrose), then 0.1 mL of diluted sample was spread-plated in triplicate on de Man/Rogosa/Sharpe (MRS) medium (Oxoid, Basingstoke, UK) supplemented with 0.17 g L^{-1} cycloheximide (Sigma-Aldrich) for the count of lactic acid bacteria (LAB) and on Dichloran/Rose Bengal/Chloramphenicol (DRBC) agar base (Oxoid) supplemented with chloramphenicol Selective Supplement (Oxoid) for the count of yeasts and molds. Additionally, 0.25 mL of each non-diluted sample was spread-plated on four plates of each substrate. Plates were incubated for 5 days at 30 °C in anaerobiosis (Anaerogen kit, Oxoid) for LAB and at 28 °C in aerobiosis for yeasts and molds. Results were expressed as log colony-forming units (CFU) mL^{-1}.

Determination of pH, turbidity, titratable acidity, soluble solids, antioxidant activity and total polyphenols

pH was measured using a Crison Basic 20 pH meter (Crison Instruments SA, Barcelona, Spain). Turbidity was measured at room temperature with a LaMotte 2020i turbidity meter (LaMotte Co., Chestertown, MD, USA). Results were expressed in nephelometric turbidity units (NTU). Titratable acidity was determined as g L^{-1} citric acid by titrating 10 mL of sample diluted in 150 mL of distilled water with 0.1 mol L^{-1} NaOH in the presence of phenolphthalein until obtaining a persistent pink coloration. The content of soluble solids (SS) was measured using a refractometer (Sper Scientific, Scottsdale, AZ, USA). Results were reported as °Brix. Total polyphenols (TP) were determined by the Folin–Ciocalteu method.[31] Briefly, 2 mL of each sample was mixed with 8 mL of distilled water, then 100 µL of the diluted juice was mixed with 0.5 mL of Folin–Ciocalteu reagent, alkalinized with 1.5 mL of 200 g L^{-1} sodium carbonate solution and finally added with 6 mL of distilled water. The mixture was allowed to stand for 60 min at room temperature, then the absorbance at 760 nm was measured using a UV–visible spectrophotometer (Shimadzu, Kyoto, Japan). The content of TP was calculated using a calibration curve obtained with gallic acid. Results were reported as mg gallic acid equivalent (GAE) L^{-1}. The antioxidant capacity (AC) of samples was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as previously described by Papillo *et al.*[32] Briefly, a DPPH solution was prepared by diluting 0.01 mmol of DPPH in 10 mL of pure methanol and adjusting the absorbance of the DPPH radical solution at 517 nm to 0.9 ± 0.02. Then 200 µL of properly diluted sample (1:50 v/v) was added to 1 mL of DPPH solution. After 10 min, the absorbance at 517 nm was measured using a UV–visible spectrophotometer (Shimadzu). Results were reported as mmol Trolox equivalent (TE) L^{-1}.

Organic acids

The analysis of organic acids was performed according to Poyrazoğlu *et al.*[33] Each sample was centrifuged at 8000 × *g* for 10 min, then the supernatant was filtered through a 0.22 µm membrane filter and properly diluted. HPLC analysis was performed on an Agilent 1100 Series HPLC system (Palo Alto, CA,

USA) equipped with a quaternary pump (G13111A) and a diode array detector (G13114B) using a 20 µL sample injection loop. Analytical separation was achieved with a Spherisorb S5 ODS2 (4.6 mm × 250 mm, 5 µm) reverse phase column (Waters Corporation, Milford, MA, USA) using an isocratic mobile phase of acidified water (pH 2.1) at a flow rate of 0.6 mL min^{-1} for 25 min. The chromatogram was monitored at 210 nm. Data acquisition was carried out with the HP-CORE ChemStation system (Agilent Technologies, Santa Clara, CA, USA). Results were expressed as g L^{-1}.

Glucose and fructose

The analysis of carbohydrates was performed according to Cabálková *et al.*[34] Each sample was centrifuged at 8000 × *g* for 10 min, then the supernatant was filtered through a 0.45 µm membrane filter and properly diluted in water/acetonitrile (25:75 v/v). HPLC analysis was performed on an Agilent 1100 Series HPLC system equipped with quaternary pump (G13111A) and a refractive index detector (G1362A) using a 20 µL sample injection loop. Analytical separation was achieved with a Zorbax NH_2 (4.6 mm × 250 mm, 5 µm) column (Agilent Technologies, Waldbronn, Germany) using an isocratic mobile phase of acetonitrile/water (75:25 v/v) at a flow rate of 1.8 mL min^{-1} for 25 min. Data acquisition was carried out with the HP-CORE ChemStation system. Results were expressed as g L^{-1}.

Hydrolysable tannins

ETs (α and β anomers of punicalin, punicalagin and pedunculagin), GA, EA and EAs (EA hexoside, EA pentoside, EA deoxyhexoside) were identified by liquid chromatography/tandem mass spectrometry (LC/MS/MS) and quantified by HPLC with diode array detection (HPLC-DAD) according to Colantuono *et al.*[20] Each sample was properly diluted in water/methanol (50:50 v/v) acidified with 5 mL L^{-1} formic acid. The HPLC system consisted of two binary pumps (LC-10 AD, Shimadzu) and a photodiode array detector (SPD-M10A, Shimadzu). A Gemini C-18 (4.6 mm × 250 mm, 5 µm) reverse phase column (Phenomenex, Torrance, CA, USA) was used. The flow rate was 0.8 mL min^{-1} and the mobile phases were water/20 mL L^{-1} formic acid (phase A) and methanol (phase B). The following gradient was applied: 0 min, 0% B; 20 min, 10% B; 30 min, 30% B; 35 min, 40% B; 40 min, 70% B; 45–48 min, 98% B; 50–52 min, 0% B. The injection volume was 20 µL. ETs were detected and quantified at 378 nm. For EAs and GA, the wavelengths used were 366 and 280 nm respectively. Punicalagin, EA and GA were identified and quantified with the corresponding standards. Punicalin anomers and pedunculagin anomers were quantified as punicalagin equivalents. EAs, i.e. EA hexoside, EA pentoside and EA deoxyhexoside, were quantified as EA equivalents. ETs were reported as the sum of α and β anomers of punicalin, punicalagin and pedunculagin. EAs were reported as the sum of EA, EA hexoside, EA pentoside and EA deoxyhexoside.

