

Research Article

Effects of extraction methods and heat treatment on total phenolic compounds and antioxidant activity in juice extracted from the Australian-grown ‘Wonderful’ pomegranate

Amir Arjmand¹, Kambiz Shamsi², and Frank Sherkat¹

¹Food Science, School of Applied Sciences, RMIT University, Melbourne, VIC 3001 Australia.

²School of Biosciences, Taylor's University, Lakeside Campus, Subang Jaya, Selangor, Malaysia.

Email: kambiz.shamsi@taylors.edu.my

Abstract

Physicochemical properties of the Australian-grown ‘Wonderful’ pomegranate juice (WPJ) including total phenolic compounds (TPC) and antioxidant activity (AA) were determined and compared with four different brands of imported pomegranate juices (IPJs). The TPC in WPJ was found to be $2,400 \pm 200$ mg/L gallic acid equivalent (GAE) while in the IPJs it ranged from 1,000 to 2,800 mg/L GAE. The AA of WPJ was 11.0 ± 1.0 mM/L Trolox equivalent antioxidant capacity (TEAC) while in IPJs it ranged from 5.5 to 14.5 mM/L TEAC. To facilitate consumer access to the health benefits of pomegranate, a probiotic product was developed from bovine milk supplemented with different levels of IPJ. Maximum IPJ supplementation level in heat-treated milk with no adverse effects on yoghurt attributes was found to be 20% (V/V), resulting in maximum TPC level of 731 ± 69 mg/L GAE in probiotic yoghurt.

Keywords: fruit, phenolic compounds, health benefits, TPC, probiotic yoghurt, *Punica granatum* L., Malaysia.

Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits widely grown in many tropical and subtropical countries [1]. Over 1,000 cultivars of ‘*Punica granatum*’ exist, originating from the Middle East, extending westward throughout the Mediterranean, and eastward to China and India, and onto the American South-West, California and Mexico [2, 3]. Pomegranate use in Australia has been primarily as a backyard ornamental tree [4]. While no official statistics are available in Australia, it is estimated that nearly 250 ha are currently grown with a similar area projected for new plantation, with expected increase to over 1,000 ha in the next 5-10 years [5].

Over the last two decades consumers have become more aware of the relationship between food intake and good health, especially from natural sources such as fruits and vegetables. Pomegranate fruit which is renowned for its health benefits has become very popular worldwide over the last few years [5]. According to Tezcan *et al.* [6], clinical research studies suggest that pomegranate juice (PJ) can reduce the level of oxidized low-density lipoprotein (LDL) cholesterol, and increase the activity of serum high-density lipoprotein (HDL)-associated paraoxonase 1 [7, 8, 9, 10]. The PJ also helps keep the prostate specific antigen (PSA) levels stable in men and even slows its rise [11], is helpful against heart disease [12, 13], Alzheimer's disease [14] and some types of cancer such as prostate and colon cancer [15, 16, 17, 18, 19]. It is also reported that PJ can improve sperm quality [20] and erectile dysfunction in male patients [21].

Studies have shown that pomegranate is a good source of flavonoids (flavonols, flavanols and anthocyanins) and hydrolyzable tannins [22, 23, 24]. Hydrolyzable tannins (HTs) are the predominant type of polyphenols in PJ [24, 25, 26] and consist of gallotannins (hydrolyzed to gallic acid and glucose), and ellagitannins (hydrolyzed to ellagic acid and glucose). Each ellagic acid consists of 2 gallic acids, so, the monomeric part of this phenolic fraction is gallic acid [26, 27, 28, 29, 30, 31, 32, 33, 34]. Chemical analyses have shown that HTs are responsible for over 92% of PJ antioxidant activity (AA) [24], which its AA is higher than red wine, green tea, cranberry, grapefruit and orange juices (3, 3, 2, 6 and 8 folds higher, respectively) [26, 35, 36, 37].

Due to increased consumer awareness of PJ's antioxidant properties, its consumption has increased dramatically around the world. There is an important body of work in the literature on pomegranate properties from different parts of the world [1, 27, 37, 38, 39, 40, 41, 42, 43]. These studies have proven that differences in varieties and environmental conditions affect the PJs attributes. This study was undertaken to characterise the juice extracted from Australian-grown pomegranate ('*Wonderful*' variety) and to evaluate the impact of processing on TPC and AA levels in probiotic yoghurt supplemented with different levels of PJ.

