Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Potential of Spanish sour–sweet pomegranates (cultivar C25) for the juice industry

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Abstract

Commercial juices of sweet pomegranates and fresh juices of sour–sweet pomegranates were analysed for organic acids, sugars, antioxidant activity, volatile composition, sensory profile and consumer liking. Organic acids and sugars were analysed by high-performance liquid chromatography, while volatiles were extracted using hydrodistillation and analysed by gas chromatography–mass spectrometry and gas chromatography–flame ionisation detector. Malic acid was predominant in sweet juices while citric acid in sour–sweet samples. Fructose and glucose were found as the predominant sugars in all juices. A total of 18 compounds were found in pomegranate aroma profile, including monoterpenes, aldehydes, alcohols, monoterpenoids and linear hydrocarbons; the most abundant compounds were 3-carene, α-terpinene and α-terpineol. The total concentration of volatiles ranged from 2.0 up to 5.1 mg/L, with commercial samples presenting higher total concentrations due to the presence of peel volatiles. The high potential of sour-sweet pomegranate fruits for the juice industry was supported by (a) the high values of positive attributes, such as colour and fresh pomegranate flavour and (b) the high overall liking of consumers.

Keywords

Antioxidant activity, hydrodistillation, sensory evaluation, *Punica granatum* L., volatile compounds

Date received: 29 September 2010; revised: 6 April 2011

INTRODUCTION

Pomegranate (PG), *Punica granatum* L., belongs to the Punicaceae family and is one of the oldest known edible fruits. It is a very interesting plant for farmers in many areas around the world; this is especially true in arid and semi-arid soils due to the fact that this fruit tree is able to adapt to poor soils without significantly reducing its yield (Melgarejo and Salazar, 2003). Nowadays, pomegranate is cultivated in Iran, Afghanistan, India, Mediterranean countries (Tunisia, Turkey, Egypt, Spain and Morocco) and to some extent in USA, China, Japan and Russia (Raisi et al., 2008; Vardin and Fenercioglu, 2003). Spain is the main European pomegranate producer and its production is mainly, more than 90%, located in the province of Alicante (Andreu-Sevilla et al., 2008; Melgarejo and Martínez, 1992).

The edible portion of pomegranates, arils, can be consumed fresh; however, there is a huge amount of fruits the quality of which is not good enough to be consumed in fresh because consumers' acceptance would be very low. For this reason, it is necessary to

Food Science and Technology International 18(2) 129–138

DOI: 10.1177/1082013211414783
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find a commercial application for the fruits which cannot be commercialised in fresh; the main option is to use them in the 'juice industry'.

The low quality of fruit appearance is many times due to the fact that pomegranate tree blooms 'in waves', which not only causes different ripening stages, but also produces fruits with low quality as the ripening happens later (Melgarejo and Salazar, 2003). Another important reason causing low-quality fruits, not meeting consumers' expectations, is that several physiopathies lead to fruits which are chapped or cracked, deteriorating fruit appearance.

The increment in scientific reports (Borochov-Neori et al., 2009) which describe the positive effects of pomegranate consumption on human health is, without any doubt, leading to a huge increment in the use of pomegranate-based products, especially juices.

The pomegranate chemical composition, as in other vegetables and fruits, depends on plant variety, cropping pattern, geographical area, environmental conditions, ripening stage, crop techniques, storage conditions and finally fruit processing. Pomegranate is a fruit rich in potassium and with a low content in sodium, for this reason it is an advisable fruit to the great hypertensive population sector.

There is a growing interest in this fruit because it is considered to be a functional product of great benefit as it contains several groups of substances that are useful in disease risk reduction (Çam et al., 2009; Martinez et al., 2006). The beneficial health qualities of pomegranate have been attributed to the exceptionally high antioxidant capacity of the fruit juice (Borochov-Neori et al., 2009; Gil et al., 2000). Phenolic concentration and composition in pomegranate are cultivar dependent, but the most abundant components are anthocyanins, catechins, ellagic tannins, gallic and ellagic acids (Poyrazoglu et al., 2002).

Commercial pomegranate juices have shown an antioxidant capacity three times higher than red wine and green tea (Gil et al., 2000) and this experimental fact help setting pomegranate juices as one of the most important in vitro antioxidants. Therefore, it can be considered that juice (due to its healthy benefits presents a great market opportunity) is a great option for the pomegranate fruits with low appearance but high sensory quality.

The main aim of this study was to evaluate the potential of sour–sweet pomegranate fruits, cultivar C25, in the juice industry. Four different pomegranate juices were studied: two of the juices are currently being marketed in Spain, while the other two samples could be potentially commercialised in the near future. More specific objectives were (a) to describe the main quality parameters (organic acids, sugars, antioxidant capacity, volatile composition and sensory profile) and (b) to study the overall liking of a consumer panel of fresh sour–sweet (cv. C25) and commercial sweet pomegranate juices.

MATERIALS AND METHODS

Materials

Chemicals. All aroma standards (2-pentanol, *cis*-3-hexenal, hexanal, *trans*-2-hexenal, *cis*-3-hexenol, hexanol, α-pinene, β-pinene, β-myrcene, 3-carene, α-terpinene, limonene, γ-terpinene, fenchone, nonanal, camphor, terpinen-4-ol, α-terpineol and dodecane) used for identification and quantification purposes were obtained from Sigma–Aldrich (Madrid, Spain).