Volatile organic compounds by gas chromatography/mass spectrometry

Volatile organic compounds (VOCs) were extracted according to Mayuoni-Kirshinbaum *et al.*[35] Briefly, 5 mL samples were mixed with 5 mL of 300 g L^{-1} NaCl solution to inhibit enzymatic degradation. Then 2 mL of the mixed solution was withdrawn and placed in a 10 mL glass vial, and 5 µL of 1-pentanol was added as internal standard. VOCs were identified by gas chromatography/mass spectrometry (GC/MS) analysis. Prior to analysis, samples were equilibrated for 5 min at 40 °C in a water bath and then incubated for 25 min at 40 °C while being gently stirred with a rod. VOCs were extracted from the vial headspace by solid phase microextraction (SPME). An SPME holder (Supelco, Bellafonte, CA, USA) containing a fused silica fiber coated with a 50/30 µm layer of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was used to trap VOCs in the vial headspace. The fiber was introduced into the splitless inlet of an Agilent 6890 N GC system (Agilent Technologies, Palo Alto, CA, USA), and thermal desorption of the analytes was performed at 250 °C for 5 min. The GC system was equipped with an HP-5MS (30 m × 0.25 mm, 0.25 µm 5% diphenyl/95% dimethylpolysiloxane) capillary column (J&W Scientific, Folsom, CA, USA). The oven was programmed to start at 50 °C (held for 1 min) and to ramp up to 160 °C at 5 °C min^{-1}, then to ramp up to 260 °C at 20 °C min^{-1} (held for 4 min). Helium (99.999%) was used as carrier gas and the flow rate was 1 mL min^{-1}. The flow was transferred from the column into an Agilent 5973 MS detector (Agilent Technologies). The ion source temperature was set at 230 °C, the ionizing electron energy was 70 eV and the mass range was 40–450 amu in full scan acquisition mode.[36] Compounds were identified using the NIST Atomic Spectra Database Version 1.6 and verified by their retention indices. Relative amounts were calculated on the basis of peak area ratios.

Determination of monomeric, polymeric and copigmented anthocyanins

Monomeric, polymeric and copigmented anthocyanins were determined according to Mazza *et al.*[37] with slight modifications. First, samples were filtered through a 0.45 µm PVDF filter. Subsequently, 40 µL of 100 mL L^{-1} acetaldehyde solution was added to 4 mL of properly diluted sample and incubated at room temperature for 45 min. The absorbance (A^{acet}) at 520 nm was measured using a UV–visible spectrophotometer (UV-1601, Shimadzu) and the final value was multiplied by 5. Additionally, 4 mL of sample was mixed with 320 µL of 50 mL L^{-1} SO_2 solution. The absorbance (A^{SO2}) at 520 nm was measured and the final value was multiplied for 5. Finally, 3.8 mL of bitartrate buffer solution in 120 mL L^{-1} ethanol (pH 3.6) was mixed with 100 µL of sample. The absorbance (A^{20}) at 520 nm was measured and the final value was multiplied by 20. The different forms of anthocyanins were expressed in absorbance units as

$$\text{copigmented anthocyanins (CPA)} = A^{acet} - A^{20}$$

$$\text{monomeric anthocyanins (MA)} = A^{20} - A^{SO_2}$$

$$\text{polymeric anthocyanins (PA)} = A^{SO_2}$$

$$\text{total anthocyanins} = A^{acet}$$

CPA, MA and PA as a proportion of total anthocyanins were calculated as

$$\text{CPA}\ (\%) = \left[\left(A^{acet} - A^{20}\right)/A^{acet}\right] \times 100$$

$$\text{MA}\ (\%) = \left[\left(A^{20} - A^{SO_2}\right)/A^{acet}\right] \times 100$$

$$\text{PA}\ (\%) = \left(A^{SO_2}/A^{acet}\right) \times 100$$

Table 1. Effect of pasteurization and microfiltration processes on microbial stability of pomegranate juice

	Raw pomegranate juice (RPJ)		Pasteurized pomegranate juice (PPJ)		Microfiltered pomegranate juice (MPJ)	
Time (days)	Yeasts and molds (log CFU mL^{-1})	LAB (log CFU mL^{-1})	Yeasts and molds (log CFU mL^{-1})	LAB (log CFU mL^{-1})	Yeasts and molds (log CFU mL^{-1})	LAB (log CFU mL^{-1})
0	2.63 ± 0.44d	2.29 ± 0.13e	ND	ND	ND	ND
7	5.04 ± 0.41c	4.37 ± 0.20d	ND	ND	ND	ND
14	5.83 ± 0.16b	5.07 ± 0.02 cd	ND	ND	ND	ND
21	6.29 ± 0.07ab	7.25 ± 0.21a	ND	1.48 ± 0.00f	ND	ND
28	6.60 ± 0.30a	6.45 ± 0.01b	ND	1.74 ± 0.06ef	ND	ND

Mean values followed by different letters within the same column are significantly different ($P \leq 0.05$) according to Tukey's test. ND, not detected (value below detectable threshold of 1 CFU mL^{-1}).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and means were compared by Tukey's test ($\alpha = 0.05$) using XLSTAT software (Addinsoft, New York, NY, USA). All experiments were performed in triplicate.

RESULTS

Microbial analysis

The initial microflora of RPJ (day 0) was represented by both LAB and yeasts/molds at levels of 2.29 and 2.63 log CFU mL^{-1} respectively (Table 1). Both pasteurization and microfiltration of PJ reduced all monitored microbial populations to below the detectable threshold (<1 CFU mL^{-1}). After 28 days of refrigerated storage, yeasts and molds in RPJ increased to a maximum of 6.60 log CFU mL^{-1}, while LAB showed their maximum level of 7.25 log CFU mL^{-1} after 21 days of storage.

After 21 days of storage at room temperature, LAB in PPJ become detectable at a level of 1.48 log CFU mL^{-1}, and only a slight increase to 1.74 log CFU mL^{-1} was observed 7 days later. The microbial population of yeasts and molds in PPJ was always below the detectable threshold over 28 days of storage. Finally, in MPJ, the monitored microbial groups always remained below the detectable threshold (<1 CFU mL^{-1}) of the method.

Physicochemical properties, total polyphenols and antioxidant capacity of RPJ, MPJ and PPJ

As shown in Table 2, the pH, amount of SS and titratable acidity of RPJ, PPJ and MPJ were not affected by storage. The turbidity of PJ was strongly influenced by the microfiltration process, being significantly lower in MPJ compared with RPJ and PPJ (Table 2). The amount of TP increased in PPJ just after the pasteurization process (Table 2). During the storage period, the concentration of TP decreased significantly in all types of juice, being 55.3, 54.4 and 40.3% lower in MPJ, RPJ and PPJ respectively after 28 days. The AC of MPJ was slightly lower than that of RPJ (−4.6%) and PPJ (−7.1%). Conversely, the AC of PPJ was similar to that of RPJ immediately after the pasteurization process. Finally, the AC of

Table 2. Physicochemical properties, total polyphenols and antioxidant capacity of raw (RPJ), pasteurized (PPJ) and microfiltered (MPJ) pomegranate juice

