

Original article

Phenolic contents, antioxidant activities and potential bioaccessibilities of industrial pomegranate nectar processing wastesEce Surek^{1,2*} & Dilara Nilufer-Erdil¹

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Summary Antioxidant potential and bioaccessibility of co-products from industrial pasteurised pomegranate nectar (PN) processing such as peel (PP), press cake (PC) and precipitate after clarification (PAC) in comparison with raw material (arils) and final products (CON and PN) were determined. Total phenolic (TPC), flavonoid (TFC), anthocyanin (TAC), tannin contents (TTC) and antioxidant activity (TAA) were determined besides identifying major phenolics and investigating *in vitro* bioaccessibility after gastrointestinal (GI) digestion. PP showed the highest values, except for TAC. Phenolics (12.7–43.0%) were found to be more stable than anthocyanins (0.6–2.1%) after *in vitro* GI digestion. PAC was found to be a better source for anthocyanins than CON and also showed higher phenolic bioaccessibility (28.8%) than PN (19.6%). PC and PAC possessed as much TPC, TFC, TTC and TAA levels as CON, with some exceptions. Therefore, these results indicated that not only PP but also PC and PAC should be valorised as a good source for phenolics and anthocyanins.

Keywords Antioxidant activity, bioaccessibility, co-product, *in vitro* digestion, nectar, peel, pomegranate, precipitate, press cake, waste.

Introduction

Pomegranate (*Punica granatum*) is a potential antioxidant source, and each of its parts (peel, seeds, arils and inner peel) have valuable compounds providing several functional and medicinal properties to this fruit. It can act as antitumoral, antidiabetic, antihepatotoxic and antimicrobial (Viuda-Martos *et al.*, 2010). It consists of 60–67% arils and 33–40% peel (Adsule & Patil, 1995). Juice can be produced from arils as well as the whole fruit (Adsule & Patil, 1995). Pomegranate contains polyphenols such as flavonoids (flavonols, flavanols, anthocyanins), condensed tannins (proanthocyanidins), hydrolysable tannins (ellagitannins and gallotannins) and other phytochemicals such as organic and phenolic acids, sterols, fatty acids, triglycerides and alkaloids (Elfalleh *et al.*, 2011). Pomegranate can be processed into many different products such as canned arils, dried arils, seeds, syrup, jam, sour sauce, concentrate and nectar in addition to its consumption as a fresh fruit (Adsule & Patil, 1995).

As pomegranate cannot be grown at all seasons, it can be consumed for longer durations when postharvest treatments such as optimum cold storage, modified atmosphere packaging and controlled atmosphere are applied (Selcuk & Erkan, 2015) or it is processed into different products. Due to the fact that bioavailability and antioxidant potential of pomegranate products should also be investigated. Although there are several studies focusing on the processing steps of pomegranate juice (Gil *et al.*, 2000; Alper *et al.*, 2005; Alighourchi *et al.*, 2008; Turfan *et al.*, 2011; Rinaldi *et al.*, 2013) or nectar (Surek & Nilufer-Erdil, 2014), there is limited research on investigating the potential antioxidant power or polyphenol contents of pomegranate co-products (Viuda-Martos *et al.*, 2011), except peel. Polyphenol content, antioxidant activity or bioaccessibility of some industrial fruit juice processing wastes have been analysed by many researchers. According to Lee & Wrolstad (2004), blueberry juice processing wastes showed high total anthocyanin and phenolic content. On the other hand, waste residues such as press cake had very low levels of the main anthocyanins during sour cherry nectar processing (Toydemir *et al.*, 2013). Suzme *et al.* (2014) investigated

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effect of juice processing on polyphenols and antioxidant capacity of black carrot. There was no difference between raw material and press cake according to total flavonoid content and antioxidant capacity; however, press cake had higher total phenolic and anthocyanin content than raw material. There is limited research on potential bioavailability of pomegranate bioactive compounds (Perez-Vicente *et al.*, 2002; Sengul *et al.*, 2014; Xuan *et al.*, 2014). Perez-Vicente *et al.* (2002) and Sengul *et al.* (2014) researched *in vitro* bioavailability of anthocyanins and total phenols of pomegranate juice and pomegranate fruit, respectively. Phenolics were found to be stable during gastric conditions; however, 29% of juice and 14% of fruit were available in the dialysed fraction (IN). Anthocyanins of pomegranate were lost in pancreatic digestion (38%), but available (12%) in IN (Sengul *et al.*, 2014). In the research by Xuan *et al.* (2014), the loss of anthocyanins of pomegranate wine was low during gastric digestion; however, 25% of anthocyanins were available after gastrointestinal (GI) digestion.

In this study, the main purpose was to determine the polyphenol contents, antioxidant activities and major phenolic compound profiles of pomegranate co-products such as peel, press cake and precipitate after clarification which is obtained from industrial scale pasteurised pomegranate nectar processing and comparing with that of arils, and products such as concentrate and pasteurised nectar. Also, the other purpose was to compare the potential bioavailability of arils, co-products and final products after simulating *in vitro* GI digestion, for better understanding the real effects in human metabolism and the potential of co-products to be used in other applications.

Materials and methods

Chemicals

Gallic acid, Quercetin, Catechin, pepsin, pancreatin, bile salts, Quercetin-3- β -D-glucoside and Pelargonidin 3-O-glucoside from Sigma-Aldrich (Steinheim, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Ferulic acid and Quercetin-3-O-galactoside from Fluka (Buchs, Switzerland); sodium bicarbonate from BDH Chemicals (Poole, UK); Cyanidin 3-O-glucoside, Pelargonidin 3,5-di-O-glucoside, Cyanidin 3,5-di-O-glucoside, Delphinidin 3-O-glucoside and Delphinidin 3,5-di-O-glucoside from Extrasynthese (Genay, France) were purchased for *in vitro* GI digestion and other analyses.

