

Article

Comparison of Fresh and Commercial Pomegranate Juices from *Mollar de Elche* Cultivar Grown under Conventional or Organic Farming Practices

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Abstract: Pomegranate juice has gained a high reputation for its health properties and consequently is now a highly demanded product. However, there is an interest in knowing if there are differences between conventional and organic agricultural practices. For that reason, total phenolic content, antioxidant capacity, punicalagin isomers and sensory analysis of pomegranate juices from *Mollar de Elche* cultivar were studied. A comparison between fresh and commercial pomegranate juice obtained by conventional and organic agricultural practices was conducted. The total phenolic content values ranged from 2285 to 2457 mg GAE L⁻¹; however, there was no significant difference among conventional and organic juices. The antioxidant capacity evaluated by DPPH and ABTS methods showed significant differences ($p < 0.05$) among conventional and organic juices. The antioxidant capacity values ranged from 17.7 to 35.9 mmol Trolox L⁻¹ for DPPH and from 5.09 to 27.9 mmol Trolox L⁻¹ for ABTS. Significant differences ($p < 0.05$) were observed among conventional and organic juices in punicalagin isomer, with the highest value found in conventional fresh pomegranate juice (0.48 g L⁻¹). Descriptive sensory

analysis showed that fresh pomegranate, fresh rind, earthy, vegetal, bitter, and astringent notes were higher in conventional fresh pomegranate juice. Cooked and mushroom notes predominated in conventional commercial pomegranate juice; while the organic juice was characterized by fresh pomegranate, fresh rind, earthy and sweet notes.

Keywords: pomegranate juice; phenolic content; antioxidant capacity; sensory analysis

1. Introduction

Conventional farming systems are experiencing a serious crisis due to the existence of a highly industrialized agriculture, using more intensively chemical and synthetic products [1]. In some cases, conventional farming is being replaced by organic farming, which does not use chemical fertilizers, pesticides and considers as “a holistic view of agriculture that aims to reflect the profound interrelationship that exists between farm biota, its production and the overall environment” [2]. This farming practice is based on rotating crops and using green manure to maintain the productivity of soil without damaging the environment, producing food free of any chemical residue [3]. It is thought that in the absence of pesticides, plants could contain higher levels of antioxidant components as a result of enhanced synthesis of active phytochemicals produced in defense against biotic and abiotic stress [4]. This concern arises because conventional agricultural practices utilize levels of pesticides and fertilizers that can result in a disruption of natural production of phenolic metabolites in the plant [5].

In the last years pomegranate fruit (*Punica granatum* L.) has taken great attention for its health benefits. The edible portion of pomegranates (arils) can be consumed in fresh. However, there is a huge amount of fruits which quality is not good enough to be consumed in fresh, mainly because of physiopathies [6]. Certainly, consumers' acceptance of these low quality fruits would be low. For this reason, it is necessary to find a commercial application for the fruits which cannot be commercialized in fresh; the main option is to use them in the “juice industry”.

Numerous studies on the antioxidant capacity and total phenolic content have shown that pomegranate juice contains high levels of antioxidants, higher than most other fruit juice and beverages [7–9]. The main antioxidant compounds in pomegranate juice are hydrolysable tannins, but anthocyanins and ellagic acid derivatives also contribute to the total antioxidant capacity of the juice [8]. Therefore, pomegranate juice has been recommended as a preventive treatment for coronary heart disease [10] and to improve the chemotherapeutic effects on human prostate cancer [11].

Pomegranate juice is being marketed as high quality or “gourmet” items because of its recent high popularity and price. The increasing concerns of food consumers on the relationship between health and diet, as well as on environmental issues have created new perspectives to the consumption of organic food [12]. There is controversy over the differences in the quality of conventional and organic pomegranate juices; while some studies [13,14] showed evidences of organic food being more nutritious, others [15,16] concluded that there were no consistent nutritional differences between both types of juices.

