Journal of Food Processing & Technology
ISSN: 2157-7110

Valero et al., J Food Process Technol 2014, 5:5
DOI: 10.4172/2157-7110.1000324

Research Article Open Access

Clarification of Pomegranate Juice at Industrial Scale

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Abstract

The effects of conventional and membrane clarification processes on some physicochemical properties of pomegranate juice such as color, turbidity, Total Soluble Solids (TSS), Total Phenolic Content (TPC), antioxidant activity and bioactive compounds (anthocyanins, ellagitannins and ellagid acid) were evaluated. Changes in color parameters such as absorbance at 520 nm (A_{520}, red color), Total Color Density (TCD) and Browning Index (BI), as well as TPC were negatively influenced by bentonite or albumin concentration in batch processes. However, both microfiltration (MF) and ultrafiltration (UF) processes at the applied conditions did not cause any significant differences on the levels of A_{520}, BI, TPC, and other parameters determined as part of the evaluation study. Moreover, the permeate flux in MF were higher than in UF, which is preferable for commercial application of tangential filtration technology in pomegranate juice industry. MF-clarified juice had physicochemical and nutritional properties similar to those of fresh juice.

Keywords: Pomegranate juice; Clarification; Microfiltration; Ultrafiltration; Bentonite; Albumin

Introduction

Pomegranate juice in its original state has a turbid appearance that makes it hard to preserve. The main purpose of the clarification is to reduce the turbidity, decrease the astringency of the product [1] and maintain the red color of pomegranate juice.

Pomegranate fruit contains considerable amounts of sugars, organic acids, amino acids and phenolic compounds such as gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, ferulic acid, o- and p-coumaric acids, catechin and quercetin [2,3] as well as enzymes, proteins, pectins and insoluble complexes as colloids (polysaccharides). The phenolic constituents give color, astringency and bitterness to the pomegranates juice [4,5]. These compounds with colloids and proteins are also responsible for the formation of the cloudy appearance of fruit juices during concentration and storage [6-8].

A number of agents including, gelatin, bentonite, activated carbon, casein, ion-exchange waxes and Poly Vinyl Poly Pyrrolidone (PVPP), have been studied for the removal of polyphenols from fruit juices [9-15], ever, these are all used in batch processes, which lead to additional costs in the existing processing line. Membrane processes have been used in the food industry because they require less manpower, are more efficient and have a shorter processing time than traditional clarification. Therefore, the operational costs of using membrane processes are considerably lower than those of more traditional processes [16-18].

Membrane filtration processes are often identified by the size range of solutes they separate. The types of filtration most commonly used to clarify and stabilize fruit juices are ultrafiltration (UF) and microfiltration (MF), which correspond to pressure-driven processes capable of separating particles in the approximate size ranges of 0.01-0.1 μm and 0.1-10 μm, respectively [19].

Colloids can produce a decrease of flow during filtration and the enzymatic treatment of the juice with pectinases, cellulase, hemicellulase, xylanase, carbohydrase, glucanase or arabinose prior to clarification may allow an increase in the permeate flow due to the reduction in the size of the particles and the subsequent decrease in viscosity [20-25].

In this study, the effect of membrane clarification on some physicochemical properties of pomegranate juice such as color, turbidity, Total Soluble Solids (TSS), Total Phenolic Content (TPC), and bioactive compounds (anthocyanins, ellagitannins and ellagid acid) in UF (0.1 μm pore size) and MF ranges (0.2 μm pore size) was evaluated. Derived changes from conventional clarification processes using bentonite or albumin as fining agents in several of these parameters were also studied and compared. The procedure helps to remove active haze precursors and thus decreases the potential for haze formation during storage. Albumin and bentonite are two fining agents used in the industry of juices. The strong negative charge of the bentonite surface exerts an important action on positively charged proteins of the juice that enables its flocculation. Albumin is a protein capable of sequestering the polyphenols present in the juice. Effective use of clarification agents requires optimization of their methods of preparation, as well as determination of the suitable concentrations needed to achieve the desired clarification.