Materials

Pomegranate (cv. Hicaznar) samples were taken from industrial scale pasteurised pomegranate nectar (PN) manufacturing plant which processed pomegranate

grown in Karaman region of Turkey. Duplicate samples were taken from two different productions. Processing steps were peeling, mashing of pomegranate arils to inactivate the enzymes (80 °C, 90 s), pressing, cooling (4 °C, 25–30 min), pasteurisation (90–95 °C, 90 s), cooling (to 50–55 °C), enzyme application (37.5 mL pectinolytic enzyme/ton juice), clarification (1.2 kg bentonite/ton juice and 780 g gelatin/ton juice at 50 °C, 1 h), ultrafiltration, evaporation (from 12.5 ° to 65 °Brix at 65–80 °C), production of nectar by adding sucrose, citric acid and water and dilution to 12.5 °Brix, and pasteurisation of nectar (95 °C, 45 s), respectively. Samples were taken from pomegranate peel (PP), pomegranate arils (PA), press cake (PC), precipitate after clarification (PAC), concentrate (CON) and PN. They were milled under liquid nitrogen using a grinder (IKA, Germany) and stored at –80 °C until analysis.

Moisture content analysis

The analysis was performed as described in Association of Official Analytical Chemists (AOAC) 934.06 method (1997) to express the results in dry matter basis (dmb).

Extraction of phenolic compounds

Extraction procedure was carried out according to Capanoglu *et al.* (2008). One gram of ground sample, except for PP (0.5 g), was extracted with 5 mL of 75% aqueous methanol including 0.1% (v/v) formic acid in an ice-cooled ultrasonic bath (Ultrasonic Cleaner-VWR) for 15 min. They were centrifuged (Hettich Universal 32, Tuttlingen, Germany) for 10 min at 2701 g under 4 °C. It was repeated four times until 20 mL of extract was collected, and extracts were stored at –20 °C until analysis.

Spectrophotometric methods

Total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), total anthocyanin content (TAC) and total antioxidant activity (TAA) analysis were carried out by spectrophotometric methods using UV-Visible spectrophotometer (Shimadzu, Japan).

Total phenolic content was determined using Folin–Ciocalteu method as described previously by Surek & Nilufer-Erdil (2014). The results were expressed as mg Gallic acid equivalents (GAE) per 100 g of sample in dmb.

Total flavonoid content was measured as described by Viuda-Martos *et al.* (2011). The results were expressed as mg Quercetin equivalents (QE) per 100 g of sample in dmb.

Total anthocyanin content and TTC were measured as described by Kar *et al.* (2011), and the results were

expressed as mg Cyanidin 3-O-glucoside (Cyn 3-O-glu) and mg Catechin equivalents (CE) per 100 g of sample in dmb, respectively.

Total antioxidant activity was determined by Cupric Reducing Antioxidant Capacity (CUPRAC), Ferric Reducing Ability of Plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid diammonium salt (ABTS) radical scavenging activity methods. The results were expressed as mg Trolox equivalents (TEAC) per 100 g of sample in dmb. CUPRAC, FRAP, ABTS and DPPH assays were performed according to Apak *et al.* (2006), Benzie & Strain (1996), Miller & Rice-Evans (1997) and Viuda-Martos *et al.* (2011), respectively.

High-performance liquid chromatography (HPLC)–photodiode array (PDA) analysis of major phenolic compounds and anthocyanins

Samples were filtered through a 0.45- μ m membrane filter and then injected into a Waters 2695 reversed phase HPLC system coupled with PDA Waters 2996 detector. Luna C18 column (150 \times 4.60 mm, pore size 100 Å, particle size 5 μ m from Phenomenex (Torrance, CA, USA)) was used as the stationary phase. The mobile phase consisted of solvent A, Milli-Q water with 0.1% (v/v) TFA, and solvent B, acetonitrile with 0.1% (v/v) TFA. The HPLC running condition was based on the study of Surek & Nilufer-Erdil (2014). Quantification was done using external standard curves.

Simulated *in vitro* gastrointestinal digestion

Gastrointestinal digestion was simulated to determine the potential bioavailability of polyphenols in samples. Bioavailability was assessed according to the protocol described by McDougall *et al.* (2005). Release of phytochemicals from pomegranate samples was analysed at different stages of digestion. These stages are the aliquots from gastric digestion (PG, postgastric) and GI digestion (IN and OUT). IN fraction (dialysed part) is the one that entered the serum while OUT fraction (undialysed fraction) is the part remaining in the colon. All fractions obtained were kept at -20°C until further analysis. Prior to analysis they were thawed and centrifuged at 23000 *g* for 20 min. TPC, TAC and TAA analysis by DPPH besides phenolic and anthocyanin profiles were determined for each fraction. The results of extracts were assumed as 100%, and % recovery was calculated for all fractions of all samples.

Statistical analysis

All of the results were reported as mean value \pm standard deviation. Data from duplicate independent samples of two treatments were analysed statistically

using one-way analysis of variance (ANOVA). When significant differences were observed ($P < 0.05$), means were subjected to Duncan's new multiple range test. Analysis was conducted using Statistical Package for the Social Sciences software (version 16.0, SPSS Inc., Chicago, IL, USA).