Therefore, the aim of the present study was to evaluate and compare the total phenolic content, antioxidant capacity, contents of punicalagin isomers, and sensory analysis of fresh and commercial

pomegranate juices from *Mollar de Elche* cultivar grown using both conventional and organic agricultural practices.

2. Materials and Methods

2.1. Plant Material and Sample Processing

Four pomegranate juices were used for the analysis: conventional fresh pomegranate juice (c-FPGJ), organic fresh pomegranate juice (o-FPGJ), conventional commercial pomegranate juice (c-CPGJ), and organic commercial pomegranate juice (o-CPGJ). The commercial pomegranate juices were directly acquired from companies in Alicante with which there is previously company-university collaboration. Commercial pomegranate juices in study (VitalGrana) were elaborated with “*Mollar de Elche*” cultivar and have two different presentations: (1) conventional and (2) organic.

Pomegranate fruits, “*Mollar de Elche*” cultivar, were harvest at fruit ripening stage (October 2014). Conventional fruits were obtained from one of the main pomegranate gene banks of the European Union, which is located at the experimental field station of the Miguel Hernandez University in Orihuela, Alicante, Spain (02°03’50’’E, 38°03’50’’N, and 25 m above sea level). Organic fruits were obtained from organic field certified by CAERM (Council Agriculture the Ecological Region of Murcia) in Alquerias (Murcia), compliance with current legislation: R (CE) 834/2007 and R (CE) 889/2008.

Both types of organic juices were produced using the farming practices approved by the CAERM (*Consejo de Agricultura Ecológica de la Región de Murcia*, <http://www.caermurcia.com/introduction>). Both types of juices were marketed with the seal of this official organism.

Ten fruits from each field were harvested in the same state of ripeness and immediately transported to the laboratory. Each husk was carefully cut at the equatorial zone with a sharp knife, and then arils were manually extracted to obtain the fresh pomegranate juice. The following parameters were analyzed in fresh and commercial juices: total phenolic content, contents of punicalagins (α and β), antioxidant capacity and sensory analysis. The chemical analyses were run in triplicate.

2.2. Extraction Procedure for Total Phenolic Content and Antioxidant Capacity (DPPH and ABTS Methods)

For a better extraction and analysis of antioxidant compounds, the method described by Chong *et al.* [17] was used. Briefly, pomegranate juice (2 mL) were mixed with 10 mL of MeOH/water (80:20 v/v) + 1% HCl, and 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 rpm for 10 min. Finally, the supernatant was collected and used for the analyses of total phenolic content and antioxidant capacity.

2.3. Total Phenolic Content

Total phenolic content (TPC) was quantified using Folin-Ciocalteu colorimetric method described previously by Gao *et al.* [18]. Juice extracts (0.1 mL) were mixed with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of ultrapure water. Then, the mixture was incubated at room temperature for 3 min and 1 mL of 20% sodium carbonate was added. TPC were determined after 1 h of incubation at room

temperature. The absorbance of the resulting blue color was measured at 765 nm using an UV-Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). Quantification was done with respect to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), mg GAE per liter of juice.

2.4. Antioxidant Capacity

The antioxidant activity (AOC) was evaluated by the DPPH and ABTS methods. The DPPH (radical 2,2-diphenyl-1-picrylhydrazyl) method was used as described by Brand-Williams *et al.* [19] with a modification in the reaction time. Briefly, 10 μL of the supernatant were mixed with 40 μL of MeOH and added to 950 μL of DPPH solution. The mixture was shaken vigorously and placed in a dark room for 10 min. The decrease in absorbance was measured at 515 nm in UV-Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France).

The ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation was employed according to [19]. The ABTS was dissolved in water to a 7 mM concentration; the radical cation ($\text{ABTS}^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and kept in the dark at room temperature for 16 h before use. Then, 10 μL of the supernatant were mixed with 990 μL of ABTS. After 10 min of reaction, the absorbance was measured at 734 nm. The absorbance was measured in UV-Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). Calibration curves for DPPH and ABTS methods were prepared with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) showing good linearity ($R^2 \geq 0.998$). The results were expressed as mean \pm standard error and units in mmol Trolox (vitamin E analog) per liter of juice.