Materials and Methods

Materials

Pomegranate 'Mollar' fruits were obtained from the Parc Natural Agrari Els Carrizals (Elche, Alicante, and Southeast Spain) under a semi-arid Mediterranean climate. They were harvested in the autumn of 2011 when fully ripened, to ensure their best flavour and color, and transported by a ventilated car to the Miguel Hernández University (UMH) Pilot Plant located in Orihuela (Alicante, Spain). The pilot plant is part of Institute of Molecular and Cell Biology (IBMC)-JBT Corp., Food Tech R&D Alliance. Fruits were washed in cold tap water

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Received February 19, 2014; **Accepted** April 21, 2014; **Published** May 02, 2014

Citation: Valero M, Vegara S, Martí N, Saura D (2014) Clarification of Pomegranate Juice at Industrial Scale. J Food Process Technol 5: 324. doi:10.4172/2157-7110.1000324

and drained. Pomegranates were cut in halves and arils were separated from the pith using an industrial stemmer (Coinme Import-Export S.L., Álava, Spain). Then juice was immediately obtained by applying pressure on arils with a vertical spindle press (Coinme Import-Export S.L.). The extracted cloudy juice contained 2% pulp and was stored at 2°C in 100 L stainless steel tanks by adding nitrogen in headspace.

Fining

Pomegranate juices were placed into 5 L decanting cones. Clarification treatments were applied by use of albumin and bentonite (Erbslöh Geisenheim AG, Geisenheim, Germany) (5 ± 1°C, 18 h). Since solubility of the fining agents is low at the pH of the fruit juice, first 10% stock solutions were prepared by dissolving them in hot water at 40-50°C. Uniform concentrations of 0.1, 0.25, 0.5, 1 and 2 g of each agent per liter of the juice were used. The resulting clarified juices were passed through a mesh and were pasteurized at 65°C for 30 s (LTP, low temperature pasteurization) or 90°C for 5 s (HTP, high temperature pasteurization) in a semi-tubular pasteurizer 25 L/h (Mipaser Prototype, Murcia Spain).

UF and MF equipment and procedures

The equipment consisted of a 50 L feed tank, a cross flow pump, feed pressure pumps, a feed meter, a thermometer and two manometers for the measure of the feed and retention pressures. All experiments were conducted at 30°C and the temperature (T) of the feed was controlled by circulating cooling water through the heat exchanger. Transmembrane pressure (TMP) was controlled by the valve on the retentate side. The retentate was recycled to the feed tank and the permeate stream was separately collected in another tank. The plant was equipped with both UF and MF membrane modules with pore sizes of 0.1 and 0.2 µm, respectively (Table 1). The experiments were performed using a semi-industrial plant. A schematic of set up used for the clarification experiments is shown in Figure 1. The operating conditions were: TMP, 2 bar; T, 30°C; feed flow, 20 L/h.

Cleaning of the membranes was done at the end of each clarification process to remove foulants and reverse the degrading effects of fouling on permeability and selectivity. Cleaning protocol consisted of three steps: washing with filtered water, chemical cleaning with alkali solutions (Ultrasil® 69 NEW, ECOLAB INC.) for removal of organic compounds and enzymatic treatment with a multi-enzyme complex containing a wide range of carbohydrases (Novozymes Viscozyme®). The complete cleaning cycle including rinses between stages may take as long as 30 minutes to complete.

TSS and color parameters

TSS, expressed as ºBrix, were measured with a WAY-S digital Abbe refractometer (Optic Ivymen® System, Biotech SL, Barcelona, Spain).

The CIE (Committee International d'Eclairage) L, a and b coordinates of the pomegranate juices were measured in glass tubes using a spectrophotocolorimeter Color Flex Citrus Meter (HunterLab, In., Reston, Virginia, USA), calibrated with a green standard tile (L = 53.52; a = -25.15; b = 13.77), illuminant D65 and 10º observer according to the CIELAB 76 convention [26]. Hue angle (h) and chroma (C) were calculated using Eqs. (1) and (2).

	Microfiltration	Ultrafiltration
Dimensions	2.4" x 40"	2.4" x 40"
Filtering area	1.8 m^2	1.8 m^2
Material	Polysulfone (PS)	Polyethersulfone (PES)
Pore size	0.2 µm	0.1 µm
Driving force (bar)	2	2

Table 1: Characteristics of filtration membranes.