Results and discussion

Total phenolic, flavonoid, anthocyanin and tannin contents

Results obtained for TPC, TAC, TFC and TTC analysis are given in Table 1. PP being the major waste of processing showed significantly highest TPC, TFC and TTC values; however, for TAC, PA was higher than all ($P < 0.05$). Nectar production step which includes addition of water and sugar resulted in significant losses in all parameters for PN, being the end product when compared to co-products ($P < 0.05$). Viuda-Martos *et al.* (2011) determined TPC, TFC and TTC of bagasse from pomegranate arils and whole fruit as 4.6 ± 0.7 and 10.0 ± 1.2 mg GAE/g fresh weight (FW); 5.7 ± 0.3 and 7.2 ± 0.5 mg Rutin equivalents/g FW; and 6.7 ± 0.3 and 8.2 ± 0.1 mg CE/g FW, respectively. In this study, TPC, TFC and TTC of PC were calculated as 14.5 ± 0.7 mg GAE/g FW, 15.1 ± 0.9 mg QE/g FW and 1.8 ± 0.2 mg CE/100 g FW, respectively, which were different than their study, due to difference in pomegranate variety and production procedure. Ardekani *et al.* (2011), Li *et al.* (2006) and Nasr *et al.* (1996) measured TPC of PP extract as 98.2 ± 4.8 – 226.6 ± 18.9 mg GAE/g DW, 249 ± 17.2 mg Tannic acid/g FW and 216 ± 7.3 mg GAE/g DW. Gullon *et al.* (2015) reported TPC of PP flour as 19.30 mg GAE/g DW. In this study, TPC of PP was found as 180.3 ± 15.7 mg GAE/g DW (55.3 ± 4.8 mg GAE/g FW), which were consistent with Ardekani *et al.* (2011), much higher than Gullon *et al.* (2015) and lower than others. TPC can be different according to several factors such as the cultivar, growing region, climate, maturity, cultivation practice, processing and storage conditions, analysis and extraction methodology used or the solvents employed (Viuda-Martos *et al.*, 2011; Gullon *et al.*, 2015). Ardekani *et al.* (2011) reported that TFC for PP in the range of 18.6 ± 0.5 to 36.4 ± 1.3 mg CE/g DW and Li *et al.* (2006) found 59.1 ± 4.8 mg Rutin/g FW. TFC of PP flour was obtained as 12.27 mg Rutin/g DW by Gullon *et al.* (2015). In this study, TFC of PP was closer to Li *et al.* (2006), but higher than Ardekani *et al.* (2011) and Gullon *et al.* (2015). Preparing calibration curves with different standards is also one of the major reasons for the different findings (Surek & Nilufer-Erdil, 2014). There was no significant difference between TAC values of PP and PC ($P > 0.05$).

Elfalleh *et al.* (2011) reported TAC of Tunisian PP as 73.9 ± 8.2 mg Cyn 3-O-glu/g DW which was higher than the value in this study (51.8 ± 6.4 mg/100 g DW). In particular, TAC in PAC was found to be very high; on the other hand, significant loss in the final product was evident ($P < 0.05$). A previous study by Turfan *et al.* (2012) reported that the reason of the loss of TAC after clarification of pomegranate juice was the interaction between anthocyanins and gelatin-tannin flocks. Mousavinejad *et al.* (2009) quantified TTC of pomegranate juices produced in laboratory as 0.01–0.03 g/100 g FW, which was lower than this study maybe because of using different raw material and producing with different techniques. This study showed that both after pressing and clarification co-products were found to possess similar amounts of TPC, TFC and TTC with CON ($P > 0.05$) and significant higher levels when compared to PN ($P < 0.05$). On the other hand, they were significantly lower than PP and PA ($P < 0.05$).

Total antioxidant activity

Total antioxidant activity analysis by DPPH, CUPRAC, FRAP and ABTS methods is shown in Table 1. In all methods used for the determination of TAA, PP showed higher antioxidant activity than other samples, statistically ($P < 0.05$). This may be explaining because PP had greater concentration of phenols, flavonoids and tannins than others. Therefore, discarding the peel, which is rich in polyphenols, caused important loss in TAA ($P < 0.05$). TAA was significantly lower in the PA, which is used as the raw material, with respect to the PP which is discarded ($P < 0.05$). On the other hand, TAA values for CON, PC and PAC were found to be 2.5-fold to 4.5-fold higher than the TAA of PN which is consumed as the product ($P < 0.05$). According to CUPRAC method, TAA of PC, CON and PAC were lower than PP and PA ($P < 0.05$). By DPPH and FRAP method, TAA was not found to be different for CON, PC and PAC ($P > 0.05$). By ABTS method differently, TAA of CON was found to be significantly different than PC and PAC ($P < 0.05$). Capanoglu *et al.* (2008) reported that TAA of seed and skin of tomato by DPPH was different from CUPRAC but similar to FRAP methods. In the current study, also DPPH results were generally consistent with FRAP. According to FRAP method, PA, CON, PC and PAC showed similar TAA values ($P > 0.05$). When overall evaluation for TAA values was done, it was obvious that CUPRAC method had given the highest responses for TAA followed by DPPH or ABTS. FRAP method generally had the lowest TAA values for those samples. Four assays were applied to assess TAA of pomegranate co-products; however, the values obtained were

Table 1 TPC, TFC, TAC, TTC and TAA of samples obtained from PN production*

Samples	TPC (mg)		TFC (mg)		TAC (mg)		TTC (mg)		TAA (mg TEAC/100 g)			
	GAE/100 g		OE/100 g		Cyn 3-O-glu/100 g		CE/100 g		DPPH	CUPRAC	FRAP	ABTS
PP	18029.2 ± 1568.9 ^a		21758.1 ± 596.8 ^a		51.8 ± 6.4 ^d		1277.3 ± 44.8 ^a		45099.6 ± 4583.1 ^a	85807.6 ± 2246.4 ^a	26622.1 ± 3473.1 ^a	51100.8 ± 2404.4 ^a
PA	4992.1 ± 84.6 ^b		6215.9 ± 1128.1 ^b		187.4 ± 15.2 ^{abf}		747.5 ± 93.4 ^b		14229.3 ± 1197.2 ^b	22209.5 ± 230.2 ^{bf}	5513.4 ± 393.2 ^{bc}	17758.9 ± 1652.8 ^b
PC	2985.4 ± 147.9 ^c		3093.5 ± 192.1 ^c		55.2 ± 4.2 ^d		369.6 ± 45.6 ^d		6147.4 ± 591.7 ^c	12725.8 ± 1487.3 ^d	3692.5 ± 118.2 ^{bc}	3940.0 ± 71.0 ^d
PAC	3492.2 ± 443.5 ^c		3887.4 ± 110.1 ^c		126.7 ± 3.1 ^b		550.4 ± 64.9 ^c		6448.7 ± 329.3 ^c	17249.3 ± 1660.4 ^c	5471.8 ± 624.4 ^{bc}	5132.2 ± 366.8 ^d
CON [†]	3631.3 ± 217.3 ^c		3629.8 ± 287.8 ^c		101.0 ± 10.9 ^c		438.5 ± 99.1 ^{cd}		7040.3 ± 945.4 ^c	16613.3 ± 1352.6 ^c	6412.7 ± 527.1 ^b	11616.4 ± 574.9 ^c
PN [‡]	1005.3 ± 115.5 ^d		949.2 ± 139.7 ^d		26.3 ± 3.3 ^e		80.7 ± 8.6 ^e		1843.5 ± 246.6 ^d	4670.7 ± 480.1 ^e	1659.9 ± 97.1 ^c	1281.4 ± 144.5 ^e