2.5. Identification and Quantification of Punicalagin Isomers

Punicalagins (α and β) were determined in commercial and fresh juice (conventional and organic juices), 5 mL diluted with 5 mL of MeOH/water (80:20 v/v) and 1% acetic acid and sonicated at 20 $^{\circ}\text{C}$ for 15 min and left for 24 h at 4 $^{\circ}\text{C}$. Then the extract was again sonicated for 15 min, and centrifuged at 15,000 rpm for 20 min. Supernatants were filtered through a 0.45- μm Millipore filter and then injected into a Hewlett-Packard HPLC series 1200 equipped with a diode-array detector (Agilent, Waldbronn, Germany). Each sample (20 μL) was analyzed on a LiChroCART 100 RP-18 reversed-phased column (250 \times 4 mm, particle size 5 μm ; Merck, Darmstadt, Germany) equipped with a pre-column C18 (LiChrospher 100 RP-18, 5 μm ; Merck, Darmstadt, Germany) using a mobile phase of 1% acetic acid in ultra-high-purity deionized water (solvent A) and 1% acetic acid in MeOH (solvent B). Elution was performed at flow rate of 1 mL/min using a gradient starting with 1% B for 5 min, and increasing to 60% B at 40 min. Punicalagins detection were conducted at 360 nm. For the identification of punicalagins, absorption spectra and retention times were employed and compared with those obtained from the chemical standards. The results were expressed as mean \pm standard error and units in g per liter of juice.

2.6. Sensory Evaluation

Sensory evaluation with trained panel was used to compare fresh and commercial pomegranate juices (conventional and organic). A panel of 8 panelists, ages 20 to 55 years (4 female and 4 male) was trained in descriptive evaluation of pomegranate juices. All panelists work at Miguel Hernandez University and have a wide expertise in sensory evaluation of fruits and juices [20].

Panelists were asked to evaluate the intensity of the next attributes: (1) *appearance and aroma*: color, fresh pomegranate, fresh rind, earthy, vegetal, mushrooms and cooked; and (2) *flavor and basic tastes*: fresh pomegranate, fresh rind, earthy, vegetal, mushrooms, sweet, sour, bitter and astringent. Panelists used for the evaluation an 11-point scale, where 0 was extremely low intensity, 5 was regular intensity, and 10 was extremely high intensity.

2.7. Statistical analysis

Data from the analyses of pomegranate commercial and fresh juices (conventional and organic juice) were examined first by analysis of variance for mean comparison. Later, the method used to discriminate among the means (Multiple Range Test) was Tukey's procedure. Significance was defined at $p \leq 0.05$. Statistical analyses were performed using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Total Phenolic Content

Total phenolic content (TPC) in fresh and commercial (conventional and organic) pomegranate juice, are presented in Table 1. The TPC values ranged from 2285 (o-CPGJ) to 2457 (c-FPGJ) mg GAE L⁻¹. The variability in the TPC values may be caused by reactions taken place during juice manufacture (*i.e.*, hydroxylation, methylation, isoprenylation, imerization and/or glycosylation) [21]. The obtained TPC values were similar with those found by Mena *et al.* [22] (1562 to 2342 mg GAE L⁻¹) in “*Mollar de Elche*” and “*Valenciana*” cultivars. In a similar way, the farming type (organic or conventional) did not affect the TPC in orange juice [23]. Contrary, Dani *et al.* [24] reported higher phenolic content in organic grape juice compared to conventional grape juice. Therefore, the effect of organic or conventional growing conditions is not clear yet. Probably, there is a combination of conditions which influences on the phenolic content such as specie, cultivar, climate conditions, *etc.* The current study was the first one on pomegranate juice phenolic content as affected by organic and conventional agricultural conditions, and not statistically significant differences were found.

Table 1. Total phenolic content and antioxidant capacity in fresh and commercial pomegranate juices obtained from conventional and organic agricultural practices: (1) total phenolic content, TPC (mg GAE L⁻¹), (2) DPPH antioxidant capacity, DPPH-AOC (mmol Trolox L⁻¹) and (3) ABTS antioxidant capacity, ABTS-AOC (mmol Trolox L⁻¹).