Time (days)	pH	Soluble solids (°Brix)	Titratable acidity (g citric acid L^{-1})	Turbidity (NTU)	Total polyphenols (mg GAE L^{-1})	Antioxidant capacity (mmol Trolox L^{-1})
RPJ						
0	3.31 ± 0.01cde	14.8 ± 0.1a	11.52 ± 0.32a	2778.0 ± 10.0a	646.88 ± 18.82c	3.73 ± 0.06ab
7	3.30 ± 0.01de	14.8 ± 0.2a	11.31 ± 1.07a	2820.0 ± 6.4a	654.69 ± 13.33c	3.48 ± 0.03bc
14	3.26 ± 0.01f	14.6 ± 0.7a	11.25 ± 0.79a	2820.0 ± 5.3a	618.75 ± 4.44 cd	3.66 ± 0.09bc
21	3.32 ± 0.01bcd	14.6 ± 0.2a	11.45 ± 0.51a	2820.0 ± 5.0a	435.79 ± 2.26f	3.53 ± 0.08c
28	3.31 ± 0.01cde	14.6 ± 0.4a	11.59 ± 0.58a	2820.0 ± 8.2a	295.00 ± 4.43 h	3.56 ± 0.05c
PPJ						
0	3.36 ± 0.01ab	14.8 ± 0.2a	10.92 ± 0.33ab	2450.0 ± 15.1b	839.84 ± 6.67a	3.83 ± 0.04a
7	3.30 ± 0.01de	14.8 ± 0.7a	11.32 ± 0.53a	2460.0 ± 8.0b	738.28 ± 11.15b	3.74 ± 0.10a
14	3.30 ± 0.01de	14.6 ± 0.9a	11.20 ± 0.57a	2450.0 ± 10.5b	752.03 ± 6.64b	3.87 ± 0.10a
21	3.33 ± 0.01bcd	14.6 ± 0.5a	11.31 ± 0.50a	2440.0 ± 11.2b	605.47 ± 16.63 cd	3.76 ± 0.07ab
28	3.33 ± 0.01bcd	14.6 ± 0.3a	11.80 ± 0.56a	2450.0 ± 9.7b	501.57 ± 4.45e	3.77 ± 0.05ab
MPJ						
0	3.36 ± 0.01ab	13.6 ± 0.6a	10.43 ± 0.48a	9.9 ± 0.5c	666.56 ± 8.81c	3.56 ± 0.04c
7	3.33 ± 0.01bcd	13.6 ± 0.8a	9.84 ± 0.69a	10.1 ± 0.2c	589.84 ± 6.62 cd	3.20 ± 0.03d
14	3.28 ± 0.01ef	13.6 ± 0.9a	9.77 ± 1.53a	10.2 ± 0.2c	576.88 ± 4.47d	3.11 ± 0.06d
21	3.34 ± 0.01abc	13.6 ± 0.5a	9.92 ± 0.81a	10.2 ± 0.7c	386.41 ± 2.29 g	3.13 ± 0.09d
28	3.36 ± 0.01ab	13.6 ± 0.4a	10.06 ± 0.79a	10.3 ± 0.3c	298.13 ± 4.53 h	3.14 ± 0.04d

Mean values followed by different letters within the same column are significantly different ($P \leq 0.05$) according to Tukey's test.

Table 3. Organic acids and sugars detected in raw (RPJ), pasteurized (PPJ) and microfiltered (MPJ) pomegranate juice

	Organic acids (g L^{-1})				Sugars (g L^{-1})	
Time (days)	Citric acid	Malic acid	Tartaric acid	Quinic acid	Glucose	Fructose
RPJ						
0	12.40 ± 0.11ab	0.48 ± 0.02a	2.09 ± 0.08a	0.93 ± 0.06a	43.25 ± 0.80a	55.38 ± 5.80a
14	12.38 ± 0.09ab	0.45 ± 0.02a	2.10 ± 0.01a	0.88 ± 0.03ab	33.66 ± 0.36cde	49.91 ± 0.01abc
28	12.35 ± 0.18ab	0.48 ± 0.03a	2.02 ± 0.02ab	0.79 ± 0.01ab	39.34 ± 0.28abc	55.31 ± 1.91a
PPJ						
0	11.98 ± 0.06b	0.47 ± 0.05a	2.00 ± 0.11ab	0.84 ± 0.13ab	31.86 ± 0.66def	48.55 ± 1.70abc
14	12.34 ± 0.01ab	0.49 ± 0.01a	2.04 ± 0.01ab	0.76 ± 0.02bc	26.03 ± 0.66f	45.36 ± 3.52bc
28	12.61 ± 0.26a	0.46 ± 0.01a	2.01 ± 0.04ab	0.70 ± 0.01bc	42.54 ± 0.45ab	58.20 ± 0.46a
MPJ						
0	11.31 ± 0.12c	0.33 ± 0.01b	1.66 ± 0.01c	0.58 ± 0.02c	16.65 ± 0.96 g	24.28 ± 0.16d
14	10.95 ± 0.24c	0.46 ± 0.04a	1.93 ± 0.21abc	0.80 ± 0.01ab	28.32 ± 1.22ef	41.76 ± 0.90c
28	10.85 ± 0.04c	0.42 ± 0.03ab	1.71 ± 0.03bc	0.69 ± 0.02bc	35.72 ± 1.29bcd	51.44 ± 0.12ab

Mean values followed by different letters within the same column are significantly different ($P \leq 0.05$) according to Tukey's test.

RPJ and MPJ decreased significantly after 4 weeks of refrigerated storage.

Organic acids and sugars

Citric acid was the major organic acid detected in RPJ, PPJ and MPJ (12.40, 11.98 and 11.31 g L^{-1} respectively), followed by tartaric acid (Table 3). Malic acid and quinic acid were present only in small amounts. The main carbohydrates detected in RPJ were glucose and fructose (43.25 and 55.38 g L^{-1} respectively). Microfiltration led to a decrease in both organic acids and sugars, with citric acid, malic acid, tartaric acid, quinic acid, glucose and fructose in MPJ decreasing by 8.8, 31.2, 20.6, 37.6, 61.5 and 56.2% respectively ($P \leq 0.05$). Pasteurization caused a 26.3% decrease in glucose concentration ($P \leq 0.05$). Over 28 days of storage, the concentrations of glucose and fructose increased in MPJ and PPJ, while they decreased slightly in RPJ; no changes in organic acid content were observed.

Hydrolysable tannins

Immediately after production, PPJ showed a significantly higher amount of GA (13.9 ± 2.1 mg L^{-1}) than RPJ (10.7 ± 0.2 mg L^{-1}) and MPJ (10.1 ± 0.2 mg L^{-1}) as well as a higher amount of EAs (4.6 ± 0.2 mg L^{-1}) than MPJ (3.8 ± 0.01 mg L^{-1}) (Fig. 1). The concentrations of ETs did not differ significantly among juices. After 28 days of storage, the amounts of ETs, EAs and GA decreased significantly in all types of juice.