Materials and Methods

Materials

Pomegranate fruit and juice

Two lots of fresh '*Wonderful*' pomegranates with average weights of 238 ± 10 and 573 ± 21 g were selected for the study. The fruits were supplied by a grower in Robinvale (between Mildura and Swan Hill in the North West of Victoria, Australia, $34^{\circ} 35'$ S latitude and $142^{\circ} 46'$ E longitude) during the harvest season in April, 2010.

Four different brands of imported PJ (IPJ) were purchased from the local market and coded as TT, TB, UP, IT. Samples TT and IT were sold in 1,000 mL tetra pack cartons, the TB was marketed in 1,000 mL bottles and UP was sold in 236 mL PET (polyethylene terephthalate) bottles. Labels on all products claimed 100% juice with no added ingredients.

Chemicals and reagents

All chemicals used were analytical grade and sourced from Sigma-Aldrich Pty. Ltd. (MO, USA). These included gallic acid, Folin-Ciocalteu reagent (F-C), ABTS (2, 2'-azinobis-(3 ethylbenzothiazoline-6-sulphonic acid) diammonium salt), potassium persulphate (dipotassium peroxodisulphate), Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

Yoghurt ingredients

Commercial homogenised, pasteurised and low-fat milk (1.3% fat, REV, Parmalat, Australia) and low-heat skim milk powder (LHSMP, 0.9% milk fat, 96% total solids, Bonlac Foods Ltd,

Melbourne, Australia) were used to produce yoghurt. The freeze-dried (FD) probiotic culture selected for this project was ABT-5-Probio-Tec™ (Chr. Hansen Pty. Ltd., Bayswater, Australia), a mixture of *Lactobacillus acidophilus* (LA-5), *Bifidobacterium bifidum* (BB-12) and *Streptococcus thermophilus* (ST). The culture was stored at -22 °C until required for yoghurt preparation as direct vat set (DVS).

Methods

Extraction of pomegranate juice

In preliminary study, arils of large and small pomegranate fruits were manually separated for juice extraction using an electric juicer (Sunbeam, model IE-AD, Italy) in two stages (the pulp from first extraction stage was passed through the juicer again for further juice extraction). The fresh juices thus extracted from the large and small fruits (LPJ and SPJ) were pooled separately and stored in a blast freezer at -28°C. The physicochemical and phytochemical properties of the thawed samples were determined and compared with those of IPJs.

To further increase the polyphenol content six different processes were designed and employed to extract PJ from small size pomegranates coded PJ1 to PJ6. In the first process juices were extracted from manually separated arils, either with an electric juicer (PJ1) in two stages to improve yield (as above) or by manually operated screw press (M-Press) (PJ2). In the second process the outer leathery skin of pomegranates were peeled off and the fruit was then segmented and PJs were extracted from these segments (the arils still inside the white pith) with electric juicer either in single stage (PJ3), or in two stages followed by manual pressing of the residual pulp (PJ4). In the third process, chopped unpeeled fruits were processed in electric juicer either in single stage (PJ5) or in two stages followed by manual pressing (PJ6). The fresh juices extracted from each step were pooled separately and used for yield calculation. Each stream was then divided into two lots, the first lot was frozen and stored in blast freezer at -28°C (PJ1 - PJ6) and the second lot was heat treated as follows before storage at refrigerated temperature (Figure 1).

Heat treatment of pomegranate juice

Different streams of juices were pasteurized at 90°C for 15 sec, immediately cooled in an ice bath to below 10°C, aseptically transferred into 450 mL glass bottles and tightly sealed. The bottles were coded PJ1P to PJ6P and stored at 4°C.

Determination of total phenolic compounds

Total phenolic compounds of PJs were determined by Folin-Ciocalteu (FC) colorimetry method which is based on chemical reduction of a mixture of tungsten and molybdenum oxides [44]. This method relies on the transfer of electrons in alkaline medium from phenolic compounds to a mixture of phosphomolybdic and phosphotungstic acids to form blue complexes readable by a spectrophotometer [45]. Frozen juices were thawed first, then 20 µL of diluted (1:10 with Milli-Q water) sample (PJ or yoghurt or gallic acid standard solution) was mixed with 1.58 mL Milli-Q water in a 2-mL plastic cuvette. A blank was prepared using only Milli-Q water. Aliquots of 100 µL FC reagent were added to each cuvette and mixed by pipetting for *ca.* 8 min at RT (20 – 25°C). Then, 300 µL of 20% sodium carbonate solution was added to all cuvettes (except those containing yoghurt) and allowed to stand for 2 h at room temperature (RT) before reading the absorbance at 765 nm in a UV/VIS Spectrometer equipped with UV Winlab software (Lambda 35, Perkin Elmer, MA, USA). The mixture of yoghurt and reagents was centrifuged (after 90 min standing at RT) at 18,500 g for 30 min at 4°C (5810R, Eppendorf Centrifuge, Hamburg, Germany), and the supernatant was used for absorbance reading as above. The results were expressed as mg gallic acid equivalent (GAE) in 1 L of sample by comparison with standard curve, which was constructed from different concentrations of gallic acid (50 to 1000 mg/L).