TPC, total phenolic content; TFC, total flavonoid content; TAC, total anthocyanin content; TTC, total tannin content; TAA, total antioxidant activity; PP, pomegranate peel; PA, pomegranate arils; PC, press cake; PAC, precipitate after clarification; CON, concentrate; PN, pasteurised nectar.

*Values are mean of triplicate measurements. Different letters in the same column present significant difference at $P < 0.05$. All contents are expressed per 100 g dry weight.

†Surek & Nilufer-Erdil (2014).

different. It is very difficult to assess TAA of a product on the basis of a single method because the antioxidant mechanism is quite complex and many factors such as solvent, temperature, chemical structure of phenolics and pH of the medium play a role in these mechanisms (Huang *et al.*, 2005; Viuda-Martos *et al.*, 2011). The DPPH assay is based on the reduction of the purple DPPH radical to 1,1-diphenyl-2-picrylhydrazine, which is applicable to hydrophobic systems. ABTS assay has the similar principle with that of DPPH, whereas it is based on the production of stable a blue/green ABTS⁺, which is applicable to both hydrophilic and lipophilic systems by reacting ABTS (pH 4.5) (Floegel *et al.*, 2011; Wootton-Beard *et al.*, 2011). On the other hand, no free radicals are involved in the FRAP and CUPRAC assays, but the reduction of Fe³⁺ to Fe²⁺ at pH 3.6 and the reduction of Cu²⁺ to Cu⁺ at pH 7.0 are observed, respectively (Apak *et al.*, 2006; Floegel *et al.*, 2011). The CUPRAC assay was claimed as more useful than FRAP by Apak *et al.* (2006) due to having end point mechanism. On the other hand, FRAP assay may not be complete even many hours, and thus, a single end point of the reaction cannot be determined (Prior *et al.*, 2005). The ABTS assay is an end point assay; however, different antioxidant compounds donate one or two electrons to reduce the radical cation (Wootton-Beard *et al.*, 2011). Therefore, the measurement of TAA cannot be evaluated sufficiently by a single method, and a mixture of assays should be used.

Major phenolic compounds

Results obtained for major phenolic compounds analysis by HPLC-PDA are shown in Table 2. Major compounds were identified as Gallic acid, Catechin, Ferulic acid, Quercetin-3-O-galactoside (Q-3-g), Quercetin-3-β-D-glucoside (Q-3-βDg), Delphinidin 3,5-di-O-glucoside (Del 3,5-dOg), Cyanidin 3-O-glucoside (Cyn 3-O-glu), Pelargonidin 3,5-di-O-glucoside (Pel 3,5-dOg) and Pelargonidin 3-O-glucoside (Pel 3-O-glu). Gallic acid, Ferulic acid, Q-3-βDg and Cyn 3-O-glu were identified in all of the samples. Catechin was not detected in CON and PAC while PC showed similar levels to PA ($P > 0.05$) and greater than PN ($P < 0.05$). Gallic acid was identified in all samples being at the highest level in PP and followed by PA and CON. Gallic acid concentration of PC was similar to PAC and CON ($P > 0.05$). Elfalleh *et al.* (2011) detected 123.79 ± 9.56 mg/100 g DW Gallic acid in Tunisian PP, which was lower than this study. Differences in phenolic acids concentration can be dependent on pomegranate variety and growth conditions. PP and PA showed the highest ferulic acid concentrations; however, PN had the lowest. Similar to gallic acid, no difference was observed between ferulic acid levels of

CON and PC ($P > 0.05$). Q-3-g was found in PP and in mostly lesser amounts in PA, PC and CON; however, in PN and PAC, it was not identified. Q-3-βDg was at the highest level in PP and followed by PA. On the other hand, no significant difference was found for its content in PC and PAC ($P > 0.05$) while PN had lowest Q-3-βDg levels as the product.

For anthocyanins; PA and CON had significantly higher values than PP ($P < 0.05$). PN had also significantly lower values than PA and CON ($P < 0.05$). PAC, CON and PC had similar values for major anthocyanins such as Cyn 3-O-glu and Pel 3,5-dOg. PA showed the highest and similar Del 3,5-dOg concentration with CON ($P > 0.05$). PN and PC showed the same Del 3,5-dOg concentrations ($P > 0.05$). Pel 3,5-dOg was only found in PA, CON, PAC and PC. Significantly important levels were retained in co-products ($P < 0.05$). Ozkal & Dinc (1993) observed that Pel 3,5-dOg and Pel 3-O-glu were present in high values in PP and in low amounts in PA. They also claimed that both Cyanidin 3,5-di-O-glucoside (Cyn 3,5-dOg) and Cyn 3-O-glu were detected in PP, but Del 3,5-dOg and Del 3-O-glu which were major anthocyanins of pomegranate juice could not be detected in PP. In this study, Pel 3,5-dOg could not be found in PP, while Pel 3-O-glu was only detected in PP. Cyn 3,5-dOg could not be found in PA and PP; however, Cyn 3-O-glu was detected for each and PA was higher than others. Delphinidin 3-O-glucoside (Del 3-O-glu) and Del 3,5-dOg could not be detected in PP as reported by Ozkal & Dinc (1993). There are also some studies in which more anthocyanins of pomegranate juice were detected. Gil *et al.* (2000) and Turfan *et al.* (2011) detected Del 3,5-dOg, Cyn 3,5-dOg, Cyn 3-O-glu, Pel 3-O-glu and Del 3-O-glu in juices. Anthocyanin concentration can be degraded or reduced during processing and storage and depends on growth conditions and variety as well (Ozkal & Dinc, 1993).