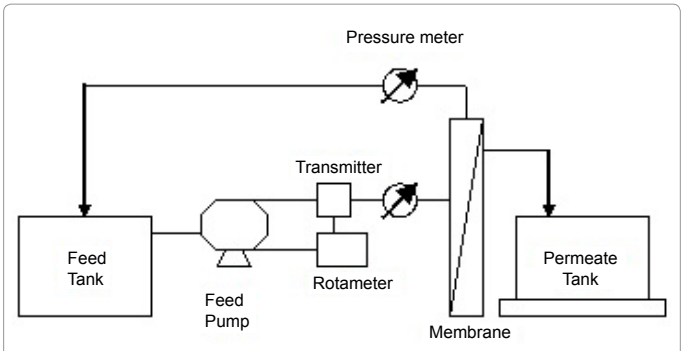

Figure 1: Schematic of the designed membrane clarification system.

$$h = \tan\text{-}1\ b/a \quad (1)$$

$$C = (a^2 + b^2)1/2 \quad (2)$$

Red color and turbidity of pomegranate juices was determined by reading the absorbance at 520 and 700 nm (A_{520} and A_{700} parameters) respectively, using a SPECTRO star Omega UV/VIS absorbance micro plate reader (BMG LABTECH GmbH, Offenburg, Germany). The pomegranate juices were previously diluted (1:2) with distilled water and then filtered through a 0.45 µm nylon syringe filter (Waters Corporation, Milford, Massachusetts, USA).

Color density is expressed as the total absorbance values of the brown compounds, which show maximum absorbance at 420 nm and absorbance of the juice that gives its maximum at 533 nm. The pomegranate juices were diluted with distilled water for each test in order to obtain an absorbance below 1.0 measured at 533 nm. The total color density (TCD) is estimated by the Eq. (3) [1,27], as a function of the absorbance at 420 nm (A_{420}), at 533 nm (A_{533}) and at 700 nm (A_{700}), and of the dilution factor (DF).

$$TCD=[(A420+A533)\_2(A700)].DF \quad (3)$$

Browning index (BI) [expressed as the absorbance ratio at 430 nm by that at 520 nm, according to Malien-Aubert, Dangles and Amiot [28] of water diluted (1:2) pomegranate juices was determined using the same microplate reader SPECTRO star Omega indicated previously.

Identification and quantification of phenolic compounds

Qualitative analysis of anthocyanins was performed by High-Performance Liquid Chromatography (HPLC) on a Model L6200 liquid chromatograph (Merck-Hitachi, Darmstadt, Germany) equipped with a SPD-M6A UV-VIS photodiode array detector (Shimadzu, Kyoto, Japan) and a Model 234 automatic sample injector (Gilson International Bv, Barcelona, Spain). Chromatograms were recorded and processed on a LC Workstation Class M10A Shimadzu PC-based chromatography data system. All samples were centrifuged at 10480g for 10 min at room temperature. The supernatant was filtered through a 0.45 µm nylon membrane.

A 20 µL sample was analyzed on a Luna® 5 µm C18 column (25 × 0.46 cm) (Phenomenex Ltd., Macclesfield, UK) with a security guard cartridge system C_{18} ODS (4 × 3 mm), using a mobile phase of water/formic acid (95:5 v/v) (solvent A) and HPLC grade methanol (solvent B). Elution was performed at a flow rate of 1 mL/min. The linear gradient started with 1% B, keeping isocratic conditions during 5 min,

reaching 20% B at 20 min, 40% B at 30 min, 95% B at 35 min and 1% B after 41 min. UV chromatograms were recorded at 520, 360 and 280 nm. The different phenols were characterized by chromatographic comparison with analytical standards and quantified by the absorbance of their corresponding peaks according to previous reports [29]. Anthocyanins were quantified as cyanidin 3-O-glucoside (Cy3G) (Polyphenols Laboratories AS, Sandnes, Norway) at 520 nm, while the gallagic-derived tannins were did as punicalagin isomers (LGC Standards, Teddington, Middlesex, UK) at 360 nm and the ellagic acid as free ellagic acid (Sigma-Aldrich Corp., Saint Louis, Missouri, USA) at 280 nm [30].