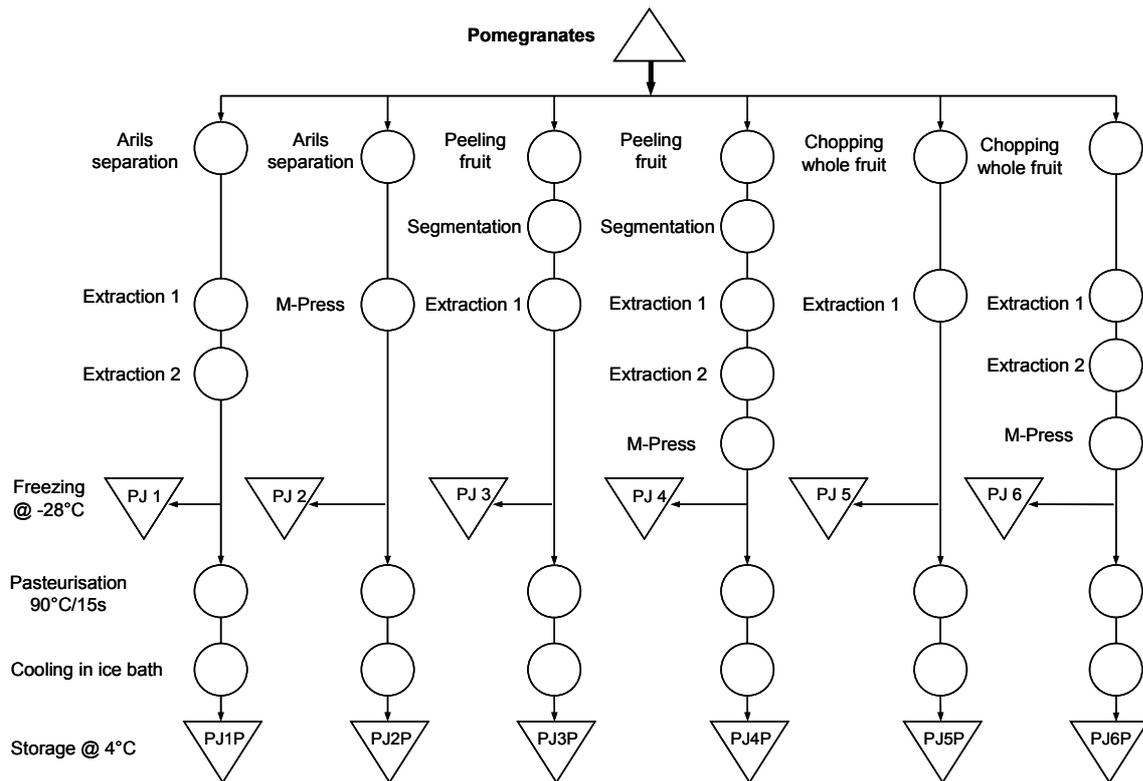


Figure 1. Process flow diagram for extraction and processing of juice from different parts of pomegranate fruit.

Determination of antioxidant activity (AA)

Total antioxidant activity of PJ was measured spectrophotometrically based on the generation of ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) di-ammonium salt) radical cation [26, 46, 47, 48, 49, 50, 51, 52].

Aliquots of 7 mM ABTS and 2.45 mM potassium persulphate aqueous solutions were mixed and kept in the dark at RT for approximately 24 h until the oxidation of ABTS was complete and the absorbance stabilised. The solution containing the generated blue/green ABTS^{•+} chromophore was diluted with Milli-Q water to an absorbance of 0.70 (± 0.020) at 734 nm [47, 53, 54].

An aliquot of 200 μ L of diluted PJ (1:50 with Milli-Q water), or Trolox[®] (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble synthetic vitamin E analogue) standard solution, or Milli-Q water (as blank) was mixed with 2.0 mL ABTS^{•+} in a plastic cuvette. The mixture was allowed to stand at RT for 10 min with continuous stirring before the absorbance was measured by UV/VIS spectrometer (Lambda 35, Perkin Elmer) at 734 nm. Absorbance values were taken to the standard curve prepared with synthetic antioxidant Trolox and results were expressed as Trolox equivalent antioxidant capacity (TEAC) [26]. The TEAC is equal to the millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to a 1.0 mM solution of the substance under investigation [55].