Volatile compound profile

A total of 13 VOCs were identified and quantified using the headspace SPME technique. The results are reported in Table 4. Ethyl acetate was the main compound in the three types of PJ. After the microfiltration process, the concentrations of β-pinene, limonene and α-bergamotene were below the limit of detection in MPJ, and the concentration of 1-hexanol was significantly lower in MPJ than in RPJ. After the pasteurization process, the concentration of limonene was significantly lower in PPJ than in RPJ and the concentration of hexanal was significantly higher in PPJ than in MPJ. After 14 days of refrigerated storage, the concentrations of limonene and hexanal in RPJ showed a significant decrease compared with the starting concentrations. After 28 days of storage, the concentrations of ethanol, ethyl ether, acetic acid, ethyl acetate and 3-methyl-butan-1-ol increased significantly in RPJ compared wvith MPJ and PPJ.

Monomeric, polymeric and copigmented anthocyanins

MA was the anthocyanin fraction present at the highest concentration in RPJ, MPJ and PPJ, being 61.8, 80.4 and 68.6% respectively at time 0 (Fig. 2). At time 0, MPJ showed the highest level of MA (80.4%) and the lowest levels of CPA and PA compared with RPJ and PPJ. Over storage, the amount of MA in MPJ decreased slightly to 67.7%, whereas the concentrations of PA and CPA increased from 6.8 and 12.8% to 15.7 and 16.6% respectively. During storage of PPJ, the MA fraction was stable while CPA decreased (from 22.6 to 18.5%) and PA increased slightly. In RPJ, the concentration of MA decreased from 61.8 to 21.7% while CPA increased to 68.3%. No significant differences in MA, CPA and PA between MPJ and PPJ over storage were found.

DISCUSSION

In this study, for the first time, the quality of a fresh MPJ was compared with that of a PPJ by measuring the physicochemical, microbiological and volatile profile characteristics over 28 days of storage.

Data showed that microfiltration was effective, just like traditional pasteurization, to guarantee the microbiological stability of PJ by removing potentially spoiling microorganisms (yeasts/molds and LAB). The results obtained for MPJ were in line with those reported by Carneiro *et al.*[38] and Laorko *et al.*[39] for microfiltered pineapple juice. To the best of our knowledge, no previous study in the literature has evaluated the microbiological stability of a microfiltered and non-thermally treated PJ over 4 weeks of storage. Data achieved for PPJ were in accordance with Mena *et al.*,[25,40] who applied a pasteurization process at 80 °C for 30 s and at 95 °C for 30 s. However, our data showed a negligible increase in LAB in PPJ after 21 days of storage at room temperature, thus indicating that pasteurization treatment did not eliminate LAB. These results are line with data reported by Vegara *et al.*,[41] who detected live microorganisms in cloudy PPJ heated at 90 °C for 5 s and stored a 5 °C. A preliminary microfiltration before the

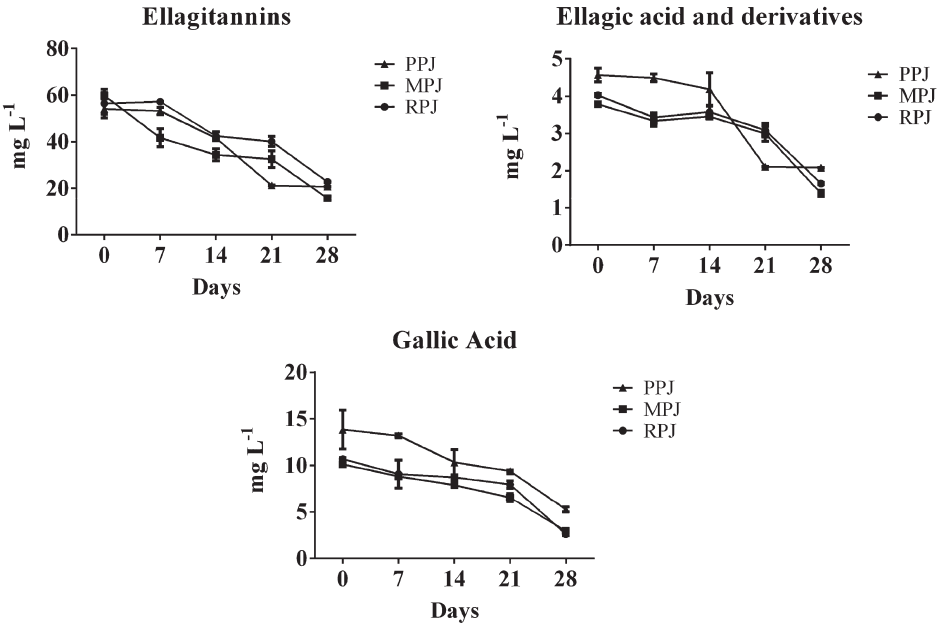

Figure 1. Ellagitannins (mg punicalagin equivalent L^{-1}), ellagic acid derivatives (mg ellagic acid equivalent L^{-1}) and gallic acid (mg L^{-1}) in raw (RPJ), pasteurized (PPJ) and microfiltered (MPJ) pomegranate juice during 28 days of storage.

Table 4. Volatile organic compounds detected in raw (RPJ), pasteurized (PPJ) and microfiltered (MPJ) pomegranate juice