Sample	TPC	DPPH-AOC	ABTS-AOC
	(mg GAE L ⁻¹)	(mmol Trolox L ⁻¹)	
c-FPGJ	2457 [†] ± 31 [‡]	17.7 ± 0.2 b	7.58 ± 0.61 b
o-FPGJ	2384 ± 7	35.9 ± 0.6 a	27.9 ± 2.3 a
c-CPGJ	2304 ± 10	17.8 ± 0.1 b	5.09 ± 0.39 b
o-CPGJ	2285 ± 12	17.9 ± 0.1 b	11.6 ± 1.2 b
ANOVA	N.S.	***	***

c-FPGJ: conventional fresh pomegranate juice; o-FPGJ: organic fresh pomegranate juice; c-CPGJ: conventional commercial pomegranate juice; o-CPGJ: organic commercial pomegranate juices; ANOVA: analysis of variance. [†] Values are the mean of 3 replications (± standard error). [‡] Values followed by the same letter within the same column were not statistically different at $p < 0.05$ (according to Tukey's multiple range test). N.S.: not significant ($p < 0.05$) and *** significant at $p < 0.001$.

3.2. Antioxidant Capacity

Table 1 shows the results of antioxidant capacity of fresh and commercial (conventional and organic) pomegranate juices by DPPH and ABTS methods. Significant differences ($p < 0.05$) were observed among conventional and organic juices. The AOC values ranged from 17.7 and 35.9 mmol Trolox L⁻¹ for DPPH and from 5.09 to 27.9 mmol Trolox L⁻¹ for ABTS. These values are higher in comparison with those found by Gil *et al.* [8] for red wine (6–7.5 mmol Trolox L⁻¹) and green tea (4.4 mmol Trolox L⁻¹). In general the values obtained for all pomegranate juices were to those previously reported by Mena *et al.* [22], which reported values in the range from 7.01 and 37.1 mmol Trolox L⁻¹.

Using the ABTS and DPPH methods, antioxidant capacity of the experimental pomegranate juices showed that fresh juice has higher antioxidant capacity than commercial pomegranate juice, specially the organic juice. Organic farming is supposed to optimize natural resources; however, conventional agriculture used non-natural products, such as fertilizers, and pesticides. Due to this supplementation, conventional farming is thought to accumulate less content of secondary metabolites, which induce the antioxidant capacity and consequently some health benefits since the most natural farming the highest content of bioactive compounds [23,24]. On the other hand, the total antioxidant capacity of pomegranate juice is attributed to polyphenols originating from the peels [8]. If whole fruits are pressed to prepare fresh juices (this is not the case of the current study, in which juices were prepared only using arils), it would be expected a large amount of bioactive compounds from the peels and consequently fresh juices would have high antioxidant capacity. Likewise, processing and pasteurization conditions play an important role in the antioxidant capacity of juice, since the bioactive compounds are affected by extrinsic factors such as oxygen, light and especially temperature [25–27].

Up to now, there are not scientific literature regarding the AOC of pomegranate juices from fruits cultivated by organic agricultural practices; however, several studies have been conducted on the TPC of different fruits. For instance, organic juices from apple, pear, blackcurrant, carrot, beetroot and

celery showed slightly higher TPC than conventional ones [28]; however, no mention was made about the AOC of these juices.

3.3. Identification and Quantification of Punicalagin Isomers

Table 2 shows the results of punicalagin isomers content in the different pomegranate juices under study. α -Punicalagin content ranged from 0.07 to 0.48 g L⁻¹, while β -punicalagin content ranged from 0.03 to 0.22 g L⁻¹. In general the c-FPGJ juice showed the highest value. The results showed that α -punicalagin was more abundant than β -punicalagin. The most abundant compounds among the pomegranate polyphenols are punicalagin isomers [29], which have important antioxidant and atherosclerotic biological properties [30]. The results from the present study showed significant difference among fresh and commercial pomegranate juice, as well as conventional and organic agricultural practices. These differences may be due to the temperatures used during industrial processing, storage conditions, cultivation techniques, and/or fertilization level [26,31,32].