Plant material. Two freshly squeezed and two commercial pomegranate juices were studied. The commercial juices (30 bottles of 200 mL per juice from three different batches) were obtained (October, 2009) from a private company (Granadas de Elche SLU; http://www.campodeelche.com), which distributes these juices in the provinces of Alicante and Murcia (Eastern Spain). Juices were freshly produced using *Mollar de Elche* fruits (sweet cultivar) and all information about manufacturing and storage was provided. One of the commercial juices is 100% pure pomegranate juice without any concentration step (GranaVida from now on PPCJ, pure pomegranate commercial juice), while the second one is a pomegranate nectar and comes from concentrated juice (Granake from now on CPN, commercial pomegranate nectar).

Fruits from a sour–sweet pomegranate cultivar (C25) were obtained from 10 trees of an experimental farm (Orihuela, Alicante, Eastern Spain). The selection of this cultivar was based on the results of earlier studies performed by Melgarejo et al. (2000) on the potential farming and industrial uses of Spanish pomegranate cultivars.

Fresh juices were prepared by manually extracting the arils from the fruits (removing all carpellar membranes) and using a pilot-plant press. No additives or preservatives were added to these fresh juices and no heat treatment was carried out. This juice was divided into two samples: (a) control sour–sweet pomegranate juice (c-SSPJ), with no addition of any additive and (b) sucrose-treated sour–sweet pomegranate juice (s-SSPJ), with approximately 5% sucrose addition to reduce its sourness. Fresh samples were stored at 4–5 °C and analysed in less than 48 h.

Methods

Analysis of organic acids and sugars. Pomegranate juices were analysed to quantify organic acids

and sugars. The juices were centrifuged at 15.000 r/min for 20 min. One millilitre extract was filtered through a 0.45 μm *Millipore* filter and injected into a Hewlett-Packard high-performance liquid chromatography (HPLC) (series 1100); the elution buffer consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL/min. Organic acids were isolated using a Supelco column (Supelcogel^{TM} C-610H column 30 cm × 7.8 mm) and Supelguard (5 cm × 4.6 mm, Supelco, Inc., Bellefonte, PA) and absorbance was measured at 210 nm using a diode array detector. These exact same HPLC conditions (elution buffer, flow rate and column) were used for the analysis of sugars. The detection was conducted using a refractive index detector.

Standards of organic acids (oxalic, citric, tartaric, malic and ascorbic) and sugars (glucose, fructose, sucrose and maltose) were obtained from Sigma (Poole, Dorset, UK). Calibration curves, obtained by triplicate injection of standard solutions, were used for quantification purposes and showed good linearity with regression coefficients being higher than 0.999. Results were expressed as g/100 mL.

Extraction of volatile compounds. Hydrodistillation (HD) using a Deryng apparatus was used for the extraction of the volatile fractions of pomegranate juices according to the method previously described by Alonso et al. (2009b). Summarising, 150 mL of juice was placed in the sample flask together with 50 mL of ultrapure water, 10 g NaCl and 10 μL of 2-pentanol (1 g/L) as internal standard. Heating of the sample flask was carried out for 1 h after reaching the boiling point. The vapours were condensed by means of a cold refrigerant, maintained at −5 °C by a cryostat, model Frigiterm (Selecta). After 60 min of extraction, the solvent, 1 mL of pentane (Panreac), containing the aroma compounds, was collected and analysed by gas chromatography–mass spectrometry (GC–MS).

Chromatographic analyses. The isolation and identification of the volatile compounds were performed on a gas chromatograph, Shimadzu GC-17A (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector GC–MS QP-5050A. The GC–MS system was equipped with a TRACSIL Meta.X5 column (Teknokroma S. Coop. C. Ltd, Barcelona, Spain; 30 m × 0.25 mm × 0.25 μm film thickness). Analyses were carried out using chromatographic conditions previously described by Vázquez-Araújo et al. (2008).

Most of the compounds were identified using three different analytical methods: (a) Kovats indices, (b) GC–MS retention times (authentic chemicals) and (c) mass spectra (authentic chemicals and Wiley spectral library collection). Identification was considered tentative when it was based on only mass spectral data (McLafferty, 2000).

The quantification of the volatile compounds was performed on a gas chromatograph, Shimadzu 2010, with a flame ionisation detector. The column and chromatographic conditions were those previously reported for the GC–MS analysis. The injector temperature was 250 °C and nitrogen was used as carrier gas (1 mL/min).

For the quantification of the volatile compounds, 2-pentanol was added as internal standard; this chemical was used as internal standard after checking that it was absent in pomegranate juices, and under the proposed conditions, it separates well from other volatile compounds. Data included in this study should be considered as semi-quantitative, because no standard curves were carried out for each one of the quantified volatile compounds. However, relative values are useful to compare differences between juices.

Antioxidant capacity. Total antioxidant activity (TAA) was quantified in triplicate according to Arnao et al. (2001), which enables the determination of TAA due to both hydrophilic-total antioxidant activity (H-TAA) and lipophilic-total antioxidant activity (L-TAA) compounds in the same extraction. Briefly, 1 mL of juice was homogenised in 5 mL of 50 mM phosphate buffer, pH 7.8 and 3 mL of ethyl acetate and then centrifuged at 15,000 min for 15 min at 4 °C. The upper fraction was used for L-TAA while the lower for H-TAA quantification. For both cases, TAA was determined using the enzymatic system composed of the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), horseradish peroxidase enzyme and its oxidant substrate (hydrogen peroxide), in which $ABTS^{\bullet+}$ radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the juice extracts was proportional to TAA of the sample. A calibration curve (0–20 nmol) was performed with Trolox ((R)-(+)-6-hydroxy-2,5,7,8-tetramethylcroman-2-carboxylic acid, (Sigma, Madrid, Spain) in both aqueous and methanolic media for H-TAA and L-TAA, respectively. The results were expressed as Trolox equivalent antioxidant capacity (TEAC); it is the concentration of Trolox required to give the same antioxidant capacity as 1 mM test substance (Gil et al., 2000).