Volatile compound (μg L^{-1})	Time 0 days			Time 14 days			Time 28 days		
	RPJ	PPJ	MPJ	RPJ	PPJ	MPJ	RPJ	PPJ	MPJ
Ethanol	108.9 ± 5.3b	115.5 ± 10.5b	93.0 ± 13.2b	122.3 ± 13.8b	102.6 ± 5.3b	100.1 ± 3.3b	862.5 ± 59.4a	115.3 ± 12.1b	120.3 ± 13.0b
Ethyl ether	37.1 ± 4.2bc	37.4 ± 13.8bc	33.0 ± 16.1bc	54.1 ± 6.4ab	33.5 ± 10.2bc	21.2 ± 8.8bc	63.5 ± 8.8a	30.6 ± 0.2bc	11.8 ± 3.5c
Acetic acid	ND	ND	ND	ND	ND	ND	17.59 ± 0.1a	ND	ND
Ethyl acetate	172.3 ± 14.7b	179.1 ± 28.4b	140.5 ± 38.8b	198.3 ± 9.9b	182.0 ± 1.8b	163.4 ± 14.4b	768.4 ± 21.6a	181.3 ± 24.4b	113.6 ± 15.9b
3-Methyl-butan-1-ol	ND	ND	ND	11.8 ± 0.7b	ND	ND	241.8 ± 24.6a	ND	ND
Hexanal	4.6 ± 0.8bc	5.6 ± 0.1ab	3.5 ± 0.1 cd	2.8 ± 0.3d	6.0 ± 0.3a	1.2 ± 0.3e	ND	ND	ND
3-Hexenol	5.4 ± 0.6abc	5.1 ± 0.7abc	4.2 ± 0.1bc	5.8 ± 0.3ab	4.7 ± 0.2abc	5.0 ± 0.1abc	6.2 ± 0.6a	5.1 ± 0.3abc	3.7 ± 0.5bc
1-Hexanol	26.7 ± 2.6b	24.9 ± 1.2bc	20.0 ± 1.4 cd	23.8 ± 1.1bcd	22.9 ± 2.3bcd	22.1 ± 0.7bcd	36.0 ± 0.0a	26.0 ± 0.6b	19.1 ± 0.8d
β-Pinene	2.8 ± 0.6a	ND	ND	2.6 ± 1.0a	ND	ND	ND	ND	ND
Limonene	7.0 ± 0.6a	4.0 ± 0.5bc	ND	4.7 ± 0.1b	3.1 ± 0.1c	ND	6.3 ± 0.0a	3.3 ± 0.3c	ND
4-Terpineol	9.5 ± 1.5ab	6.8 ± 0.9b	6.6 ± 0.3b	8.8 ± 1.6ab	7.9 ± 1.0b	7.9 ± 0.7b	11.7 ± 1.0a	7.4 ± 0.3b	6.5 ± 0.4b
α-Terpineol	6.9 ± 1.3ab	6.4 ± 0.3ab	5.5 ± 0.8b	6.8 ± 1.3ab	6.9 ± 1.2ab	6.6 ± 1.5ab	9.8 ± 1.0a	6.7 ± 0.4ab	4.4 ± 0.6b
α-Bergamotene	7.8 ± 3.4a	5.8 ± 1.4a	ND	4.2 ± 0.4ab	5.9 ± 2.0a	ND	8.1 ± 1.0a	6.9 ± 0.9a	ND

Mean values followed by different letters within the same row are significantly different ($P \leq 0.05$) according to Tukey's test. ND, not detected.

pasteurization process could contribute to keeping the microbial count unchanged over storage.

Concerning the physicochemical properties (pH, titratable acidity, turbidity and SS content) of RPJ, the results were in accordance with Poyrazoğlu *et al.*[33] and Rinaldi *et al.*[42] According to Mirsaeedghazi *et al.*,[43] microfiltration reduced the turbidity of the final product about 280-fold compared with the raw juice. The turbidity of fruit juices is mainly associated with the presence of pectins. A reduction of fruit juice turbidity results in improved clarity and color of the juice. As expected, the reduction of turbidity in MPJ is due to the enzymatic pretreatment, centrifugation and microfiltration of PJ. Pectinase hydrolyses pectins, thus decreasing the turbidity of PJ and speeding up the filtration process.[42,44] Moreover, pectolytic enzyme solutions can increase the amount of SS, proteins and polyphenols in enzyme-treated PJ compared with control PJ.[42] In accordance with previous evidence, data indicated that microfiltration produced lower AC, organic acids and sugars compared with the raw juice.[43,45,46] It is likely that the partial loss of some soluble compounds in MPJ was caused by blocking mechanisms that can take place during PJ membrane filtration. Cake deposition was demonstrated to be the main cause of membrane fouling during ultrafiltration and microfiltration of PJ.[47] Fouling is a serious drawback related to membrane applications in food processing and bioprocessing. To overcome the shortcomings of microfiltration membranes, different solutions have been proposed. The retention of substances causing fouling can be limited by using vibrating systems, by selecting the most adequate membrane material and by adopting efficient cleaning programs.[48] Moreover, pretreatment of the fluid prior to the microfiltration step could improve the permeation efficiency, for

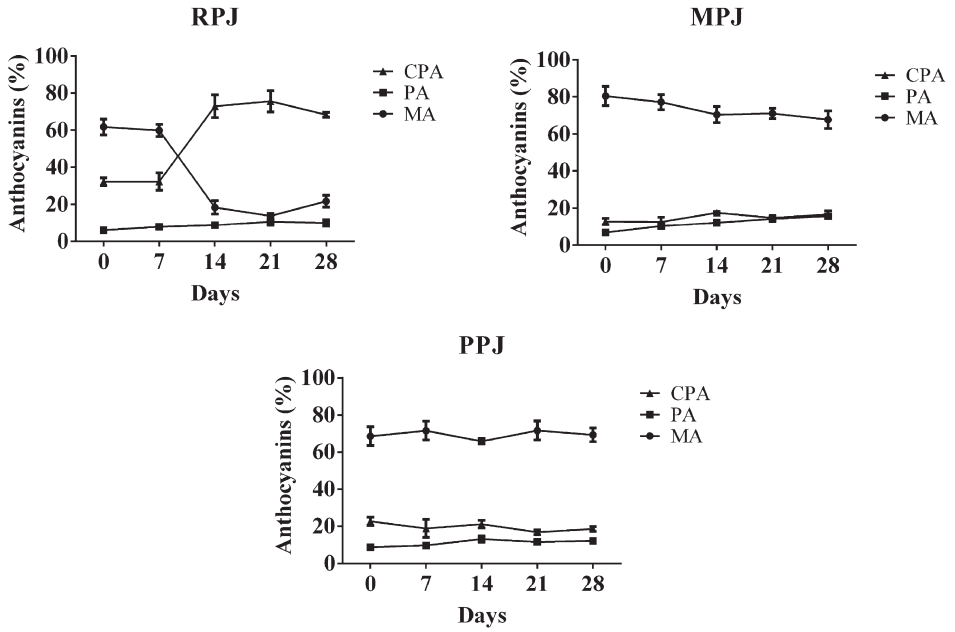

Figure 2. Monomeric (MA), polymeric (PA) and copigmented (CPA) anthocyanins (% of total anthocyanins) in raw (RPJ), pasteurized (PPJ) and microfiltered (MPJ) pomegranate juice during 28 days of storage.

example by application of ultrasound or enzymatic pretreatment with a mixture of pectinase and protease in order to degrade pectins and proteins.[30,49,50] Additionally, pre-centrifugation of fruit juices can improve the efficiency of filtering systems.[51]

The decrease in glucose and fructose concentrations in cloudy PPJ can be associated with the chemical reactions taking place in acid foods rich in reducing sugars during thermal processes such as Maillard reaction and caramelization. These thermally induced reactions can lead to the formation of potentially toxic undesirable compounds (e.g. hydroxymethylfurfural and furan) as well as induce non-enzymatic browning of the juice.[52–54]

Based on this evidence, the development of non-thermal strategies useful to sanitize and stabilize PJ is a challenge for the juice industry, and further optimization of microfiltration conditions is warranted that can lead to the reduction of fouling effects and ameliorate the nutrient composition of minimally treated MPJ.

