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The effect of fruit processing and enzymatic treatments on pomegranate juice composition, antioxidant activity and polyphenols content

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ABSTRACT

Pomegranate fruit and pomegranate juices have recently received great attention for their health benefits, mainly due to their high polyphenols content and the related antioxidant capacity. Nevertheless, the pomegranate processing and its effects on juice composition has been only little investigated. In this paper two important steps of pomegranate juice production, the separation of arils from peels (cleaning) and the enzymatic clarification of the juice, were considered, and the characterization (pH, acidity, total soluble solids, protein, polyphenols and antioxidant activity) of pomegranate juices obtained in different cleaning and clarification conditions was reported. Results showed that the cleaning procedures largely affect the juice composition and its organoleptic properties, and a significant positive correlation was found among peels content before pressing, total polyphenols, antioxidant capacity and perceived bitterness of the juice. Juice with endocarp and mesocarp content of 2 and 10 g/100 g and only mesocarp of 10 g/100 g showed a TEAC value of 14.1 and 13.1 mmol/L, respectively, significantly higher compared to juice with no peels (9.5 mmol/L). Treatment with pectolytic enzymes resulted in a better extraction of all the fruit components, as polyphenols (about 2-fold) and a decrease of turbidity (−40%).

1. Introduction

Pomegranate (*Punica granatum* L., Punicaceae) is one of the oldest edible fruits that widely grows in many tropical and subtropical countries, but only in the last ten years a great research and commercial interest have been focused on its composition and biological properties. Nowadays, pomegranate juice (PJ) is often marketed in health shops and groceries with claims related to well-being, mainly rising from its antioxidant properties, contributing to the prevention of cardiovascular diseases and cancers (Basu & Penugonda, 2009; Esmaillzadeh, Tahbaz, Gaieni, Alavi-Majd, & Azadbakht, 2004; Heber, 2008; Kim et al., 2002; Lansky & Newman, 2007; Sartippour et al., 2008).

After the health beneficial properties of pomegranate compounds had been well-established by scientific researches, market demand for pomegranate and products thereof showed a considerable increase, especially for pomegranate juice. At the same time, together with industries producing PJ, also an increase of pharmaceutical companies and food supplements producers, which extracted health beneficial compounds from this fruit, was worldwide observed (Seeram, Zhang, & Heber, 2006).

Pomegranate juice is primarily considered an important source of phenolic compounds: the soluble polyphenols content varies from 0.2 to 1.0 g/100 g, being anthocyanins one of the most important (Du, Wang, & Francis, 1975; Miguel, Fontes, Martins, Neves, & Antunes, 2007) together with lignans (Bonzanini, Bruni, Palla, Serlataite and Caligiani. 2009), gallagyl-type tannins, ellagic acid derivatives, and other hydrolysable tannins which contribute to the antioxidant activity of the juice (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000; Heber, 2008).

The high polyphenols content resulted in a high antioxidant activity: in general, common fruit juices or extracts show an antioxidant activity in vitro very lower than that of the pomegranate (Lansky & Newman, 2007). Gil et al. (2000) reported that the antioxidant capacity of commercial pomegranate juices was three times higher than that of red wine and green tea. However, great variability on antioxidant activity and related polyphenols content was observed among different cultivars (Cam, Hisil, & Durmaz, 2008).

The composition of pomegranate juice depends on many factors, such as cultivar type (over thousand cultivars of *P. granatum* are known), environment, postharvest, storage and, last but not

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least, processing conditions (Heshi, Garande, Wagh, & Katore, 2001; Melgarejo, Salazar, & Artés, 2000).

Even if exhaustive works can be found in the literature on the health effects of pomegranate juice, few studies about the processing effect on the composition and variability of PJ are available, as well as studies aimed to improve the production process in order to enhance the health characteristics.

The classic production process of concentrated pomegranate juice involves peduncle removal, fruit washing, separation of arils from peels (cleaning), pressing, clarification, concentration and finally pasteurization. From a technological point of view, pomegranate is one of the most difficult fruits to process, due to the difficult to separate the arils from the peels (mesocarp and endocarp) and to the risk that an incorrect technology could contaminate the juice with tannic elements, with great quality damages. Nevertheless, the contact with peels and membranes during arils squeezing may result in a major extraction of the polyphenol and tannin fraction, mainly present in these fruits parts (Rosenblat & Aviram, 2006).

Generally, untreated pomegranate juices present an excessive turbidity, and a clarification is required during processing. Clarification can be obtained by means of several methods: simple filtration, precipitation of polyphenols and/or enzymation processes.