Sensory evaluation with trained panel. Sensory evaluation with trained panel was used to describe pomegranate juices. A panel of eight panellists, aged 20–55 (4 female and 4 male) was trained in descriptive evaluation of fresh fruits, including pomegranates.

All panellists work at Miguel Hernández University and have a wide expertise in sensory evaluation of foods. Details about panel selection and training could be found in Ruiz et al. (2005).

The study was carried out at UMH facilities (individual booths with controlled illumination and temperature) during three different sessions; samples were evaluated in triplicate. In each session, panellists tested all four juices; the samples order for each panellist was randomised (AENOR, 1997). Approximately 20 mL of juice were served, at 10 °C together with the appropriate questionnaire, one at a time and waiting 5 min between samples. Unsalted crackers and water were provided to consumers for palate cleansing between samples.

In each questionnaire, panellists were asked, using 11-point scales (0 = extremely low intensity; 5 = regular intensity of fresh pomegranate juice; and 10 = extremely high intensity), the intensity of the main pomegranate juice attributes: colour, fresh pomegranate odour, caramel odour, sweetness, sourness, fresh pomegranate flavour, caramel flavour and astringency.

Sensory evaluation with consumer panel. A sample group of 50 consumers was recruited at the Orihuela campus of Miguel Hernández University (UMH). The group consisted of 30 women and 20 men, aged between 18 and 57. Consumers were from the Communities of Valencia and Murcia (provinces: Valencia, Alicante and Murcia); the main requirement for their recruitment was that they consume fruit juices at least twice a week.

The consumers study was carried out at UMH, consumers tested all four pomegranate juices; the samples order for each consumer was randomised. Approximately, 20 mL of pomegranate juice were served, at 10 °C together with the appropriate questionnaire, one at a time and waiting 5 min between samples. Unsalted crackers and water were provided to consumers for palate cleansing between samples.

In each questionnaire, consumers were asked, using 11-point hedonic scales [from 0 to 10, with 5 (the central point of the scale) meaning neither like nor dislike it], about the overall liking of the sample.

Statistical analyses. Data from pomegranate analyses were examined by one-way analysis of variance (ANOVA) using STATGRAPHICS Plus 5.0 software (Manugistics, Inc., Rockville, MD). Wherever F values were significant, Tukey's multiple range test was used to separate the mean effects. Significance was defined at $p \leq 0.05$. Graphics were created using Sigma Plot 9.0 (SPSS Science, Chicago, IL, USA).

RESULTS AND DISCUSSION

Organic acids

The organic acids profile could be an important source of information about the sensory properties of pomegranate juices because each acid grants different organoleptic attributes to the final product. Malic and citric acids were the main organic acids in all juices under study. These results agree with previous data reported in the literature (Melgarejo et al., 2000; Ozgen et al., 2008; Poyrazoglu et al., 2002).

The malic acid content was slightly higher than the citric acid in the commercial samples because of the pomegranate variety used, *Mollar de Elche*. The composition of organic acid found in the commercial samples agreed with those from previous studies carried out by Mirdehghan et al. (2006, 2007) in fruits from the Indian cultivar *Malas Yazdi*. On the other hand, in fresh sour–sweet pomegranate juices, citric acid predominated over malic acid as reported previously by Melgarejo et al. (2000) for several Spanish sour–sweet and sour cultivars.

The values of malic acid contents ranged, in all four juices under study, between 0.61 ± 0.04 and 1.24 ± 0.16 g/100 mL, with a mean value of 0.88 g/100 mL, while citric acid ranged from 0.33 ± 0.03 up to 1.89 ± 0.01 g/100 mL and presented a mean value of 1.11 g/100 mL. Finally, three other organic acids were also detected but occurred at much lower concentrations, 0.03–0.13, 0.02–0.17 and 0.10–0.23 g/100 mL for oxalic, tartaric and ascorbic acids, respectively (Table 1).

Finally, the total concentration of organic acids ranged from 1.15 up to 3.36 g/100 mL, and as expected, the total concentration of organic acids was significantly ($p < 0.01$) lower in the commercial nectar, 1.15 g/100 mL, than in all other juices under study, mean of 2.76 g/100 mL.

Sugars

Fructose and glucose were the main sugars found in the pomegranate juices under study. Fructose contents ranged from 2.52 ± 0.02 to 17.6 ± 0.07 g/100 mL while glucose from 2.42 ± 0.02 to 13.8 ± 0.01 g/100 mL (Table 2). In this study, fructose values were higher than glucose contents, as previous studies reported (Melgarejo et al., 2000; Tezcan et al., 2009; Zhang et al., 2009), although differences were not always statistically significant ($p > 0.05$). However, other studies have reported that glucose was slightly higher than fructose (Al-Maiman and Ahmad, 2002; Miguel et al., 2004; Ozgen et al., 2008). These differences could be related to, for example, fruit cultivar, climatic conditions and irrigation management, among other factors.