Table 2. Contents of α -punicalagin and β -punicalagin (g L⁻¹) in fresh and commercial pomegranate juices obtained from conventional and organic agricultural practices.

Sample	α -Punicalagin	β -Punicalagin
	(g L ⁻¹)	
c-FPGJ	0.48 ± 0.01 a	0.22 ± 0.01 a
o-FPGJ	0.21 ± 0.04 b	0.12 ± 0.01 b
c-CPGJ	0.12 ± 0.01 b	0.08 ± 0.01 c
o-CPGJ	0.07 ± 0.01 c	0.03 ± 0.01 c
ANOVA	***	***

c-FPGJ: conventional fresh pomegranate juice; o-FPGJ: organic fresh pomegranate juice; c-CPGJ: conventional commercial pomegranate juice; o-CPGJ: organic commercial pomegranate juices; ANOVA: analysis of variance. † Values are the mean of 3 replications (\pm standard error). ‡ Values followed by the same letter within the same column were not statistically different at $p < 0.05$ (according to Tukey's multiple range test). *** significant at $p < 0.001$.

3.4. Sensory Analysis

Regarding appearance and aroma attributes during descriptive sensory analysis, c-FPGJ and o-FPGJ showed higher scores of desirable attributes (color, fresh pomegranate aroma, fresh rind aroma and vegetal notes) for fresh juices than those processed juices (Figure 1A). Similar situations took place when comparing fresh and commercial pomegranate juices in previous study [32]. c-CPGJ showed the highest scores in undesirable attributes, such as cooked and mushrooms notes; these attributes are typical from commercial juices after heat treatment [32]. o-CPGJ obtained lower scores of cooked notes showing closer values to desirable attributes of appearance and aroma of fresh juices.

Taking into account, flavor and basic tastes, c-FPGJ showed higher scores in fresh pomegranate, earthy, bitterness, and astringency (Figure 1B). These results were well correlated with the highest values found in the total phenolic content of c-FPGJ. Phenolic content is related to the astringency and bitterness of the juice, so high polyphenols content can cause unpleasant levels of bitterness and astringency [33]. The o-FPGJ was characterized by fresh pomegranate, fresh rind and earthy notes.

The c-CPGJ has the highest values in mushrooms, cooked and sour notes. Finally, the o-CPGJ showed the highest values in sweet notes.