MPJ and RPJ showed the same amount of TP, while PPJ showed a higher amount of TP probably due to the breakdown of polymeric polyphenols.[25,55] During storage, the major decrease in TP was in RPJ and MPJ. This result can be associated with the activity of polyphenol oxidases (PPO) that are still present in the juice after microfiltration.[56] The content of TP in PPJ was coherent with a partial inactivation of PPO induced by heating.[57]

Useful strategies to limit PPO activity in MPJ could be the use of proteolytic enzymes (e.g. papain, bromelain and ficin) in association with pectolytic enzymes before microfiltration, or the addition of PPO inhibitors to the juice.[58]

In line with the TP concentration decline, data showed that the concentrations of ETs, EAs and GA in RPJ, MPJ and PPJ decreased over storage. Just after pasteurization, the concentrations of GA and EA increased significantly in PPJ compared with MPJ. This finding indicated that heating PJ caused a partial breakdown of complex ETs with release of EAs and GA.[59,60] Moreover, the degradation of ETs during storage could also cause the partial release of reducing sugars from the complex ETs.[61,62]

Flavor and color are two important attributes of foods and beverages, being key quality parameters influencing consumer sensory acceptance. Thirteen key aroma compounds were identified in all types of PJ. Data showed that ethyl acetate and ethanol were the most abundant VOCs in RPJ. These data may be explained by considering the postharvest storage conditions, i.e. a partial fermentative process of pomegranate fruits squeezed for the juice used in this study, according to Mayuoni-Kirshinbaum and co-workers.[35,63] β-Pinene detected in RPJ was not identified in either PPJ or MPJ, whereas α-bergamotene was detected only in PPJ, thus indicating retention of this compound during the microfiltration process.[64] Limonene was significantly reduced in PJ after pasteurization. The decline of hexanal concentration in all types of juice during storage was in line with the evidence of a reduced intensity of green aroma in stored PJ.[65] On the other hand, the increased concentrations of VOCs derived from microbial fermentation (e.g. ethanol, acetic acid, ethyl acetate, ethyl ether and 3-methyl-butan-1-ol) in RPJ (but not in PPJ and MPJ) mirrored the ongoing fermentative processes.[66,67]

The brilliant red color of PJ is due to the occurrence of cyanidin and delphinidin glycosides, and several extrinsic and intrinsic factors such as concentrations of organic acids and sugars, oxygen and light exposure, pH value as well as heat treatments may influence their chemical stability.[53,68] The color deterioration of fruit juices containing anthocyanins occurs during heating and storage as a result of the degradation of MA and their polymerization with formation of brown pigments.[69] Similarly to ETs breakdown, the degradation of MA can also result in the release of glycosidic moieties.[69] Conversely, copigmentation mechanisms lead to the formation of intramolecular and intermolecular complexes that contribute to enhancing the chemical stability and color intensity of anthocyanins during storage. Intramolecular copigmentation is defined as the covalent interaction between copigment and anthocyanin chromophore. On the other hand, intermolecular copigmentation can result from weak interactions (van der Waals forces, hydrophobic and ionic interactions) between colored anthocyanins and colorless flavonoids or other phenolic compounds.[68–70] Just after the microfiltration process, MPJ showed the highest level of MA and the lowest levels of CPA

and PA compared with PPJ and RPJ, probably because CPA were partially removed during microfiltration. After 14 days of storage, the CPA fraction in RPJ doubled (from 32.2 to 68.3%) and the MA fraction decreased threefold (from 61.8 to 21.7%) owing to the fermentative process causing degradation and changes in the chemical structure of MA and their involvement in the copigmentation mechanism.[70–73] MPJ showed a slight decrease in MA (from 80.4 to 67.7%) accompanied by an increase in PA, indicating that MA polymerized during storage. It can be assumed that MA were enzymatically degraded by the residual activity of PPO.[69]

CONCLUSION

In this study, physicochemical and microbiological parameters determining the overall quality of a PJ produced by microfiltration and pasteurization were compared over 4 weeks of storage. Data demonstrated that microfiltration was effective to guarantee the microbiological stability of the juice, avoiding microbial growth and spoilage of the final product. The optimization of enzyme pretreatments, membrane filtration properties and operating conditions could reduce the impact of fouling effects and lead to the design of an industrial scale-up for production of high-quality minimally processed PJ.