Bioaccessibility evaluation by *in vitro* gastrointestinal digestion

Evaluations of bioaccessibility for the samples (PG, IN and OUT fractions) after *in vitro* GI digestion are given in Table 3. PP and PN had the highest and the lowest TPC values for all fractions, respectively. No significant difference was found between CON and PC according to PG fraction which is representing the part leaving the stomach ($P > 0.05$). For the IN fraction, TPC of PA and PAC were statistically similar ($P > 0.05$). There was no significant difference between TPC of OUT fractions of PA and PAC, and CON and PC ($P > 0.05$). Most phenolics were discarded as by products or wastes during pomegranate processing. However, very low bioaccessibility was obtained for PN. % recovery values in PG fraction were very high

Table 2 Major phenolic compound analysis by HPLC-PDA of samples obtained from PN production (mg/100 g DW)*

Samples	Gallic Acid	Catechin	Ferulic Acid	Q-3-g	Q-3-βDg
PP	213.8 ± 57.4 ^a	6954.6 ± 865.0 ^a	65.0 ± 7.0 ^a	117.1 ± 12.4 ^a	400.5 ± 5.9 ^a
PA	156.8 ± 6.7 ^b	506.5 ± 96.5 ^b	47.6 ± 4.2 ^b	9.1 ± 0.0 ^b	326.5 ± 26.7 ^{b†}
PC	71.2 ± 0.1 ^{cd}	359.9 ± 52.0 ^b	28.8 ± 1.7 ^c	13.7 ± 0.9 ^b	108.4 ± 14.0 ^c
PAC	61.9 ± 5.9 ^d	ND	20.6 ± 1.9 ^d	ND	88.0 ± 8.2 ^c
CON [†]	100.4 ± 10.4 ^c	ND	31.8 ± 4.1 ^c	4.9 ± 0.1 ^b	61.0 ± 0.0 ^d
PN [†]	26.1 ± 2.3 ^e	10.5 ± 0.0 ^b	5.4 ± 0.5 ^e	ND	21.0 ± 1.6 ^e

Anthocyanins					
Samples	Cyn 3-O-glu	Del 3,5-dOg	Pel 3,5-dOg	Pel 3-O-glu	
PP	15.2 ± 0.3 ^c	ND	ND	12.4 ± 0.8	
PA	42.6 ± 4.9 ^{a†}	41.0 ± 4.1 ^{a†}	103.4 ± 28.6 ^a	ND	
PC	24.9 ± 0.1 ^b	10.9 ± 1.5 ^c	14.6 ± 1.4 ^b	ND	
PAC	20.5 ± 1.0 ^{bc}	23.0 ± 5.2 ^b	34.6 ± 5.3 ^b	ND	
CON [†]	21.0 ± 0.2 ^b	35.6 ± 0.0 ^a	38.3 ± 3.7 ^b	ND	
PN [†]	5.5 ± 0.7 ^d	13.0 ± 2.1 ^c	ND	ND	

PP, pomegranate peel; PA, pomegranate arils; PC, press cake; PAC, precipitate after clarification; CON, concentrate; PN, pasteurised nectar; Q-3-g, Quercetin-3-O-galactoside; Q-3-βDg, Quercetin-3-β-D-glucoside; Cyn 3-O-Glu, Cyanidin 3-O-glucoside; Del 3,5-dOg, Delphinidin 3,5-di-O-glucoside; Pel 3,5-dOg, Pelargonidin 3,5-di-O-glucoside; Pel-3-O-glu, Pelargonidin 3-O-glucoside; ND, not detected.

*Values are mean of triplicate measurements. Different letters in the same column present significant difference at $P < 0.05$.

†Surek & Nilufer-Erdil (2014).

Table 3 The concentrations and per cent recoveries of TPC, TAC and TAA by DPPH of samples after *in vitro* GI digestion*

Samples	TPC (mg GAE/100 g DW)						TAC (mg Cyn 3-O-glu/100 g DW)					
	PG	%	IN	%	OUT	%	PG	%	IN	%	OUT	%
PP	14511.5 ± 1271.3 ^a	80.5	2295.6 ± 54.5 ^a	12.7	20242.3 ± 1068.2 ^a	112.3	40.7 ± 4.3 ^d	78.6	0.3 ± 0.0 ^b	0.6	8.8 ± 1.0 ^d	17.0
PA	6444.1 ± 432.0 ^b	129.1	1113.1 ± 52.2 ^c	22.3	6540.4 ± 499.3 ^b	131.0	118.2 ± 11.4 ^a	63.1	2.1 ± 0.1 ^a	1.1	14.2 ± 1.9 ^c	7.6
PC	2909.3 ± 108.3 ^d	97.5	512.0 ± 63.2 ^d	17.2	4480.7 ± 650.1 ^c	150.1	22.3 ± 5.7 ^e	40.4	0.6 ± 0.1 ^b	1.1	-	-
PAC	4575.1 ± 198.9 ^c	131.0	1004.6 ± 86.8 ^c	28.8	7163.2 ± 345.4 ^b	205.1	67.8 ± 6.1 ^c	53.5	2.0 ± 0.7 ^a	1.6	32.9 ± 0.0 ^a	26.0
CON	3569.3 ± 67.1 ^{cd}	98.3	1559.7 ± 192.3 ^b	43.0	3719.0 ± 250.7 ^c	102.4	87.0 ± 3.6 ^b	86.1	2.1 ± 0.2 ^a	2.1	24.9 ± 2.9 ^b	24.7
PN	968.3 ± 169.5 ^e	96.3	196.6 ± 11.4 ^e	19.6	54.8 ± 9.5 ^d	5.5	2.4 ± 0.4 ^f	9.1	0.4 ± 0.1 ^b	1.5	2.7 ± 0.9 ^e	10.3