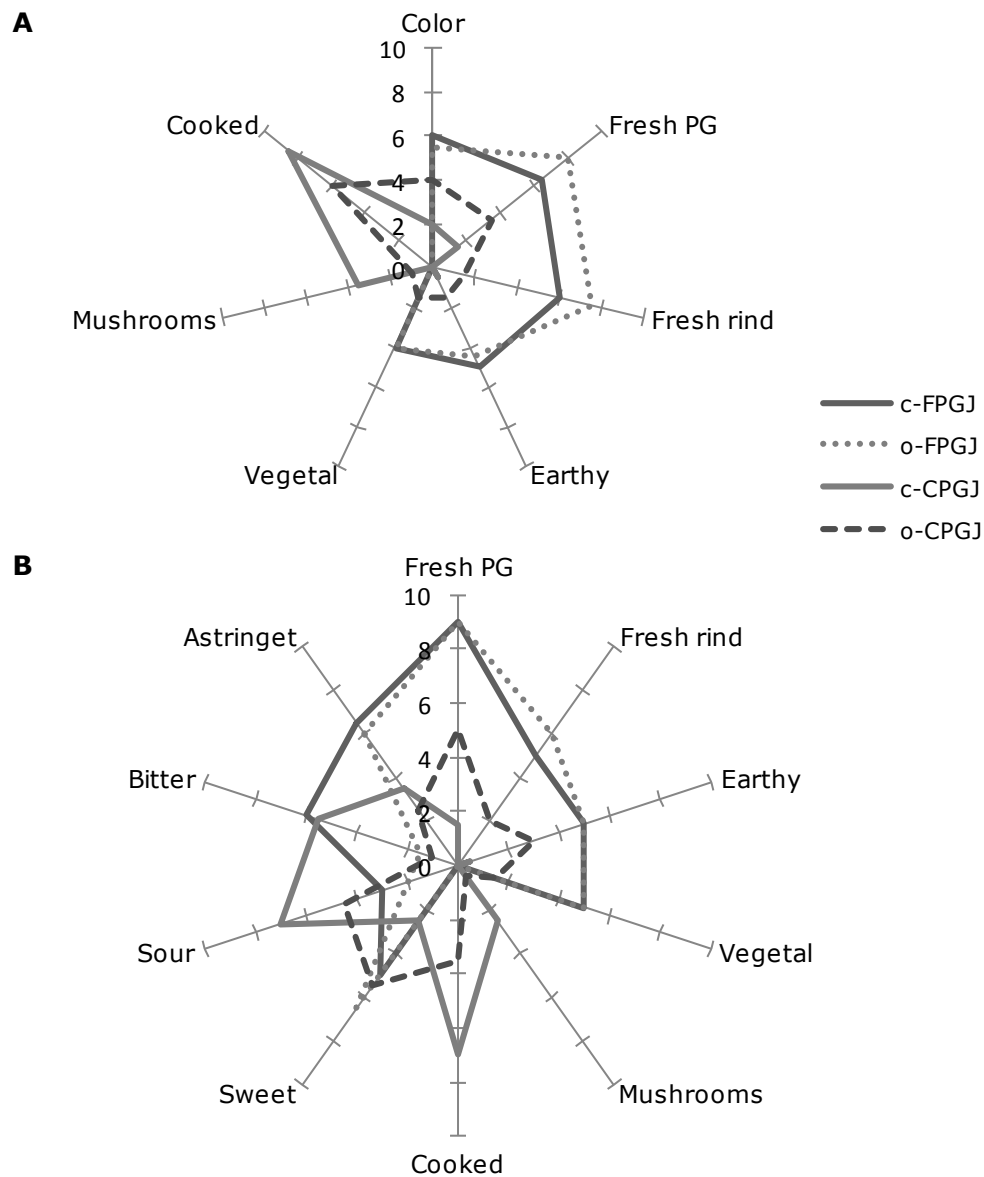


Figure 1. Descriptive Sensory Analysis by a trained panel of fresh and commercial pomegranate juices obtained from conventional and organic agricultural practices (A = appearance and aroma; B = flavor and basic tastes).

4. Conclusions

This first study comparing fresh and commercial pomegranate (cultivar *Mollar de Elche*) juices from organic and conventional agricultural practices provided useful information about the antioxidant capacity, total phenolic content, punicalagina isomers content and sensory profiles of these samples. Total phenolic content did not show significant differences in all the juices under study, while antioxidant capacity was significantly higher for organic fresh pomegranate juice. In the case of punicalagin isomers, the content was significantly higher in conventional fresh juice. With these results, it can be concluded that organic pomegranate juices is a good option for pomegranate

processing because of two main reasons: (1) the quality, functional and sensory, of fresh and processed juices is optimal, and (2) organic agricultural practices are considered to be more respectful with the environment. Since all fruits used in this study are from the same cultivar, *Mollar de Elche*, the main differences were attributed to the farming type, showing that phenolic compounds were not affected by conventional or organic farming, whereas pomegranate juice antioxidant capacity decreased by using chemical fertilizers and pesticides.

Author Contributions

N.N.J., M.C.L., Á.A.C.B., F.H., and Á.C.S. planned and designed the experiments; N.N.J. and M.C.L. performed the experiments; N.N.J., M.C.L. and Á.C.S. analyzed the data; N.N.J., M.C.L., Á.A.C.B., F.H., and Á.C.S. wrote the manuscript and Á.C.S. edited the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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