REFERENCES

1 Johanningsmeier SD and Harris GK, Pomegranate as a functional food and nutraceutical source. *Annu Rev Food Sci Technol* **2**:181–201 (2011).
2 Tomás-Barberán FA, García-Villalba R, González-Sarrías A, Selma MV and Espín JC, Ellagic acid metabolism by human gut microbiota: consistent observation of three urolithin phenotypes in intervention trials, independent of food source, age, and health status. *J Agric Food Chem* **62**:6535–6538 (2014).
3 Selma MV, Romo-Vaquero M, García-Villalba R, González-Sarrías A, Tomás-Barberán FA and Espín JC, The human gut microbial ecology associated with overweight and obesity determines ellagic acid metabolism. *Food Funct* **7**:1769–1774 (2016).
4 Fuster-Muñoz E, Roche E, Funes L, Martínez-Peinado P, Sempere JM and Vicente-Salar N, Effects of pomegranate juice in circulating parameters, cytokines, and oxidative stress markers in endurance-based athletes: a randomized controlled trial. *Nutrition* **32**:539–545 (2016).
5 Díaz-Rubio ME, Pérez-Jiménez J, Martínez-Bartolomé MÁ, Álvarez I and Saura-Calixto F, Regular consumption of an antioxidant-rich juice improves oxidative status and causes metabolome changes in healthy adults. *Plant Foods Hum Nutr* **70**:9–14 (2015).
6 Shema-Didi L, Sela S, Ore L, Shapiro G, Geron R, Moshe G *et al.*, One year of pomegranate juice intake decreases oxidative stress, inflammation, and incidence of infections in hemodialysis patients: a randomized placebo-controlled trial. *Free Radic Biol Med* **53**:297–304 (2012).
7 González-Sarrías A, Giménez-Bastida JA, García-Conesa MT, Gómez-Sánchez MB, García-Talavera NV, Gil-Izquierdo A *et al.*, Occurrence of urolithins, gut microbiota ellagic acid metabolites and proliferation markers expression response in the human prostate gland upon consumption of walnuts and pomegranate juice. *Mol Nutr Food Res* **54**:311–322 (2010).
8 Kapoor R, Ronnenberg A, Puleo E, Chatterton Jr RT, Dorgan JF, Seeram NP *et al.*, Effects of pomegranate juice on hormonal biomarkers of breast cancer risk. *Nutr Cancer* **67**:1113–1119 (2015).
9 Banihani SA, Makahleh SM, El-Akawi Z, Al-Fashtaki RA, Khabour OF, Gharibeh MY *et al.*, Fresh pomegranate juice ameliorates insulin resistance, enhances β-cell function, and decreases fasting serum glucose in type 2 diabetic patients. *Nutr Res* **34**:862–867 (2014).
10 Kojadinovic MI, Arsic AC, Debeljak-Martacic JD, Konic-Ristic AI, Kardum ND, Popovic TB *et al.*, Consumption of pomegranate juice decreases blood lipid peroxidation and levels of arachidonic acid in women with metabolic syndrome. *J Sci Food Agric* **97**:1798–1804 (2017).
11 Sohrab G, Ebrahimof S, Sotoudeh G, Neyestani TR, Angoorani P, Hedayati M *et al.*, Effects of pomegranate juice consumption on oxidative stress in patients with type 2 diabetes: a single-blind, randomized clinical trial. *Int J Food Sci Nutr* **68**:249–255 (2017).
12 Ekhlasi G, Shidfar F, Agah S, Merat S and Hosseini AF, Effects of pomegranate and orange juice on antioxidant status in non-alcoholic fatty liver disease patients: a randomized clinical trial. *Int J Vitam Nutr Res* **14**:1–7 (2016).
13 Al-Muammar MN and Khan F, Obesity: the preventive role of the pomegranate (*Punica granatum*). *Nutrition* **28**:595–604 (2012).
14 Moazzen H and Alizadeh M, Effects of pomegranate juice on cardiovascular risk factors in patients with metabolic syndrome: a double-blinded, randomized crossover controlled trial. *Plant Foods Hum Nutr* **72**:126–133 (2017).
15 ISTAT, Article [Online]. (2016). Available: http://agri.istat.it/sag_is_pdwout/jsp/dawinci.jsp?q=plC20000010000012000&an=2016&ig=1&ct=271&id=15A|21A|30A. [2 May 2017].
16 Akhtar S, Ismail T, Fraternale D and Sestili P, Pomegranate peel and peel extracts: chemistry and food features. *Food Chem* **174**:417–425 (2015).
17 Kulkarni AP and Aradhya SM, Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chem* **93**:319–324 (2005).
18 Iqbal S, Haleem S, Akhtar M, Zia-ul-Haq M and Akbar J, Efficiency of pomegranate peel extracts in stabilization of sunflower oil under accelerated conditions. *Food Res Int* **41**:194–200 (2008).
19 Kanatt SR, Chander R and Sharma A, Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. *Int J Food Sci Technol* **45**:216–222 (2010).
20 Colantuono A, Ferracane R and Vitaglione P, *In vitro* bioaccessibility and functional properties of polyphenols from pomegranate peels and pomegranate peels-enriched cookies. *Food Funct* **7**:4247–4258 (2016).
21 Altunkaya A, Hedegaard RV, Brimer L, Gökmen V and Skibsted LH, Antioxidant capacity *versus* chemical safety of wheat bread enriched with pomegranate peel powder. *Food Funct* **4**:722–727 (2013).
22 Colantuono A, Vitaglione P, Ferracane R, Campanella OH and Hamaker BR, Development and functional characterization of new antioxidant dietary fibers from pomegranate, olive and artichoke by-products. *Food Res Int* **101**:155–164 (2017).
23 Maskan M, Production of pomegranate (*Punica granatum* L.) juice concentrate by various heating methods: colour degradation and kinetics. *J Food Eng* **72**:218–224 (2006).
24 Ferrari G, Maresca P and Ciccarone R, The application of high hydrostatic pressure for the stabilization of functional foods: pomegranate juice. *J Food Eng* **100**:245–253 (2010).
25 Mena P, Martí N, Saura D, Valero M and García-Viguera C, Combinatory effect of thermal treatment and blending on the quality of pomegranate juices. *Food Bioprocess Technol* **6**:3186–3199 (2013).
26 Guo M, Jin TZ, Geveke DJ, Fan X, Sites JE and Wang L, Evaluation of microbial stability, bioactive compounds, physicochemical properties, and consumer acceptance of pomegranate juice processed in a commercial scale pulsed electric field system. *Food Bioprocess Technol* **7**:2112–2120 (2014).
27 Varela-Santos E, Ochoa-Martinez A, Tabilo-Munizaga G, Reyes JE, Pérez-Won M, Briones-Labarca V *et al.*, Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovat Food Sci Emerg Technol* **13**:13–22 (2012).
28 Kovačević DB, Putnik P, Dragović-Uzelac V, Pedisić S, Jambrak AR and Herceg Z, Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chem* **190**:317–323 (2016).
29 Chen Y, Yu LJ and Rupasinghe HPV, Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: a mini-review. *J Sci Food Agric* **93**:981–986 (2013).
30 Echavarría AP, Torras C, Pagán J and Ibarz A, Fruit juice processing and membrane technology application. *Food Eng Rev* **3**:136–158 (2011).
31 Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* **16**:144–158 (1965).
32 Papillo VA, Vitaglione P, Graziani G, Gokmen V and Fogliano V, Release of antioxidant capacity from five plant foods during a multistep enzymatic digestion protocol. *J Agric Food Chem* **62**:4119–4126 (2014).