Determination of TPC

For TPC determination, 1:10 dilutions of the juices were used. TPC were determined with Folin–Ciocalteu reagent (Sigma-Aldrich Corp.) in a Spectrostar Omega UV/VIS absorbance microplate reader (BMG LABTECH GmbH). 10 µL of sample dilution, 50 µL Folin-Ciocalteu reagent, 100 µL of aqueous 20% Na_2CO_3 (Panreac Química S.A., Barcelona, Spain) and 100 µL of distilled water were mixed. The mixture was allowed to stand for 30 min at room temperature before measuring absorbance at 750 nm. Gallic acid (Sigma-Aldrich Corp.) was used as standard. Results were expressed as gallic acid equivalents (mg GAE/L).

Antioxidant capacity

Antioxidant capacity of pomegranate juice was evaluated by the ABTS [2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid); Sigma-Aldrich Corp.] radical scavenging assay. The radical cation was prepared by the reaction between a 7 mM solution of ABTS in water mixed with a 2.45 mM solution of potassium persulfate. The mixture was incubated 24 h in the dark at room temperature. Then this solution was diluted with water to reach an absorbance of 0.7 ± 0.02 at 734 nm, measured in a microplate reader Spectro star Omega (BMG LabTech GmbH). To determine the antioxidant capacity of pomegranate juice, 200 µL of the ABTS+ dissolution were mixed with 20 µL of juice and after 6 min the absorbance was measured at 734 nm, obtaining the value of the decrease in absorbance. This determination was carried out with a 1:50 dilution of juice. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Fluka Chemika, Neu-Ulm, Germany) was used as standard and results were expressed as Trolox equivalents (mM Trolox/L).

Statistical analysis

Treatments were performed in triplicate, and all the parameters studied were also determined in triplicate. Statgraphics® Plus for Windows 3.0 (Statistical Graphic Corp. and Graphic Software Systems Inc., Rockville, Maryland, USA) was used for Statistical Analysis of data, including Analysis of Variance (ANOVA), Fisher's least significant difference (LSD) procedure to discriminate among the means, and Regression Analysis to describe the relationship between variables.

Results and Discussion

Fining treatments

According to the P-values ($P < 0.05$), the type of fining agent and the concentration of this were the main factors affecting all tested parameters in pomegranate juices. As shown in Tables 2 and 3, results of analyses of pomegranate juices clarified with bentonite and albumin showed a similar behavior in terms of red color and color intensity determined as A_{520} and TCD. Concentration of fining agent had a statistically significant effect on both parameters at the 95% confidence level. When the clarified concentration increased, the red color of pomegranate juice decreased up to 60.98% and 63.80% with the use of 2 g/L bentonite or albumin, while the TCD did up to 57.10% and 53.46% respectively.

Changes in pomegranate juice turbidity were also influenced by concentration of fining agent. For bentonite, when the concentration increased, the turbidity of pomegranate juice decreased. However, for albumin a concentration of 0.25 g/L resulted in a decrease in turbidity which did not vary at concentrations equal or higher. Both bentonite and albumin decreased 77.36% and 73.19% turbidity (determined as A700) at the highest dose used. According to Vardin and Fenercioglu [31], the natural clarification of pomegranate juice resulted in a decrease in turbidity of 67.7% at 16 h and 85% at 96 h, however, 2.5 g/L of gelatin produced a reduction of 80.2% at 16 h.

BI of pomegranate juice increased when the concentration of bentonite did, while it decreased with increasing concentrations of albumin. Thus the addition of 1.0 g/L bentonite caused a statistically

Bentonite (g/L)	A_{520}	A_{700}	BI	TCD	ABTS(mM Trolox/L)	TPC(mg GAE/L)
0	1.489 ± 0.012 a	0.552 ± 0.006 a	4.540 ± 0.007 c	7.613 ± 0.079 a	15.218 ± 0.267 c	1870.982 ± 22.955 a
0.1	1.338 ± 0.036 b	0.471 ± 0.019 b	4.562 ± 0.005 c	7.371 ± 0.091 b	16.086 ± 0.298 b	1878.138 ± 68.116 a
0.25	1.036 ± 0.038 c	0.347 ± 0.011 c	4.513 ± 0.143 c	7.007 ± 0.049 c	16.707 ± 0.675 ab	1832.292 ± 50.794 ab
0.5	0.865 ± 0.021 d	0.241 ± 0.009 d	4.614 ± 0.005 bc	6.740 ± 0.043 d	16.802 ± 0.423 a	1769.667 ± 13.930 bc
1	0.681 ± 0.010 e	0.176 ± 0.008 e	4.694 ± 0.011 b	6.495 ± 0.081 e	16.230 ± 0.203 ab	1727.827 ± 62.604 c
2	0.581 ± 0.009 f	0.125 ± 0.012 f	4.937 ± 0.045 a	6.121 ± 0.058 f	16.664 ± 0.147 ab	1624.533 ± 55.005 d

