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Effect of extraction method on chemical, volatile composition and antioxidant properties of pomegranate juice

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ABSTRACT

The study investigated chemical, volatile composition and bioactive compounds extracted from different fruit fractions of pomegranate (*Punica granatum* L.) cv. Wonderful. Juice variants evaluated included juice extracted without crushing the seeds using a juice extractor (arils), juice extracted by crushing the seeds using a blender (arils plus seed), juice extracted by pressing a whole fruit using a squeezer (whole fruit) and juice extracted from halved fruit using a commercial hand press juicer (halved fruit). There were no significant differences ($P > 0.05$) in total soluble solids (°Brix) content in pomegranate juice obtained from different fruit fractions. Juice extracted from halved fruit had higher titratable acidity (1.78 mg citric acid/100 mL), lower pH content (1.58) and juice yield (28.01%). The lowest citric acid content was observed in blended juice (18.96 g/L) and high juice colour (2.69). Fructose content did not vary in all extraction methods. Catechin and epicatechin were the most dominant flavonoids whereas gallic acid was the dominant phenolic acid identified in all extraction methods. The total phenolics, tannins, flavonoids and anthocyanin content in the investigated juice ranged from 138.36 to 289.94 mg gallic acid equivalent/100 mL, 120.00 to 267.10 mg gallic acid equivalent/100 mL, 23.35 to 50.39 mg catechin equivalent/100 mL, and 10.96 to 13.91 mg cyanidin 3-glucoside equivalent/100 mL crude juice, respectively. Furthermore, halved fruit juice had high radical scavenging activity and ferric reducing antioxidant power. The most abundant volatile compounds were ethyl acetate (21.35–31.45%) and 3-octanone (8.12–18.74%) in all the juice variants. Principal component analysis (PCA) also revealed that the chemical, volatile and bioactive compounds separated the investigated juice extraction method. The results of the study provide information on the importance of methods of extraction on the quality of pomegranate juice.

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest recognized edible fruit belonging to Punicaceae family. To date, pomegranate is widely grown in areas such as Iran, India, Egypt, Lebanon, China, Spain, France, USA, Oman, Syria, Tunisia, Italy, Greece, Cyprus, Israel, Turkey, Chile, Portugal and South Africa (Al-Said et al., 2009; Holland et al., 2009; Fawole and Opara, 2013a,b). Currently, South Africa's commercial production of pomegranate fruit stands at 758,330 cartons (Hortgro, 2015). Pomegranate fruit has gained popularity in the past 15 years due to its valuable source of polyphenols when equated with other compound rich beverages such as wine and green tea (Gil et al., 2000; Fischer et al., 2013).

Pomegranate fruit is a rich source of bioactive compounds including phenolic acids, tannins, flavonols and anthocyanins (Viuda-Martos et al., 2010), and consumption has intensified because of their role in promoting health by reducing the risk of atherosclerosis, cancer, diabetes and neurodegenerative disorders (Miguel et al., 2010; Viuda-Martos et al., 2010). Moreover, these bioactive compounds (phenolic acids, flavonoids and hydrolysable tannins) were found to be present in higher amounts, in particular, high content of hydrolysable tannins. These are reported to be mainly located in the fruit peel and mesocarp (Fischer et al., 2011). Research has shown that these compounds may be scavengers of reactive species, thus exhibiting antioxidant activity (Fawole et al., 2012; Fischer et al., 2013).

Generally, pomegranate similar to any other fruit is not only available as fresh arils but also widely distributed as processed products such as juice, jams, anardana, carbonated drinks, garnish and deserts (Al-Maiman and Ahmad, 2002; Opara et al., 2009). The edible parts of

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pomegranate fruit (50%) comprised 40% arils (juice sacs) and 10% seeds. Arils contain 85% water, 10% total sugars (fructose and glucose), organic acid (ascorbic acid, citric acid, and malic acid), and bioactive compounds such as phenolics and flavonoids (anthocyanins) (Viuda-Martos et al., 2010).

The desire of the consumers to maintain a diet which promotes better health has increased the demand of juices that preserve their natural nutritive value. Therefore, alternative processing methods which potentially increase nutritive properties are necessary. Bioactive concentration and composition of pomegranate juice are strongly influenced by cultivar, climatic conditions, maturity status and juice extraction methods (Turfan et al., 2011; Caleb et al., 2012; Rajasekar et al., 2012; Fawole and Opara, 2013a,b; Mphahlele et al., 2014a,b). More recently, several methods of juice extraction such as juice processing from the whole and separated aril sacs have been explored (Miguel et al., 2004; Muhacir-Güzel et al., 2014). These researchers have shown that high amount of polyphenolic compounds were found in juice extracted from the whole fruit whereas juice from arils only had the least. Similarly, Fischer et al. (2011) found higher total polyphenol and hydrolysable tannins contents in juice from whole fruit than those from arils only due to migration of phenolic compounds from rind during pressing the fruit. However, the varietal differences on the polyphenol contents were also observed amongst the studies. Tzulker et al. (2007) reported 20 and 6.5-fold higher antioxidant activity in juice obtained from the whole fruit and aril only juice, respectively.