Table 1. Organic acid contents in commercial sweet and fresh sour–sweet pomegranate juices

	Acids (g/100 mL, mean ± SE, $n = 3$)				
	Oxalic	Citric	Tartaric	Malic	Ascorbic
Commercial sweet juices					
PPCJ	0.05 ± 0.02 a	0.68 ± 0.11 c	0.02 ± 0.01 b	0.72 ± 0.10 c	0.15 ± 0.03 b
CPN	0.03 ± 0.02 a	0.33 ± 0.03 d	0.08 ± 0.01 b	0.61 ± 0.04 c	0.10 ± 0.02 b
Fresh sour–sweet pomegranate (cv. C25) juices					
c-SSPJ	0.10 ± 0.04 a	1.54 ± 0.10 b	0.17 ± 0.04 a	1.24 ± 0.16 a	0.23 ± 0.02 a
s-SSPJ	0.13 ± 0.01 a	1.89 ± 0.01 a	0.16 ± 0.01 a	0.96 ± 0.02 b	0.22 ± 0.01 a
ANOVA	N.S.	***	**	***	**

PPCJ: pure pomegranate commercial juice; CPN: commercial pomegranate nectar; c-SSPJ: control sour–sweet pomegranate juice; s-SSPJ: sucrose-treated sour–sweet pomegranate juice; and ANOVA: analysis of variance.
Values followed by the same letter, within a column, are not significantly different at $p < 0.05$.
N.S.: not significant F ratio ($p < 0.05$) and *** and ** significant at $p < 0.001$ and 0.01, respectively.

Table 2. Sugars content in commercial sweet and fresh sour–sweet pomegranate juices

	Sugars (g/100 mL, mean ± SE, $n = 3$)		
Juices	Fructose	Glucose	Sucrose
Commercial sweet juices			
PPCJ	8.58 ± 0.29 c	6.54 ± 0.22 c	0 c
CPN	2.52 ± 0.02 d	2.42 ± 0.02 d	0 c
Fresh sour–sweet pomegranate (cv. C25) juices			
c-SSPJ	11.1 ± 0.03 b	9.05 ± 0.02 b	1.15 ± 0.01 b
s-SSPJ	17.6 ± 0.07 a	13.8 ± 0.01 a	1.60 ± 0.01 a
ANOVA	***	***	***

PPCJ: pure pomegranate commercial juice; CPN: commercial pomegranate nectar; c-SSPJ: control sour–sweet pomegranate juice; s-SSPJ: sucrose-treated sour–sweet pomegranate juice; and ANOVA: analysis of variance.
Values followed by the same letter, within a column, are not significantly different at $p < 0.05$.
***significant at $p < 0.001$.

Sugar analysis also revealed the presence of sucrose in low, but significant levels in juices from sour–sweet fruits; no sucrose was detected in the commercial juices. Besides, a significant portion of the added sucrose to the juice s-SSPH was hydrolysed and converted into fructose and glucose (Table 2); therefore, increasing the contents of these two sugars in s-SSPJ compared to the control samples (c-SSPJ).

Finally, the total concentration of sugars ranged from 4.94 up to 33.0 g/100 mL, and as expected, the total concentration of sugars was significantly ($p < 0.001$) lower in the commercial nectar, 4.94 g/100 mL, than in all other juices under study, mean of 23.1 g/100 mL. However, the label of the pomegranate nectar indicates that sweetener was added to improve the sweetness of the product.

Antioxidant capacity

The total antioxidant capacity (AOC) was measured separately as H-TAA and L-TAA fractions by ABTS analytic method. It could be observed that, H-TAA (mean of 3.02 ± 0.11 TEAC for all juices under study) was significantly higher ($p < 0.001$) than L-TAA (mean of 0.58 ± 0.03 TEAC), showing that the major contributors to antioxidant activity of pomegranate juices were hydrophilic compounds (Figure 1). The highest value of AOC in the hydrophilic extract was obtained in the control sour–sweet juice, 3.24 ± 0.09 TEAC; even though there were statistically significant differences ($p < 0.05$) among the antioxidant activities of the four pomegranate juices under study, the differences only represented a maximum value of 14% (0.55 TEAC).

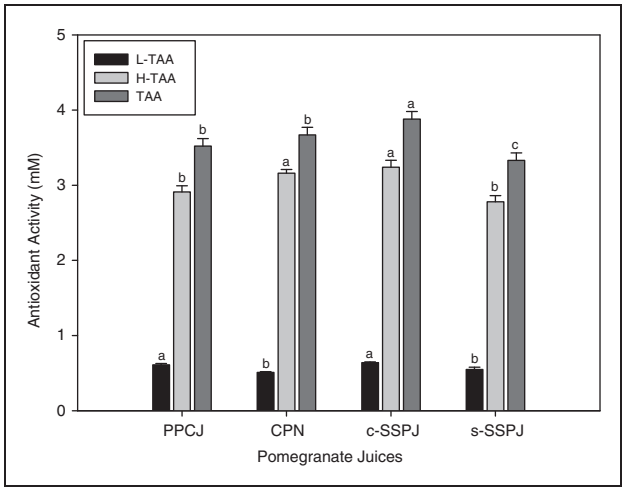

Figure 1. Antioxidant activity in H-TAA and L-TAA extracts of commercial sweet and fresh sour–sweet pomegranate juices.
H-TAA: hydrophilic total antioxidant activity; L-TAA: lipophilic total antioxidant activity; TAA: total antioxidant activity PPCJ: pure pomegranate commercial juice; CPN: commercial pomegranate nectar; c-SSPJ: control sour–sweet pomegranate juice; and s-SSPJ: sucrose-treated sour–sweet pomegranate juice.