Table 2: Characteristics of pomegranate juice clarified by sodium bentonite at 5°C for 18 h [mean and standard deviation (SD) are given] (A_{520}: Absorbance at 520 nm; A700: Absorbance at 700 nm; BI: Browning index; TCD: Total color density; ABTS [2,2'-az ino-bis(3-ethyl-benzothiazoline-6-sulfonic acid)]: ABTS radical scavenging assay; TPC: Total phenolic content. a-fMeans within same column followed by different letters are significantly different ($P \leq 0.05$).

Albumin (g/L)	A_{520}	A_{700}	BI	TCD	ABTS (mM Trolox/L)	TPC (mg GAE/L)
0	1.489 ± 0.012 a	0.552 ± 0.006 a	4.540 ± 0.007 a	7.613 ± 0.079 a	15.218 ± 0.267 b	1870.982 ± 22.955 a
0.1	0.947 ± 0.037 b	0.255 ± 0.003 b	4.520 ± 0.004 ab	6.837 ± 0.063 b	16.874 ± 0.188 a	1736.458 ± 7.272 b
0.25	0.731 ± 0.013 c	0.151 ± 0.005 c	4.495 ± 0.004 bc	6.514 ± 0.042 c	16.959 ± 0.054 a	1745.685 ± 79.506 b
0.5	0.656 ± 0.018 d	0.126 ± 0.030 c	4.474 ± 0.019 c	6.415 ± 0.067 cd	16.915 ± 0.277 a	1741.781 ± 113.970 b
1	0.615 ± 0.015 e	0.119 ± 0.024 c	4.485 ± 0.046 bc	6.321 ± 0.053 de	17.121 ± 0.173 a	1629.315 ± 90.109 b
2	0.539 ± 0.017 f	0.148 ± 0.034 c	4.489 ± 0.015 bc	6.216 ± 0.078 e	16.775 ± 0.281 a	1402.232 ± 17.244 c

Table 3: Characteristics of pomegranate juice clarified by albumin at 5°C for 18 h [mean and standard deviation (SD) are given] (A_{520}: Absorbance at 520 nm; A700: Absorbance at 700 nm; BI: Browning index; TCD: Total color density; ABTS [2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid)]: ABTS radical scavenging assay; TPC: Total phenolic content. a-fMeans within same column followed by different letters are significantly different ($P \leq 0.05$).)

significant increase in BI values compared to the control. In contrast, only 0.25 g/L albumin was necessary to achieve the maximum reduction level of BI. Color intensity of pomegranate juices can be determined by taking into consideration its absorbance values at 520 nm (A_{520}), the wavelength of maximum absorbance of the present monomeric anthocyanins, which give the juice the characteristic red color [32]. The negative charge of bentonite could also interact with the positive charge of anthocyanins, causing a decrease in the A_{520} value in pomegranate juice.

TPC was significantly influenced by albumin and bentonite concentration. TPC in pomegranate juice decreased up to 25.05% with the use of 2 g/L albumin. A minor reduction of 13.17% was observed with the highest concentration of bentonite. These results are similar than those obtained by Alper, Onsekizoglu and Acar [33] and Vardin and Fenercioglu [31].

On the other hand, a statistically significant increase in antioxidant activity of pomegranate juice was observed after fining treatments irrespective of the agent applied. Specifically 0.5 g/L bentonite was necessary to achieve the maximum level of antioxidant activity (16.802 ± 0.423 mM Trolox/L). For 0.1 g/L albumin, a similar value (16.874 ± 0.188 mM Trolox/L) that is not statistically different from those obtained for higher albumin concentrations was measured. Hence, the clarification process had a positive effect on the antioxidant capacity of pomegranate juice despite inducing a reduction in the TPC, the main source of bioactive compounds [34-36]. The interactive antioxidant capacity of phenolic compounds within fruit juices has not been well explored. In spite of this, synergistic and antagonistic interactions of two or more antioxidants have been documented in biological and model systems [37,38]. A possible explanation for the antioxidant potentiation could be the removal of the antagonism between constituents by the conventional clarification process with bentonite and albumin.