In this paper, experimental pomegranate juices obtained from different cleaning and enzymatic clarification steps were analysed for their polyphenols and protein content and for other quality parameters including pH, total soluble solids and acidity, in order to correlate the technology to the composition of the juices, and find the better processing conditions to increase the extraction of polyphenol and other compounds with health beneficial effects without exceeding the bitterness threshold accepted by consumers.

2. Materials and methods

2.1. Pomegranate juices preparation

Pomegranates fruits (*P. granatum* cv. Wonderful) of uniform size and appearance were washed and manually peeled, by separating and weighting the four components: arils (83.2 ± 13.4 g/100 g), endocarp, mesocarp (14.2 ± 2.6 g/100 g) and epicarp (2.6 ± 0.7 g/100 g). Then, the fruit components were manually mixed in different proportions, as reported in Table 1, on the basis of the relative content of components and previous cleaning tests on industrial plant.

The pomegranate juices were extracted by pressing the various mixtures (Vincent Corporation, Florida, USA) and immediately stored at −20 °C in the dark.

In addition, the juice obtained from 100 g/100 g of arils (PJ7) from a second lot of fruits was further treated with a pectolytic enzymes solution (ERBSLÖH Geisenheim, Germany) for 120 min at three different concentrations (10, 12.5 or 15 μL of enzyme solution per litre of PJ), at 25 °C. Samples in the text are named PJ7E0 for the non treated juice (control), PJ7E1, PJ7E2 and PJ7E3 for samples treated with the reported increasing amounts of enzyme solution.

Table 1
Composition (g/100 g) of the raw materials utilized for pomegranate juice preparation. Separation and recombination of the fruit parts were performed manually.

Raw material	Arils	Endocarp	Mesocarp	Epicarp	Corresponding juices
1	52.5	2	17	28.5	PJ1[a]
2	86	1	13	0	PJ2
3	88	2	10	0	PJ3
4	90	0	10	0	PJ4
5	94	1	5	0	PJ5
6	98	2	0	0	PJ6
7	100	0	0	0	PJ7

[a] PJ1 juice was obtained from whole fruit.

2.2. Determination of pH, titrable acidity, soluble solids and proteins

pH was measured by means of a pH meter (Jenway, Staffordshire, United Kingdom). Titrable acidity was calculated as percentage of citric acid by titrating 10 mL of the pomegranate juice with NaOH (0.1 mol/L) reaching pH 8.1. The level of soluble solids was measured as Brix degrees by a refractometer (Optika, Ponteranica, Bergamo, Italy). Protein content was determined on 10 mL of juice, according to the Kjeldahl method (AOAC, 1995), utilizing 6.25 as nitrogen conversion factors.

2.3. Total polyphenols and antioxidant activity determination

Total phenolic compounds were determined by using the Folin-Ciocalteu method (Singleton & Rossi, 1965). Briefly, 1 mL of pomegranate juice was exactly diluted to 10 mL with distilled water, then 200 μL of the diluted juice was mixed with 0.5 mL of Folin–Ciocalteu reagent, alkalinized with 1 mL of 20 g/100 g sodium carbonate solution and taken to 10 mL with distilled water. The mixture was allowed to stand for 60 min at room temperature, then the absorbance was measured by a UV/vis spectrophotometer (Perkin-Elmer Waltham, Massachusetts, USA) at 760 nm. Total phenolics were calculated against a calibration curve obtained with gallic acid and results were reported as gallic acid equivalents (mg/L).

The antioxidant activity was measured by Trolox-Equivalent Antioxidant Capacity (TEAC) assay according to Pellegrini et al. (2003). Results were expressed as TEAC in mmol of Trolox per litre of sample.

2.4. Turbidity analysis

The turbidity of pomegranate juices was measured with a turbidity-meter (Aqualytic GmbH, Dortmund, Germany) at room temperature according to the instructions. Results are expressed as Nephelometric Turbidity Unit (NTU).

2.5. Sensorial analysis

A consumer preference test was performed over two consecutive days with a total of 48 subjects recruited from the University of Parma and familiar with sensory tests (28 males, 20 females: average age 23 ± 2). The participants were asked to refrain from eating, smoking, drinking or chewing gum for 1 h prior to testing.

A triangle forced choice procedure (Meilgaard, Civille, & Carr, 1999) was used to determine if subjects could distinguish between samples of pomegranate juice from different peeling technologies. Four consecutive triangle tests were performed and each subject was presented with a set of three samples, two identical ones and a different one. Each sample was identified by a 3-digit code and the order of presentation was randomized and could have been any of six possible combinations (XXY, XYY, XYX, YXX, YYX or YXY). Participants were requested to determine which sample was the odd one on the basis of bitterness. Pomegranate juice obtained from 100 g/100 g arils (PJ7) was used as reference sample for all triangle tests. Participants were also asked to wait for 10 min between each triangle test.