TAA by DPPH (mg TEAC/100 g DW)						
Samples	PG	%	IN	%	OUT	%
PP	2332.3 ± 168.4 ^a	5.2	22.9 ± 1.2 ^a	0.1	2024.6 ± 293.7 ^a	4.5
PA	2184.7 ± 116.7 ^a	15.4	126.3 ± 0.5 ^a	0.9	1329.5 ± 245.7 ^b	9.3
PC	1158.7 ± 8.0 ^b	18.8	45.1 ± 4.0 ^c	0.7	611.5 ± 4.7 ^c	9.9
PAC	2337.8 ± 292.4 ^a	36.3	114.2 ± 7.9 ^b	1.8	2011.9 ± 31.0 ^a	31.2
CON	1012.6 ± 70.3 ^b	14.4	33.8 ± 2.4 ^d	0.5	661.3 ± 60.2 ^c	9.4
PN	2013.7 ± 117.1 ^a	109.2	44.4 ± 3.3 ^c	2.4	350.4 ± 34.0 ^c	19.0

TPC, total phenolic content; TAC, total anthocyanin content; TAA, total antioxidant activity; PP, pomegranate peel; PA, pomegranate arils; PC, press cake; PAC, precipitate after clarification; CON, concentrate; PN, pasteurised nectar; PG, the fraction leaving the stomach; OUT, the fraction going into colon; IN, the fraction absorbed in serum; %, recovery values were calculated by assuming the extract values as 100%.

*Values are mean of triplicate measurements. Different letters in the same column present significant difference at $P < 0.05$.

for PA and PAC. Although PP showed the greatest level of TPC for the extract, and even IN, PG and OUT fractions, it had the lowest % recovery in IN fraction (12.7%) when compared to the extract. For OUT fraction, recovery values were found to be so

low (5.5%) for the final product although all other samples had very high recovery values. For TAC values, PA had the highest values in PG fraction whereas for other fractions, especially, for IN no significant difference was obtained between PA, PAC and CON

($P > 0.05$); PP, PC and PN as the anthocyanins passed into serum at very low levels ($P > 0.05$). PN had the lowest values in PG and OUT fractions. IN values were lower than the OUT for all samples. When % recovery for TAC is evaluated for IN fraction, all samples had low recoveries (0.6–2.1%), whereas for PG fraction, recoveries were determined in the range of (9.1–86.1%) CON being the highest, and thus, potential anthocyanin bioavailability was at very low levels. According to TPC results by Perez-Vicente *et al.* (2002), pepsin digestion did not change the values; however, 29% of TPC were detected in IN after pancreatin–bile salt digestion. Similar results were found for PA and PAC in this study; and for arils, 129% of TPC were found in PG. There was an increase in TPC compared to initial values for some samples after gastric digestion. This increase can be due to acidic hydrolysis of the phenolic glycosides to their aglycons during gastric digestion Perez-Vicente *et al.* (2002) reported that % recovery values for anthocyanin bioaccessibility in IN and in OUT fractions were 2.4 and 15.3%, respectively. These values were also in agreement with the ones detected in this study. McDougall *et al.* (2005) reported that only 5.3% of TAC and 10.3% of TPC of raspberry were recovered after digestion. Fazzari *et al.* (2008) determined increase in TPC at PG fraction for frozen sweet cherries; and in IN fraction, TPC % recovery values were about 26–30%. Some of pomegranate samples in this study showed higher TPC % recovery and lower TAC % recovery than McDougall *et al.* (2005) and Fazzari *et al.* (2008). The reason for high loss of anthocyanins is not completely known, but there can be some factors such as chemical conversions of anthocyanins (Perez-Vicente *et al.*, 2002). It could be due to release of phenolic compounds from matrix after pepsin digestion. Anthocyanin stability is dependent on pH conditions; at low pH, anthocyanins occur in the red form of flavylium cation. On the other hand, comparisons of *in vitro* studies are not easy because of the differences in material and *in vitro* digestion applied (Fazzari *et al.*, 2008). The bioavailability of pomegranate polyphenols is affected by many factors such as production conditions of juice, analysis methods and variety of the fruit used (Basu & Penugonda, 2009).

Total antioxidant activity obtained by DPPH method after GI digestion showed that for PG fraction PP, PA, PAC and PN had similar TAA values ($P > 0.05$). PA showed also higher TAA values in IN fraction ($P < 0.05$). For IN fraction, PC and PN showed similar TAA values ($P > 0.05$). PP had the lowest TAA value and % recovery of IN fraction. For OUT fraction, PP and PAC and CON, PC and PN were not significantly different ($P > 0.05$). IN % recovery values for all samples were very low (0.1–2.4%). PG % recovery of PN, PAC and PC was high,

respectively. Although CON showed higher total phenolic bioaccessibility, it had low TAA.

Results for major phenolic compound analysis by HPLC-PDA of samples after *in vitro* digestion are given in Table 4. Anthocyanins were not detected for any IN and OUT of samples, so phenolic bioaccessibility was detected higher than anthocyanin bioaccessibility. PP had the highest values of PG, IN and OUT of Gallic acid, Catechin, Ferulic acid, Q-3-g and Q-3- β Dg due to also showing the highest values for extracts. IN values were lower than OUT for all samples. As reported by Perez-Vicente *et al.* (2002), the reason of the significant loss of anthocyanins is not completely known, but they can be metabolised to noncoloured forms, oxidised or degraded into other chemicals which cannot be detected under these conditions. It is also important that no aglycons occur after pancreatin–bile (intestinal) digestion (Perez-Vicente *et al.*, 2002). Gallic acid was only detected for IN of PP and PA. Catechin values in PG of PP, PA, PC, PAC, CON and PN were measured as 1405.3 ± 291.9 , 668.5 ± 13.5 , 236.1 ± 16.4 , 297.9 ± 16.2 , 379.1 ± 16.2 and 121.3 ± 11.4 mg/100 g DW, respectively. There was no Catechin in any IN and PP showed the only OUT value as 21390.2 ± 4500.2 mg/100 g DW.