33 Poyrazoğlu E, Gökmen V and Artık N, Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. *J Food Compos Anal* **15**:567–575 (2002).
34 Cabálková J, Žídková J, Přibyla L and Chmelík J, Determination of carbohydrates in juices by capillary electrophoresis, high-performance liquid chromatography, and matrix-assisted laser desorption/ionization-time of flight-mass spectrometry. *Electrophoresis* **25**:487–493 (2004).
35 Mayuoni-Kirshinbaum L, Daus A and Porat R, Changes in sensory quality and aroma volatile composition during prolonged storage of 'Wonderful' pomegranate fruit. *Int J Food Sci Technol* **48**:1569–1578 (2013).
36 Huang B, Lei Y, Tang Y, Zhang J, Qin L and Liu J, Comparison of HS-SPME with hydrodistillation and SFE for the analysis of the volatile compounds of Zisu and Baisu, two varietal species of *Perilla frutescens* of Chinese origin. *Food Chem* **125**:268–275 (2011).
37 Mazza G, Fukumoto L, Delaquis P, Girard B and Ewert B, Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J Agric Food Chem* **47**:4009–4017 (1999).
38 Carneiro L, dos Santos Sa I, dos Santos Gomes F, Matta VM and Cabral LMC, Cold sterilization and clarification of pineapple juice by tangential microfiltration. *Desalination* **148**:93–98 (2002).
39 Laorko A, Tongchitpakdee S and Youravong W, Storage quality of pineapple juice non-thermally pasteurized and clarified by microfiltration. *J Food Eng* **116**:554–561 (2013).
40 Mena P, Vegara S, Martí N, García-Viguera C, Saura D and Valero M, Changes on indigenous microbiota, colour, bioactive compounds and antioxidant activity of pasteurised pomegranate juice. *Food Chem* **141**:2122–2129 (2013).
41 Vegara S, Martí N, Mena P, Saura D and Valero M, Effect of pasteurization process and storage on color and shelf-life of pomegranate juices. *LWT – Food Sci Technol* **54**:592–596 (2013).
42 Rinaldi M, Caligiani A, Borgese R, Palla G, Barbanti D and Massini R, The effect of fruit processing and enzymatic treatments on pomegranate juice composition, antioxidant activity and polyphenols content. *LWT – Food Sci Technol* **53**:355–359 (2013).
43 Mirsaeedghazi H, Emam-Djomeh Z, Mousavi SM, Ahmadkhaniha R and Shafiee A, Effect of membrane clarification on the physicochemical properties of pomegranate juice. *Int J Food Sci Technol* **45**:1457–1463 (2010).
44 Cerreti M, Liburdi K, Benucci I and Esti M, The effect of pectinase and protease treatment on turbidity and on haze active molecules in pomegranate juice. *LWT – Food Sci Technol* **73**:326–333 (2016).
45 Mirsaeedghazi H, Emam-Djomeh Z, Mousavi SM, Aroujalian A and Navidbakhsh M, Clarification of pomegranate juice by microfiltration with PVDF membranes. *Desalination* **264**:243–248 (2010).
46 Cassano A, Conidi C and Drioli E, Clarification and concentration of pomegranate juice (*Punica granatum* L.) using membrane processes. *J Food Eng* **107**:366–373 (2011).
47 Mirsaeedghazi H, Emam-Djomeh Z, Mousavi SM, Aroujalian A and Navidbakhsh M, Changes in blocking mechanisms during membrane processing of pomegranate juice. *Int J Food Sci Technol* **44**:2135–2141 (2009).
48 Mohammad AW, Ng CY, Lim YP and Ng GH, Ultrafiltration in food processing industry: review on application, membrane fouling, and fouling control. *Food Bioprocess Technol* **5**:1143–1156 (2012).
49 De Bruijn J and Bórquez R, Analysis of the fouling mechanisms during cross-flow ultrafiltration of apple juice. *LWT – Food Sci Technol* **39**:861–871 (2006).
50 Yu J and Lencki RW, Effect of enzyme treatments on the fouling behavior of apple juice during microfiltration. *J Food Eng* **63**:413–423 (2004).
51 Yousefnezhad B, Mirsaeedghazi H and Arabhosseini A, Pretreatment of pomegranate and red beet juices by centrifugation before membrane clarification: a comparative study. *J Food Process Preserv* **41**:e12765 (2017).
52 Onsekizoglu P, Production of high quality clarified pomegranate juice concentrate by membrane processes. *J Membr Sci* **442**:264–271 (2013).
53 Fischer UA, Carle R and Kammerer DR, Thermal stability of anthocyanins and colourless phenolics in pomegranate (*Punica granatum* L.) juices and model solutions. *Food Chem* **138**:1800–1809 (2013).
54 Kus S, Gogus F and Eren S, Hydroxymethyl furfural content of concentrated food products. *Int J Food Prop* **8**:367–375 (2005).
55 Yildiz H, Bozkurt H and Icier F, Ohmic and conventional heating of pomegranate juice: effects on rheology, color, and total phenolics. *Food Sci Technol Int* **15**:503–512 (2009).
56 Vaillant F, Cisse M, Chaverri M, Perez A, Dornier M, Viquez F *et al.*, Clarification and concentration of melon juice using membrane processes. *Innovat Food Sci Emerg Technol* **6**:213–220 (2005).
57 Jaiswal V, DerMarderosian A and Porter JR, Anthocyanins and polyphenol oxidase from dried arils of pomegranate (*Punica granatum* L.). *Food Chem* **118**:11–16 (2010).
58 Holzwarth M, Wittig J, Carle R and Kammerer DR, Influence of putative polyphenoloxidase (PPO) inhibitors on strawberry *(Fragaria* × *ananassa* Duch.) PPO, anthocyanin and color stability of stored purées. *LWT – Food Sci Technol* **52**:116–122 (2013).
59 Bakkalbaşi E, Menteş Ö and Artik N, Food ellagitannins – occurrence, effects of processing and storage. *Crit Rev Food Sci Nutr* **49**:283–298 (2008).
60 González MJ, Torres JL and Medina I, Impact of thermal processing on the activity of gallotannins and condensed tannins from *Hamamelis virginiana* used as functional ingredients in seafood. *J Agric Food Chem* **58**:4274–4283 (2010).
61 Häkkinen SH, Kärenlampi SO, Mykkänen HM, Heinonen IM and Törrönen AR, Ellagic acid content in berries: influence of domestic processing and storage. *Eur Food Res Technol* **212**:75–80 (2000).
62 Aguilera-Carbo A, Augur C, Prado-Barragan LA, Favela-Torres E and Aguilar CN, Microbial production of ellagic acid and biodegradation of ellagitannins. *Appl Microbiol Biotechnol* **78**:189–199 (2008).
63 Mayuoni-Kirshinbaum L and Porat R, The flavor of pomegranate fruit: a review. *J Sci Food Agric* **94**:21–27 (2014).
64 Olle D, Baron A, Lozano YF, Sznaper C, Baumes R, Bayonove C *et al.*, Microfiltration and reverse osmosis affect recovery of mango puree flavor compounds. *J Food Sci* **62**:1116–1119 (1997).
65 Cadwallader KR, Tamamoto LC and Sajuti SC, Aroma components of fresh and stored pomegranate (*Punica granatum* L.) juice. *ACS Symp Ser* **1036**:93–101 (2010).
66 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).
67 Lan Y, Wu J, Wang X, Sun X, Hackman RM, Li Z *et al.*, Evaluation of antioxidant capacity and flavor profile change of pomegranate wine during fermentation and aging process. *Food Chem* **232**:777–787 (2017).
68 Cavalcanti RN, Santos DT and Meireles MAA, Non-thermal stabilization mechanisms of anthocyanins in model and food systems – an overview. *Food Res Int* **44**:499–509 (2011).
69 Patras A, Brunton NP, O'Donnell C and Tiwari BK, Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Sci Technol* **21**:3–11 (2010).
70 Eiro MJ and Heinonen M, Anthocyanin color behavior and stability during storage: effect of intermolecular copigmentation. *J Agric Food Chem* **50**:7461–7466 (2002).
71 Mousavi ZE, Mousavi SM, Razavi SH, Hadinejad M, Emam-Djomeh Z and Mirzapour M, Effect of fermentation of pomegranate juice by *Lactobacillus plantarum* and *Lactobacillus acidophilus* on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. *Food Biotechnol* **27**:1–13 (2013).
72 Davies AJ and Mazza G, Copigmentation of simple and acylated anthocyanins with colorless phenolic compounds. *J Agric Food Chem* **41**:716–720 (1993).
73 Rein M, *Copigmentation reactions and color stability of berry anthocyanins*. Dissertation, University (2005).