Pomegranate fruit have different fractions including pith, carpellary membrane and the peel. These fractions have broad group of compounds with health beneficial effects than part considered edible by consumer. There have been research findings on preharvest and postharvest management of pomegranate cv. Wonderful grown in South Africa but less attention has been given to individual phenolic concentrations and volatile composition resulting from juice processing of pomegranate fruit. The objective of the study was to investigate the effect of extraction method on the chemical properties, volatile organic compounds and bioactive compounds of pomegranate juice cv. 'Wonderful'.

2. Materials and methods

2.1. Plant material

Pomegranate fruit (cv. Wonderful) were obtained during commercial harvest from Sonlia Pack-house (33°34′851″S, 19°00′360″E) in the Western Cape, South Africa. Fruit were transported in an air-conditioned car to the Postharvest Technology Research Laboratory at Stellenbosch University. Fruit were stored at 7.5 ± 0.5 °C, 92 ± 3% RH for less than five days before processing.

2.2. Sample preparation

Fruit of the same size without any physical defects were randomly selected and washed with tap water before processing. Four extraction methods were employed as illustrated in Table 1. A total of 30 fruit were used for each extraction method. Fruit weight, peel, aril and seed proportion are highlighted in Table 2. All the extraction were performed three times and then immediately stored at −80 °C until analysis. Juice yield was calculated according to Türkyılmaz et al. (2013) using Eq. (1).

$$\text{Juice yield} = (\text{weight of unclarified pomegranate juice} \div \text{weight of pomegranate with rinds}) \times 100 \quad (1)$$

2.3. Chemical composition

2.3.1. Total soluble solids (TSS), titratable acidity (TA), pH and juice colour

Pomegranate juice total soluble solid in (°Brix) was measured using digital refractometer (Atago, Tokyo, Japan, calibrated with distilled water) at 20 °C. A metrohemn 862 compact titrosampler (Herisau, Switzerland) was used to determine titratable acidity (g citric acid (CA)/100 mL). A juice sample of approximately 2 mL was diluted with 70 mL of distilled water and titrated with 0.1 N of NaOH to the endpoint of pH 8.2. The pH was carried at room temperature with a pH metre (Crison, Barcelona, Spain). Juice colour absorbance was measured at a wavelength of 520 nm using spectrophotometer (Thermo Scientific, Madison, USA). Fruit maturity index was determined as the ratio between TSS and TA.

Table 1
Extraction techniques and fruit fractions used to obtain juice from pomegranate fruit cv. Wonderful.

Fruit fraction	Type of equipment and manufacturer	Description
1. Arils	LiquaFresh juice extractor (Mellerware, South Africa)	Juice was extracted from the arils by spinning at a minimum speed the arils without crushing the seeds (kernels)
2. Arils plus seeds	Electronic blender (AEG, Germany)	Arils were blended at a maximum speed using electronic blender (AEG) for approximately 30 s which ensured that the seeds were broken together with the arils
3. Whole fruit	A Lansmont squeezer compression (Lansmont Corporation, Monterey, CA, USA)	Juice was obtained by pressing the whole fruit (without cutting the fruit) at a force of 15,000 N for 5 min
4. Halved fruit	Commercial juicer press (hand press) (Jupiter, China)	Fruit halves were individually hand pressed. In this case, the pith, carpellary membrane and arils were consistently included during the extraction process

2.3.2. Sugars and organic acids

A Thermo Scientific Arena 20XT random access chemistry analyser was used for enzyme robot assays. The organic acids including $_{L}$-malic, succinic and citric and sugars (D-glucose, D-fructose and sucrose) contents were determined by enzymatic test kits (R-Biopharm AG, Germany), measuring the formation of NADPH at 340 nm, according to the described protocol of the kits.

2.3.3. Determination of phenolic acid, flavonoids and individual anthocyanin content

LC-MS and LC-MS^{E} analyses were conducted on a Waters Synapt G2 quadrupole time-of-flight mass spectrometer system (Milford, MA, USA). The instrument was connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) and Acquity photo diode array (PDA) detector. The gradient for the analysis of phenolic compounds started with 100% using 0.1% (*v*/*v*) formic acid (solvent A) and kept at 100% for 0.5 min, followed by a linear gradient to 22% acetonitrile (solvent B) over 2.5 min, 44% solvent B over 4 min and finally to 100% solvent B over 5 min. The column was subjected to 100% solvent B for an extra 2 min. The column was then re-equilibrated over 1 min to yield a total run time of 15 min. Reference standards (Sigma-Aldrich, South Africa) of flavonoids and phenolic acids were used for the

Table 2
Average fruit fractions of pomegranate cv. Wonderful.

Fruit fraction	Weight (g)	Peel proportion (%)	Aril proportion (%)	Seed proportion (%)
Arils	331.92 ± 6.83	–	53.79 ± 0.82	–
Arils plus seeds	341.78 ± 15.04	–	52.74 ± 3.10	29.62 ± 1.44
Whole fruit	367.82 ± 22.63	50.38 ± 0.25	48.89 ± 0.50	–
Halved fruit	343.43 ± 3.95	49.33 ± 2.51	49.00 ± 3.29	–

Average of 30 fruit were used for juice extraction.