Pel 3,5-dOg for PN and Pel 3-O-glu for PA, CON, PAC and PC were detected for PG samples, although they were not found for extracts. Generally, there was an increase in PG for phenolic compounds and anthocyanins, however, excessive decrease in IN and OUT for anthocyanins. This was probably due to enhancer effect of enzymes such as pepsin and acidic pH of the medium in gastric conditions, and effect of pancreatin–bile mixtures and alkaline pH of the medium in intestinal conditions during digestion (Bermudez-Soto *et al.*, 2007). Gastric conditions are effective on the release of some bound anthocyanins by acidic hydrolysis.

Conclusion

In general, phenolic content, antioxidant activities and potential bioaccessibilities of co-products obtained from industrial PN processing were determined in this study. This is the first study to investigate antioxidant potential and bioaccessibilities of co-products such as PC and PAC in comparison with raw materials and final products. PA and PAC were good sources for anthocyanins. It is evident that the results obtained with our *in vitro* GI method has shown that co-products such as PP, PC and PAC have significant levels of phenolics and antioxidant activity as much as or even higher than products. They can be used as a functional ingredient in dietary supplements or food formulations. Although *in vitro* digestion methods are preferred because of their advantages such as low cost, rapidity and simplicity for investigating potential

Table 4 The concentrations and per cent recoveries of major phenolic compounds by HPLC-PDA analysis of samples after *in vitro* GI digestion (mg/100 g DW)*

Samples	Gallic Acid						Ferulic Acid						O-3-g						O-3-βDg									
	PG		OUT		IN		PG		OUT		IN		PG		OUT		IN		PG		OUT		IN		PG		OUT	
	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT	PG	OUT		
PP	1405.3 ± 291.9 ^a	1441.8 ± 76.1 ^a	39.5 ± 8.2	1441.8 ± 76.1 ^a	63.1 ± 25.7 ^a	383.0 ± 25.7 ^a	452.8 ± 18.8 ^a	432.3 ± 10.4 ^a	48.2 ± 1.5 ^a	531.4 ± 50.5 ^a	1464.3 ± 345.1 ^a	188.8 ± 26.1 ^a	2636.8 ± 207.0 ^a	1405.3 ± 291.9 ^a	1441.8 ± 76.1 ^a	39.5 ± 8.2	1441.8 ± 76.1 ^a	63.1 ± 25.7 ^a	383.0 ± 25.7 ^a	452.8 ± 18.8 ^a	432.3 ± 10.4 ^a	48.2 ± 1.5 ^a	531.4 ± 50.5 ^a	1464.3 ± 345.1 ^a	188.8 ± 26.1 ^a	2636.8 ± 207.0 ^a		
PA	668.5 ± 13.5 ^b	50.4 ± 1.7 ^c	28.0 ± 5.7	50.4 ± 1.7 ^c	13.8 ± 1.3 ^c	149.2 ± 9.3 ^b	40.4 ± 7.2 ^{cd}	23.1 ± 1.4 ^{bc}	9.1 ± 0.2 ^c	15.2 ± 0.0 ^c	252.8 ± 13.4 ^b	65.2 ± 6.6 ^b	336.1 ± 20.8 ^c	668.5 ± 13.5 ^b	50.4 ± 1.7 ^c	28.0 ± 5.7	50.4 ± 1.7 ^c	13.8 ± 1.3 ^c	149.2 ± 9.3 ^b	40.4 ± 7.2 ^{cd}	23.1 ± 1.4 ^{bc}	9.1 ± 0.2 ^c	15.2 ± 0.0 ^c	252.8 ± 13.4 ^b	65.2 ± 6.6 ^b	336.1 ± 20.8 ^c		
PC	236.1 ± 16.4 ^{cd}	151.7 ± 4.4 ^b	ND	151.7 ± 4.4 ^b	8.1 ± 0.5 ^d	44.1 ± 2.6 ^d	59.3 ± 3.9 ^c	27.7 ± 1.0 ^b	4.1 ± 0.2 ^d	4.1 ± 0.2 ^c	192.6 ± 8.8 ^b	22.3 ± 2.4 ^c	356.5 ± 39.5 ^c	236.1 ± 16.4 ^{cd}	151.7 ± 4.4 ^b	ND	151.7 ± 4.4 ^b	8.1 ± 0.5 ^d	44.1 ± 2.6 ^d	59.3 ± 3.9 ^c	27.7 ± 1.0 ^b	4.1 ± 0.2 ^d	4.1 ± 0.2 ^c	192.6 ± 8.8 ^b	22.3 ± 2.4 ^c	356.5 ± 39.5 ^c		
PAC	297.9 ± 16.2 ^{cd}	45.8 ± 0.0 ^c	ND	45.8 ± 0.0 ^c	38.5 ± 0.7 ^b	84.8 ± 6.3 ^c	137.1 ± 28.2 ^b	15.8 ± 0.2 ^{cd}	18.8 ± 1.8 ^b	74.1 ± 3.6 ^b	244.4 ± 20.0 ^b	67.1 ± 8.4 ^b	613.0 ± 77.8 ^b	297.9 ± 16.2 ^{cd}	45.8 ± 0.0 ^c	ND	45.8 ± 0.0 ^c	38.5 ± 0.7 ^b	84.8 ± 6.3 ^c	137.1 ± 28.2 ^b	15.8 ± 0.2 ^{cd}	18.8 ± 1.8 ^b	74.1 ± 3.6 ^b	244.4 ± 20.0 ^b	67.1 ± 8.4 ^b	613.0 ± 77.8 ^b		
CON	379.1 ± 16.2 ^c	91.5 ± 11.0 ^{bc}	ND	91.5 ± 11.0 ^{bc}	5.7 ± 0.7 ^{de}	83.0 ± 3.2 ^c	24.6 ± 3.5 ^{de}	5.5 ± 0.0 ^{de}	3.1 ± 0.0 ^d	9.8 ± 0.0 ^c	193.8 ± 15.4 ^b	48.9 ± 4.9 ^b	361.3 ± 15.2 ^c	379.1 ± 16.2 ^c	91.5 ± 11.0 ^{bc}	ND	91.5 ± 11.0 ^{bc}	5.7 ± 0.7 ^{de}	83.0 ± 3.2 ^c	24.6 ± 3.5 ^{de}	5.5 ± 0.0 ^{de}	3.1 ± 0.0 ^d	9.8 ± 0.0 ^c	193.8 ± 15.4 ^b	48.9 ± 4.9 ^b	361.3 ± 15.2 ^c		
PN	121.3 ± 11.4 ^d	ND	ND	ND	2.4 ± 0.4 ^e	26.0 ± 2.2 ^d	5.0 ± 0.6 ^e	3.1 ± 0.0 ^e	ND	ND	87.4 ± 1.2 ^b	20.4 ± 2.4 ^c	43.9 ± 4.4 ^b	121.3 ± 11.4 ^d	ND	ND	ND	2.4 ± 0.4 ^e	26.0 ± 2.2 ^d	5.0 ± 0.6 ^e	3.1 ± 0.0 ^e	ND	ND	87.4 ± 1.2 ^b	20.4 ± 2.4 ^c	43.9 ± 4.4 ^b		