Pomegranate juice clarification by UF and MF

Measurements of permeate flux with water previously of juice filtration were 103.54 ± 6.75 and 150.46 ± 7.26 L/hm^2 for MF and UF processes, respectively. In general, permeate flux increases with the membrane pore size while retention decreases as the membrane pore size increases. The high anomalous value of permeate flux for UF process maybe could be due to that membrane was new whereas the MF-membrane had been regenerated and showed filtration efficiency loss.

The evolution of the permeate flux during UF and MF of pomegranate juice in the selected operating conditions is illustrated in Figure 2. In MF, the initial permeate flux of about 88 ± 2 L/hm^2 decreased gradually with operating times due to polarization concentration and fouling phenomena up to reach a steady-state value of about 11.50 ± 1.63 L/hm2. In the case of UF, the initial permeate flux was 100 L/hm^2 and slowed to around 7.20 ± 2.9 L/hm^2.

During the first stages of the process, the permeate flux in UF was much greater than that in MF. However, no statistically significant differences between the two processes in the final stages of the membrane processing were found at the 95% confidence level. This is due to the fact that the permeate flux in the early stages of processing is dependent on the membrane pore size. However, after this stage, the thicknesses of cake layers on the membrane surfaces play the major role in the permeate flux [39].

Membrane clarification processes of pomegranate juice have been

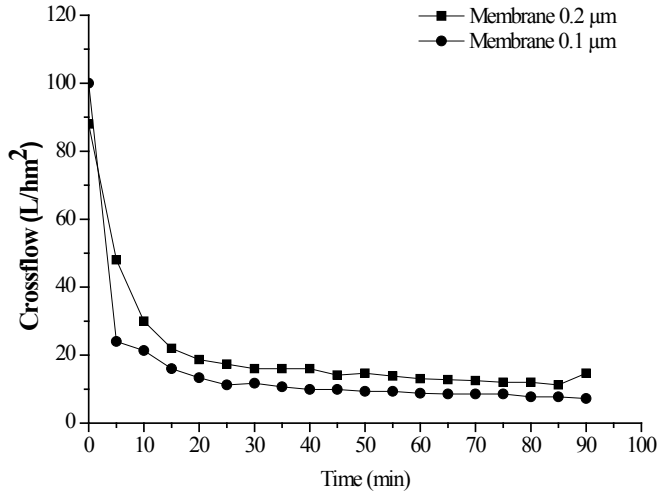

Figure 2: Permeate flux evolution throughout time.

evaluated in term of productivity (permeate flux) by different authors. In particular, Cassano, Conidi and Drioli [40], reported for the UF of pomegranate juice in different operating conditions a permeate flux of about 20 L/hm^2, while Mirsaeedghazi et al. [39] showed values of about 2.5 and 3 L/hm^2 using MF and UF, respectively.

Effect of clarification in pomegranate juice

The characteristics of the pomegranate juices clarified by MF and UF are shown in Table 4. Among color parameters, statistically significant decreases in CIE L (lightness, brightness), a (redness) and b (yellowness) values were the characteristic changes in the clarified samples. MF-treated pomegranate juice showed CIE L, a and b values decreased by 12.7%, 85.6% and 102.7%, respectively. For the UF-treated juice, the percentages of variation in CIE Lab parameters were 13.9%, 82.4 and 97.3%. Consequently, fresh juice showed a higher average value of C and lower of h than the clarified ones.

Moreover, changes in A_{520} (red color), A_{700} (turbidity) and BI among juices were not statistically significant. It should be noted however that spectrophotometric determinations of turbidity in UF-clarified juices decreased by approximately 36.4% compared to untreated juices. No close correlation was found between the A_{520} and CIE parameters at the 90% or higher confidence level ($P > 0.10$) as well as between A700 and CIE L.