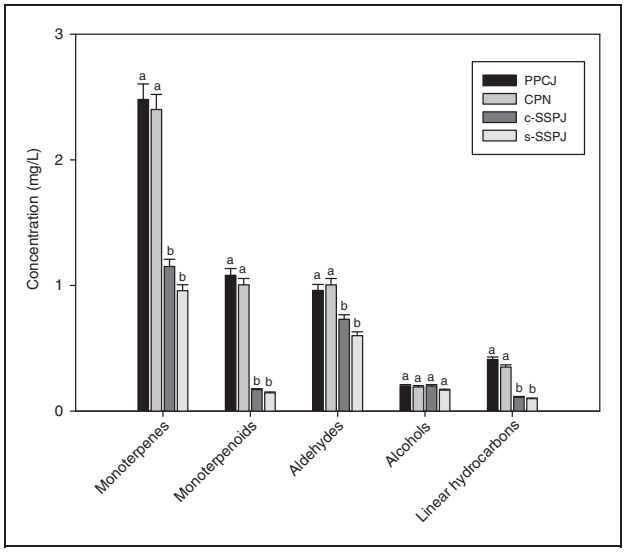

Figure 2. Grouping of volatile compounds in commercial sweet and fresh sour–sweet pomegranate juices.
PPCJ: pure pomegranate commercial juice; CPN: commercial pomegranate nectar; c-SSPJ: control sour–sweet pomegranate juice; and s-SSPJ: sucrose-treated sour–sweet pomegranate juice.

Arnao et al. (2001) showed that H-TAA was the fraction responsible for 70–90% of the TAA in vegetable soups. In this study, H-TAA represented about 84% of the TAA of pomegranate juices.

The values of antioxidant activity found in this study (3.3–3.9 TEAC) were significantly lower than those found by previous researchers; for instance, Gil et al. (2000) reported values of 18–20 TEAC for commercial pomegranate juices from *Wonderful* pomegranates. However, the AOC values found here for pomegranate juices are similar to those reported for products such as green tea (4.4 TEAC), which is well known by its high antioxidant activity (Gil et al., 2000). Results are in agreement with those previously published by other research groups working with *Mollar de Elche* fruits; for instance, Sayyari et al. (2011) reported values at harvest of 3.40 and 0.64 TEAC for H-TAA and L-TAA, respectively. Also, results are within the range of values, 3–11 TEAC, reported by Tzulker et al. (2007) in 29 different pomegranate accessions from Israel. This wide range in the antioxidant activity can be justified by the fact that climatic conditions and cultural factors can affect the nutritional composition of foods, including bioactive compounds (Scalzo et al., 2005; Tomás-Barberán and Espín, 2001).

The initial total AOC of freshly squeezed juices from Mollar de Elche fruits was 4.1 TEAC. It is interesting to point out that even though commercial juices had a thermal treatment (98 °C for 20 s), the antioxidant activity was only slightly reduced down to 3.52 TEAC.

The correlation between antioxidant activity and phenolic compounds has been considered in several studies assessing a wide range of fruits and vegetables (Kaur and Kapoor, 2001; Wu et al., 2004). For instance, a highly significant and positive correlation was obtained among antioxidant activity and both phenolic and anthocyanin contents in different sweet cherry cultivars (Díaz-Mula et al., 2009; Serrano et al., 2005). The existence of this correlation in Spanish commercial pomegranate juices has not been yet established but it will be evaluated in future experiments.

Volatile composition

Before starting to discuss the volatile composition of the pomegranate juices, it is important to consider that their main problem is the low intensity of both odour and aroma. This is the main reason why there are only few manuscripts describing the volatile composition of pomegranate and/or pomegranate juices. For instance, Raisi et al. (2008) studied the possibility of using pervaporation to recover the pomegranate aroma compounds and were able to identify only nine compounds in juices from Iran.

The volatile compounds found in pomegranate juices (Table 3) can be grouped in five chemical families: (a) *monoterpenes*: α-pinene, β-pinene, β-myrcene, 3-carene,

Table 3. Identification and characteristics of volatile compounds found in commercial sweet and fresh sour-sweet pomegranate juices

		Kovats Indexes		
Compound	RT (min)	NIST data	Experimental	Descriptor
cis-3-Hexenal	6.29	794	784	Apple, floral, pear, green, vegetable
Hexanal	6.34	795	798	Fatty, green, grassy, powerful
trans-2-Hexenal	8.74	844	854	Fruity, green, leafy, apple, vegetable
cis-3-Hexenol	9.06	851	856	Fresh, green grass
Hexanol	9.80	866	864	Mint, grass
α-Pinene	12.87	925	933	Sharp, pine
β-Pinene	15.21	967	966	Woody, pine
β-Myrcene	16.21	985	991	Sweet, balsamic, plastic
3-Carene	17.20	1003	1007	Lemon
α-Terpinene	17.43	1007	1015	Berry, lemon, vegetable, woody
Limonene	18.18	1020	1029	Mild, citrus, sweet, orange, lemon
γ-Terpinene	19.87	1051	1060	Herbaceous, citrus
Fenchone	21.29	1077	1087	Sweet, vanilla
Nonanal	22.41	1098	1103	Floral, citrus, orange, rose, fatty
Camphor	24.35	1135	1143	Aromatic, woody, medicinal
Terpinen-4-ol	26.26	1171	1177	
α-Terpineol	27.06	1187	1192	Fragrant, floral, lilac
Dodecane	27.45	1194	1200	

RT: retention time and NIST: National Institute of Standards and Technology (2010).