Samples	Anthocyanins - PG						Del 3,5-dOg						Pel 3,5-dOg						Pel 3-O-glu									
	Cyn 3-O-Glu		Del 3,5-dOg		Pel 3,5-dOg		Cyn 3-O-Glu		Del 3,5-dOg		Pel 3,5-dOg		Cyn 3-O-Glu		Del 3,5-dOg		Pel 3,5-dOg		Cyn 3-O-Glu		Del 3,5-dOg		Pel 3,5-dOg		Cyn 3-O-Glu		Del 3,5-dOg	
	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg	Cyn 3-O-Glu	Del 3,5-dOg		
PP	111.9 ± 6.5 ^b	ND	111.9 ± 6.5 ^b	ND	56.7 ± 4.9 ^b	ND	56.7 ± 4.9 ^b	360.1 ± 41.9 ^a	ND	360.1 ± 41.9 ^a	61.6 ± 0.2 ^a	21.2 ± 2.6 ^b	111.9 ± 6.5 ^b	ND	111.9 ± 6.5 ^b	ND	111.9 ± 6.5 ^b	ND	56.7 ± 4.9 ^b	ND	56.7 ± 4.9 ^b	360.1 ± 41.9 ^a	ND	360.1 ± 41.9 ^a	61.6 ± 0.2 ^a	21.2 ± 2.6 ^b		
PA	184.9 ± 2.8 ^a	35.1 ± 3.1 ^c	184.9 ± 2.8 ^a	35.1 ± 3.1 ^c	27.5 ± 0.1 ^c	66.0 ± 2.1 ^b	27.5 ± 0.1 ^c	45.2 ± 2.1 ^{cd}	96.5 ± 6.0 ^{bc}	45.2 ± 2.1 ^{cd}	7.8 ± 0.2 ^d	9.7 ± 1.1 ^{cd}	184.9 ± 2.8 ^a	35.1 ± 3.1 ^c	184.9 ± 2.8 ^a	35.1 ± 3.1 ^c	184.9 ± 2.8 ^a	35.1 ± 3.1 ^c	27.5 ± 0.1 ^c	66.0 ± 2.1 ^b	27.5 ± 0.1 ^c	45.2 ± 2.1 ^{cd}	96.5 ± 6.0 ^{bc}	45.2 ± 2.1 ^{cd}	7.8 ± 0.2 ^d	9.7 ± 1.1 ^{cd}		
PAC	108.5 ± 12.2 ^b	100.5 ± 9.0 ^b	108.5 ± 12.2 ^b	100.5 ± 9.0 ^b	89.5 ± 5.8 ^a	33.8 ± 3.0 ^c	89.5 ± 5.8 ^a	146.1 ± 6.8 ^b	28.3 ± 0.0 ^d	146.1 ± 6.8 ^b	11.8 ± 1.0 ^c	ND	108.5 ± 12.2 ^b	100.5 ± 9.0 ^b	108.5 ± 12.2 ^b	100.5 ± 9.0 ^b	108.5 ± 12.2 ^b	100.5 ± 9.0 ^b	89.5 ± 5.8 ^a	33.8 ± 3.0 ^c	89.5 ± 5.8 ^a	146.1 ± 6.8 ^b	28.3 ± 0.0 ^d	146.1 ± 6.8 ^b	11.8 ± 1.0 ^c	ND		
CON	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	ND	ND	26.0 ± 1.1 ^c	ND	ND	ND	ND	ND	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	ND	ND	ND	ND	ND	ND	ND	ND	ND	
PN	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	ND	ND	26.0 ± 1.1 ^c	ND	ND	ND	ND	ND	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	26.0 ± 1.1 ^c	ND	ND	ND	ND	ND	ND	ND	ND	ND	

PP, pomegranate peel; PA, pomegranate arils; PC, press cake; PAC, precipitate after clarification; CON, concentrate; PN, pasteurised nectar; PG, the fraction leaving the stomach; OUT, the fraction going into colon; IN, the fraction absorbed in serum; O-3-g, Quercetin-3-O-galactoside; O-3-βDg, Quercetin-3-β-D-glucoside; Cyn 3-O-Glu, Cyanidin 3-O-glucoside; Del 3,5-dOg, Delphinidin 3,5-di-O-glucoside; Pel 3,5-dOg, Pelargonidin 3,5-di-O-glucoside; Pel-3-O-glu, Pelargonidin 3-O-glucoside; ND, not detected.

*Values are mean of triplicate measurements. Different letters in the same column present significant difference at $P < 0.05$.

bioaccessibility, they cannot exactly predict the human *in vivo* conditions. Therefore, further *in vivo* studies should also be performed to support the findings of *in vitro* studies.

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