UF-clarified pomegranate juice showed a discoloration of approximately 25.8% compared to its original color, while that MF-treated lost 13.2%. Probably, this was due to the reduction of tannin-protein complexes in the finished juice [16,41]. TCD takes into account the contribution of both brown (tannins) and red (anthocyanins) pigments to the color of pomegranate juice. A significant positive correlation was noted between TCD and BI at the 95% confidence level (correlation coefficient r = 0.6746). On the contrary, no statistically significant relationship between TCD and A_{520} was found at the 90% of higher confidence level.

UF-clarified pomegranate juices exhibited significantly lower TSS content compared to those MF-clarified and untreated. TPC in pomegranate juices showed slight decreases after tangential filtration, being this loss greater when a membrane of smaller pore size (UF) was used. However, these results were not statistically significant.

Parameters	Initial	MF	UF
	Mean ± SD	Mean ± SD	Mean ± SD
L	$16.66 \pm 0.03_a$	$14.54 \pm 0.01_b$	$14.34 \pm 0.03_c$
a	$2.16 \pm 0.05_a$	$0.31 \pm 0.04_b$	$0.38 \pm 0.04_b$
b	$1.12 \pm 0.01_a$	$-0.03 \pm 0.04_c$	$0.03 \pm 0.04_b$
C	$4.24 \pm 0.08_a$	$0.57 \pm 0.06_b$	$0.70 \pm 0.08_b$
h	$29.46 \pm 0.44_b$	$34.27 \pm 1.10_a$	$33.00 \pm 0.00_a$
A_{520}	$1.24 \pm 0.21_a$	$1.38 \pm 0.04_a$	$1.08 \pm 0.03_a$
A_{700}	$0.22 \pm 0.06_a$	$0.19 \pm 0.02_a$	$0.14 \pm 0.04_a$
BI	$4.96 \pm 0.54_a$	$4.48 \pm 0.52_a$	$4.16 \pm 0.08_a$
TCD	$7.73 \pm 0.11_a$	$6.71 \pm 0.14_b$	$5.73 \pm 0.12_c$
TSS (°Brix)	$15.26 \pm 0.42_a$	$15.00 \pm 0.00_a$	$13.87 \pm 0.12_b$
TPC (mg GAE/L)	$2581.37 \pm 62.76_a$	$2569.43 \pm 209.90_a$	$2370.85 \pm 47.39_a$
Dp3,5dG (mg/L)	$0.95 \pm 0.12_c$	$1.42 \pm 0.02_a$	$1.18 \pm 0.01_b$
Cy3,5dG (mg/L)	$17.96 \pm 0.88_a$	$18.92 \pm 0.06_a$	$17.53 \pm 0.51_a$
Pg3,5dG (mg/L)	$1.82 \pm 0.26_a$	$1.93 \pm 0.05_a$	$1.71 \pm 0.02_a$
Dp3G (mg/L)	$1.06 \pm 0.17_{ab}$	$1.21 \pm 0.02_a$	$0.91 \pm 0.02_b$
Cy3G (mg/L)	$6.71 \pm 1.06_b$	$8.30 \pm 0.01_a$	$7.30 \pm 0.03_{ab}$
Pg3G (mg/L)	$1.00 \pm 0.37_a$	$1.20 \pm 0.01_a$	$0.93 \pm 0.02_a$
Total anthocyanins (mg/L)	$29.50 \pm 2.59_b$	$32.97 \pm 0.13_a$	$29.56 \pm 0.46_b$
Punicalin α (mg/L)	$427.04 \pm 31.14_a$	$428.90 \pm 0.10_a$	$402.89 \pm 1.83_a$
Punicalin β (mg/L)	$276.27 \pm 22.29_a$	$280.78 \pm 0.72_a$	$260.08 \pm 0.88_a$
Total punicalins (mg/L)	$703.30 \pm 53.40_a$	$709.67 \pm 0.71_a$	$662.97 \pm 2.67_a$
Punicalagin α (mg/L)	$210.02 \pm 16.59_a$	$104.62 \pm 0.54_b$	$101.38 \pm 1.51_b$
Punicalagin β (mg/L)	$344.86 \pm 18.72_a$	$258.28 \pm 2.84_b$	$214.09 \pm 0.36_c$
Total punicalagins (mg/L)	$554.87 \pm 13.39_a$	$362.91 \pm 3.39_b$	$315.48 \pm 1.21_c$
Ellagic acid (mg/L)	$322.26 \pm 61.65_a$	$111.47 \pm 1.38_b$	$19.16 \pm 0.05_c$