α-terpinene, limonene and γ-terpinene, (b) *monoterpenoids*: fenchone, camphor, terpinen-4-ol and α-terpineol, (c) *aldehydes*: *cis*-3-hexenal, hexanal, *trans*-2-hexenal and nonanal, (d) *alcohols*: *cis*-3-hexenol and hexanol and (e) linear hydrocarbons: dodecane. The most abundant group was that of monoterpenes followed by monoterpenoids, aldehydes, alcohols and linear hydrocarbons (Figure 2). The total concentrations of volatile compounds ranged from 5.2 mg/L for the PPCJ and 1.97 mg/L for s-SSPJ. The amount of volatile compounds was always higher in commercial juices from sweet fruits than in freshly squeezed samples from sour–sweet fruits, even though fresh juices were not exposed to any thermal treatment. The explanation for this experimental observation is simple and it is that commercial juices might include volatile compounds from both arils and peel, while the fresh juices were only prepared using arils; the pomegranate peel is richer in antioxidant and volatile compounds than the arils. In general, monoterpenes can be related to pine and citrus notes, while aldehydes are more related to green, grassy and herbaceous notes; it is possible that the different predominance of chemical groups have some influence on consumers' preference for pomegranate juices.

Vázquez-Araújo et al. (2011) found up to 83 aromatic compounds in pomegranate juices from USA. These authors concluded that freshly squeezed pomegranate juice was mainly characterised by the presence of terpenes and aldehydes, while furans were important contributors in the aroma of commercial juices. The fact that no furans were found in the volatile composition of the commercial juices studied in the current experiment shows that the intensity of the thermal treatment (98 °C for 20 s) to which they were subjected to was minimal and did not significantly changed the volatile profile of freshly squeezed juices.

A total of 18 compounds were isolated, identified and quantified using the HD technique (Table 3); this technique has been previously used by our research group to successfully describe the volatile composition of different products, for instance Spanish tomatoes (Alonso et al., 2009a) and Spanish pomegranates (Calín-Sánchez et al., 2011). Several of the volatiles identified in pomegranate juices were present in very low concentrations, for instance hexanol, β-myrcene, fenchone, camphor and terpinen-4-ol (Table 4). In general, the most abundant compounds were 3-carene (sensory descriptor: lemon), α-terpinene (lemon, vegetable, woody), limonene (mild, citrus, sweet, orange, lemon) and α-terpineol (floral). In very recent studies, the volatile compositions of nine Spanish cultivars were studied using different isolation techniques: (a) headspace solid phase microextraction (SPME; Melgarejo et al., 2011)

Table 4. Volatile compounds obtained after hydrodistillation, in commercial sweet and fresh sour-sweet pomegranate juices

		Commercial sweet PG juices		Fresh sour–sweet pomegranate (cv. C25) juices	
		PPCJ	CPN	c-SSPJ	s-SSPJ
Compound	ANOVA	(mg/L, mean ± SE, $n = 3$)			
cis-3-Hexenal	***	0.23	0.25	0.16	0.14
Hexanal	N.S.	0.18	0.16	0.22	0.18
trans-2-Hexenal	*	0.35	0.37	0.31	0.25
cis-3-Hexenol	N.S.	0.14	0.13	0.14	0.11
Hexanol	N.S.	0.06	0.07	0.06	0.05
α-Pinene	N.S.	0.05	0.05	0.03	0.02
β-Pinene	N.S.	0.06	0.07	0.02	0.02
β-Myrcene	N.S.	0.03	0.03	0.03	0.02
3-Carene	***	0.84	0.75	0.33	0.30
α-Terpinene	***	0.86	0.95	0.32	0.26
Limonene	*	0.45	0.40	0.37	0.30
γ-Terpinene	**	0.19	0.16	0.05	0.05
Fenchone	**	0.10	0.11	0.03	0.03
Nonanal	***	0.20	0.22	0.04	0.03
Camphor	N.S.	0.05	0.06	0.01	0.01
Terpinen-4-ol	***	0.10	0.11	0.01	0.01
α-Terpineol	***	0.83	0.73	0.12	0.10
Dodecane	***	0.41	0.35	0.11	0.10
Total	***	5.13	4.95	2.36	1.97

ANOVA: analysis of variance; PPCJ: pure pomegranate commercial juice; CPN: commercial pomegranate nectar; c-SSPJ: control sour–sweet pomegranate juice; and s-SSPJ: sucrose-treated sour–sweet pomegranate juice.
Values followed by the same letter, within a raw, are not significantly different at $p < 0.05$.
N.S.: not significant F ratio ($p < 0.05$) and *** and ** significant at $p < 0.001$ and 0.01, respectively.

Table 5. Main sensory attributes of commercial sweet and fresh sour–sweet pomegranate juices evaluated by a trained panel and overliking evaluated by a consumer panel

		Commercial juices		Fresh sour–sweet pomegranate (cv. C25) juices	
Attribute	ANOVA	PPCJ	CPN	c-SSPJ	s-SSPJ
Trained panel					
Colour	***	3.3 d	4.5 c	8.8 a	7.4 b
Fresh PG odour	N.S.	5.3	5.5	5.8	4.3
Caramel odour	**	8.6 a	7.6 a	1.0 b	2.0 b
Fresh PG flavour	***	6.3 b	3.5 c	7.0 a	6.0 a
Caramel flavour	***	6.8 b	7.5 a	1.3 c	1.8 c
Sweetness	**	5.8 b	6.5 a	3.6 c	7.3 a
Sourness	**	4.8 b	3.3 c	7.0 a	6.3 a
Astringency	**	7.0 a	4.8 b	3.3 c	3.1 c
Consumer panel					
Overall liking	***	6.6 a	4.3 b	7.6 a	6.8 a

ANOVA: analysis of variance; PPCJ: pure pomegranate commercial juice; CPN: commercial pomegranate nectar; c-SSPJ: control sour–sweet pomegranate juice; s-SSPJ: sucrose-treated sour–sweet pomegranate juice; and PG: pomegranate.
N.S.: not significant F ratio ($p < 0.05$) and *** and ** significant at $p < 0.001$ and 0.01, respectively. Values followed by the same letter, within a raw, are not significantly different at $p < 0.05$.

and (b) HD (Calín-Sánchez et al., 2011) and different compounds predominated in SPME: hexanal, limonene, *trans*-2-hexenal and *cis*-3-hexenol and HD: trans-2-hexenal, 3-carene, α-terpinene and α-terpineol. These differences reported in these studies proved how difficult is to obtain a true image of the volatile compositions of foods.

Unfortunately, the compounds with low odour thresholds and positive sensory descriptors, such as α-pinene and β-myrcene, were also found in the pomegranate juices in very low concentrations. According to the literature, a compound positively contributes to the global odour of a food if the log of the odour units (concentration/odour threshold) is positive (Alonso et al., 2009b; Baldwin et al., 2000); only 3-carene and α-terpineol were present in concentrations high enough to positively contribute to the aroma of pomegranate juices.

Sensory evaluation

Even though HD did not allow the isolation of furans or pyrazines, the descriptive sensory analysis of juices showed that commercial samples were characterised by significantly higher ($p < 0.001$) intensities of caramel odour and flavour than freshly squeezed juices (Table 5); these caramel notes could be originated during the soft pasteurisation of juices. Besides, commercial juices from *Mollar de Elche* fruits were characterised by high intensities of sweetness, astringency and low levels of sourness.

Sensory results from the trained panel (Table 5) proved that fresh juices prepared using Spanish sour–sweet pomegranate fruits (c-SSPJ) led very good sensory results and that these juices were characterised by *high intensities* of colour (8.8, intense garnet colour), fresh pomegranate odour (5.8), fresh pomegranate flavour (7.0) and sourness (7.0), *medium intensity* of sweetness (3.6) and astringency (3.3) and *low scores* of caramel odour (1.0) and caramel flavour (1.3). The addition of sucrose to the juice of sour–sweet fruits resulted in higher intensities of sweetness but at the same time in lower intensities of colour and fresh pomegranate odour and flavour, finally leading to slightly lower but not statistically significant overall liking by consumers.

The *overall liking* of the consumer panel regarding sour–sweet fresh pomegranate juices (c-SSPJ) was 7.6 in a 0 to 10 scale (Table 5). Fresh pomegranate juice with sucrose addition (s-SSPJ) was the sample with the second overall liking score (6.8). In general, Spanish and USA consumers are not willing to consume juices from sour or sour–sweet varieties (Vázquez-Araújo et al., 2010); however, fresh juices from selected sour–sweet cultivars of high quality (such as C25) have proved to be useful for the manufacturing of commercial pomegranate juices. The level of sourness of C25 fruits was useful in improving the typical freshness, colour, odour and flavour of pomegranate juices, but it was low enough not to cause rejection by consumers.

CONCLUSIONS

Spanish sour–sweet pomegranates could be useful for juice manufacturing because they presented high levels of antioxidant activity, proper volatile composition, high intensities of positive sensory attributes and high overall liking by consumers. The sourness of the sour–sweet pomegranate juices could also be useful in reducing the sometimes excessive sweetness of the most cultivated sweet Spanish pomegranate cultivar, *Mollar de Elche*.

FUNDING

This study was funded by the UMH Research Group "Calidad y Seguridad Alimentaria".

REFERENCES

AENOR (Asociación Española de Normalización y Certificación). (1997). *Sensory evaluation of food*. UNE Standards. Madrid: AENOR.

Al-Maiman SA and Ahmad D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. *Food Chemistry* 76(4): 437–441.

Alonso A, García-Aliaga R, García-Martínez S, et al. (2009a). Characterization of Spanish tomatoes using aroma compostion and discriminant analysis. *Food Science and Technology International* 15(1): 47–55.

Alonso A, Vázquez-Araújo L, García-Martínez S, et al. (2009b). Volatile compounds of traditional and virus-resistant breeding lines of Muchamiel tomatoes. *European Food Research and Technology* 203(2): 315–323.

Andreu Sevilla AJ, Signes Pastor AJ and Carbonell Barrachina AA. (2008). La granada y su zumo. Producción, composición y propiedades beneficiosas para la salud. *Alimentación, Equipos y Tecnología* 234: 36–39.

Arnao MB, Cano A and Acosta M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry* 73(2): 239–244.

Baldwin EA, Scott JW, Shewmaker C, et al. (2000). Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. *HortScience* 35(6): 1013–1022.

Borochov-Neori H, Judeinstein S, Tripler E, et al. (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. *Journal of Food Composition and Analysis* 22(3): 189–195.