Table 4: Analyses of pomegranate juice clarified by microfiltration (MF) and ultrafiltration (UF) technologies [mean and standard deviation (SD) are given] (L, a and b: CIE (Committee International d'Eclairage) parameters; C: Chroma and h: Hue angle; A_{520}: Absorbance at 520 nm; A700: Absorbance at 700 nm; BI, Browning index; TCD, Total color density; TSS, total soluble solids; TPC, Total phenolic content; Dp3,5dG: delphinidin 3,5-diglucoside; Cy3,5dG: cyanidin 3,5-diglucoside; Pg3,5dG: pelargonidin 3,5-diglucoside; Dp3G: delphinidin 3-O-glucoside; Cy3G: cyanidin 3-O-glucoside; Pg3G: pelargonidin 3-O-glucoside. a-cMeans within same row followed by different letters are significantly different ($P \leq 0.05$).)

Just after clarification by MF and UF, contents of individual anthocyanins in permeates were practically equal to those from untreated juice except for delphinidin 3,5-diglucoside (Dp3,5dG) and Cy3G. These two monomeric anthocyanins showed slight rises that led to a statistically significant increase in the total anthocyanin content (11.8%) in the clarified juice by MF. Total anthocyanins were not reduced using a UF system for the clarification of pomegranate juice. Contradicting, these data differ from the results reported by Cassano, Conidi and Drioli [40] who showed that there were decreases around 12% in the concentration of total anthocyanins in pomegranate juice after UF. According to these authors, monoglucoside anthocyanins were the compounds that most decreased during UF.

To investigate color quality in a systematic manner, it is necessary to objectively measure color as well as pigment concentration [42]. The chromatic parameters correlate closely with the anthocyanin content and indicate that color measurements can be used for estimation of the anthocyanin content [43]. In the present study, there is an apparent conflict between great variation in CIE a parameter and the constancy of anthocyanin content. Pérez-Vicente et al. [44] reported that total anthocyanin concentration of pomegranate juices highly correlated with CIE a value, but the best correlation in another study [45] was found for the total color difference (ΔE). Reactive phenolics could play a major role in the color of pomegranate juices clarified by MF and UF, in accordance with previous studies on color deterioration of strawberry preserves [46]. Additionally, clarification of fresh juice by membrane processes essentially enables the removal of suspended solids which remain totally concentrated in the retentate stream. These suspended solids could significantly increase CIE a values and, in general, all chromatic parameters.

The results indicated that ellagitannin punicalin α was the major constituent in pomegranate juice, followed in decreasing order by punicalagin β, ellagic acid, ellagitannin punicalin β, and punicalagin α. Total punicalins, punicalagins, and ellagid acid content in the pomegranate juice were 703.30 ± 53.40, 554.87 ± 13.39, and 322.26 ± 61.65 mg/L, respectively. Clarification process had not a statistically significant effect on punicalin concentration in pomegranate juice. In contrast, changes in punicalagins and ellagid acid content were influenced by both MF and UF. Total punicalagins decreased 34.6% and 43.1% by MF and UF processes, respectively. Hence concentration of punicalagins in juice was more affected by UF than by MF, as expected for a membrane of smaller pore size. Finally, the tangential filtration caused a large decrease in ellagic acid content, being also this loss greater by UF (94.1%) than by MF (65.4%).

Conclusions

According to results, the use of bentonite or albumin as fining agents in batch processes for clarification of pomegranate juice had a negative effect on color parameters such as A_{520} (red color), TCD (color intensity) and BI (browning), as well as TPC. However, membrane processes (both MF and UF) at the applied conditions did not cause any significant differences on the levels of A_{520}, BI, TPC, and other parameters determined as part of the evaluation study. Moreover, the permeate flux in MF were higher than in UF, which is preferable for commercial application of tangential filtration technology in pomegranate juice industry. MF-clarified juice had physicochemical and nutritional properties similar to those of fresh juice.

Acknowledgements

This research was funded by AGROALIMED Foundation from Conselleria d'Agricultura, Pesca i Alimentació, Generalitat Valenciana (Spain), Project 'Producción de Zumo de Caqui (*Diospyros kaki* L.) y de Industrialización de la Granada (*Punica granatum* L.)'.

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