Calín-Sánchez A, Martínez JJ, Vázquez-Araújo L, et al. (2011). Volatile composition and sensory quality of Spanish pomegranates (*Punica granatum* L.). *Journal of the Science of Food and Agriculture* 91(3): 586–592.

Çam M, Jisil Y and Durmaz G. (2009). Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food Chemistry* 112(3): 721–726.

Díaz-Mula HM, Castillo S, Martinez-Romero D, et al. (2009). Sensory, nutritive and functional properties of sweet cherry as affected by cultivar and ripening stage. *Food Science and Technology International* 15(6): 535–543.

Gil MI, Tomas-Barberan FA, Hess-Pierce B, et al. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry* 48(10): 4581–4589.

Kaur C and Kapoor HC. (2001). Antioxidant in fruits and vegetables - the millenium's health. *International Journal of Food Science and Technology* 36(7): 703–725.

Martinez JJ, Melgarejo P, Hernandez F, et al. (2006). Seed characterisation of five new pomegranate (*Punica granatum* L.) varieties. *Scientia Horticulturae* 110(3): 241–246.

McLafferty F. (2000). *Wiley Registry of Mass Spectral data*, 7th edn. New York, NY: John Wiley & Sons Inc.

Melgarejo P, Calín-Sánchez A, Vázquez-Araújo L, et al. (2011). Volatile composition of pomegranates from 9 Spanish cultivars using headspace solid phase Microextraction. *Journal of Food Science* 76(1): S114–S120.

Melgarejo P and Martínez R. (1992). *El Granado*. Madrid, Spain: Mundi-Prensa.

Melgarejo P and Salazar DM. (2003). *Tratado de Fruticultura para Zonas Áridas y Semiáridas*. Madrid, Spain: Mundi-Prensa.

Melgarejo P, Salazar DM and Artés F. (2000). Organic acids and sugars composition of harvested pomegranate fruits. *European Food Research and Technology* 211(3): 185–190.

Miguel G, Dandlen S, Antunes D, et al. (2004). The effect of two methods of pomegranate (*Punica granatum* L) juice extraction on quality during storage at 4 °C. *Journal of Biomedicine and Biotechnology* 2004(5): 332–337.

Mirdehghan SH, Rahemi M, Serrano M, et al. (2006). Prestorage heat treatment to maintain nutritive and functional properties during postharvest cold storage of pomegranate. *Journal of Agricultural and Food Chemistry* 54(22): 8495–8500.

Mirdehghan SH, Rahemi M, Serrano M, et al. (2007). The application of polyamines by pressure or immersion as tool to maintain functional properties in stored pomegranate arils. *Journal of Agricultural and Food Chemistry* 55(3): 755–760.

NIST. (2010). Search for species data by chemical name. Available at: http://webbook.nist.gov/chemistry/name-ser.html (accessed March 2011).

Ozgen M, Durgaç C, Serçe S, et al. (2008). hemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. *Food Chemistry* 111(3): 703–706.

Poyrazoglu E, Gokmen V and Artik N. (2002). Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. *Journal of Food Composition and Analysis* 15(3): 567–575.

Raisi A, Aroujalian A and Kaghazchi T. (2008). Multicomponent pervaporation for volatile aroma compounds recovery from pomegranate juice. *Journal of Membrane Science* 322(2): 339–348.

Ruiz JJ, Cuenca L, García-Martínez S, et al. (2005). Reclutamiento, entrenamiento y validación de un panel de catadores especializado en tomate en fresco. *Agrícola Vergel* 286(Octubre): 475–485.

Sayyari M, Castillo S, Valero D, et al. (2011). Acetyl salicylic acid alleviates chilling injury and maintains nutritive and bioactive compounds and antioxidant activity during postharvest storage of pomegranates. *Postharvest Biology and Technology* 60(2): 136–142.

Scalzo J, Politi A, Pellegrini N, et al. (2005). Plant genotype affects 387 total antioxidant capacity and phenolic content in fruit. *Nutrition* 21(2): 207–213.

Serrano M, Guillén F, Martínez-Romero D, et al. (2005). Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *Journal of Agricultural and Food Chemistry* 53(7): 2741–2745.

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

Tomás-Barberán FA and Espín JC. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture* 81(9): 853–876.

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

Vardin H and Fenercioglu H. (2003). Study on the development of pomegranate juice processing technology: clarification of pomegranate juice. *Nahrung* 47(5): 300–303.

Vázquez-Araújo L, Chambers IV E, Adhikari K, et al. (2010). Sensory and physico-chemical characterization of juices made with pomegranate and blueberries, blackberries and raspberries. *Journal of Food Science* 75(7): S398–S404.

Vázquez-Araújo L, Koppel K, Chambers IV E, et al. (2011). Instrumental and sensory aroma profile of pomegranate juices from the USA: differences between fresh and commercial juice. *Flavour and Fragrance Journal* 26(2): 129–138.

Vázquez-Araújo L, Verdú A and Carbonell-Barrachina AA. (2008). Aroma volatiles of "a la piedra" turrón. *Flavour and Fragrance Journal* 23(2): 84–92.

Wu X, Beecher GR, Holden JM, et al. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry* 52(12): 4026–4037.

Zhang Y, Krueger D, Durst R, et al. (2009). International multidimensional authenticity specification (IMAS) algorithm for detection of commercial pomegranate juice adulteration. *Journal of Agricultural and Food Chemistry* 57(6): 2550–2557.