Colour measurement

Aliquots of 25 mL of experimental and commercial PJs were transferred into disposable plastic petri dishes, covered and the colour parameters were determined using a Chroma Meter CR-400 (Konica Minolta, Sensing, INC, Japan) according to Shwartz *et al.* [42, 43] and expressed in

dimensions of L^* , a^* , b^* , C and H° . The mean values of triplicate readings were reported for each sample. Values of L^* - indicate darkness and L^* + indicate lightness of sample colour, while a^* - indicates green colour and a^* + indicates red colour. The b^* + indicates a more yellow colour and b^* - indicates blue colour. The chroma (C) value is calculated as $C = (a^{*2} + b^{*2})^{1/2}$ and indicates the colour intensity or saturation. Hue angle H° is a parameter that is effective in evaluating visual colour appearance and is calculated as $H^\circ = \tan^{-1}(b^*/a^*)$ (Solomon et al., 2006). The colour index was calculated from $(180 - H^\circ)/(L^* + C)$ [37, 42, 43].

Yoghurt preparation

Plain yoghurt was made according to Pasephol *et al.* [56]. Briefly, the low-fat milk (2.1.3) was standardised with LHSMP to a total solid content of 16% and heat-treated at 90°C for 10 min, then cooled to 43°C and inoculated with freeze-dried ABT-5-Probio-Tec™ culture at a level recommended by the supplier (50U/250L). After gentle stirring to distribute the culture evenly, the inoculated milk samples were aseptically transferred into 100-mL plastic containers, tightly sealed and incubated at 43°C. At pH 4.7 the samples were transferred to a cold room at 4°C. The commercial IPJ sample IT was selected for preliminary supplementation trials to produce probiotic yoghurt. Four supplementation levels of 9, 13, 17 and 20% were trialled before or after heat treatment while keeping solids content constant at 16%

Physico-chemical analyses

Soluble solids of fresh PJ was determined according to AOAC [57] refractive index method with a Shibuya hand-held refractometer (Japan) and reported as degree Brix (°B). Total solids of milk and yoghurt samples were determined using oven method according to Australian Standard (AS 2300.1.1-2008) [58]. pH values of all samples were measured using a pH-meter (HI 8424, Hanna instruments, USA). Titratable acidity (TA) of PJ samples was determined potentiometrically using 0.1M NaOH to the end point of pH 8.1 according the AOAC [59] and reported as % citric acid (g per 100 mL). pH and TA of yoghurt and milk samples were determined according to Australian Standards (AS 2300.2.10-2008, AS 2300.1.6-2010) [60, 61].

Statistical analyses

All tests were conducted in triplicate and the mean values \pm standard deviation (SD) are reported. Statistical analyses were performed by applying one-way analysis of variance (ANOVA) to determine the significance of the 95% confidence interval and correlation coefficient using Minitab software (Version 14, Minitab Inc., State College, PA, USA).

Results and Discussion

Physicochemical properties of pomegranate juice

The average weight of pomegranates was found to range between 238 ± 10 g for small fruit and 573 ± 21 g for the large ones. The yield of arils from small pomegranates was $61.44 \pm 2.11\%$ but only $45.58 \pm 2.71\%$ from the large fruits that appeared larger and more red than the small fruits arils. Arils' juice yield was comparable at $74.18 \pm 3.19\%$ and $76.54 \pm 2.38\%$ for small and large fruit respectively, however, on whole fruit basis the yield from larger fruit was expectedly lower due to their thicker skin, i.e. $34.89 \pm 1.15\%$ vs. $45.58 \pm 1.96\%$ from small fruit.

Soluble solids in LPJ were significantly ($P < 0.05$) higher (16.8 ± 0.2 °B) than that of SPJ (15.2 ± 0.2 °B). In comparison, only one of the IPJs showed high soluble solids content (16.1 ± 0.1 °B) while others were significantly ($P < 0.05$) lower and ranged from 13.3 to 14.5 °B (Table 1). The SPJ was slightly more acidic than the LPJ ($1.58 \pm 0.07\%$ vs. $1.15 \pm 0.09\%$). The acidity of SPJ was closer ($P < 0.05$) to that of IPJ coded TB. Likewise, the acidity of the LPJ and the IPJ coded IT was not significantly different ($P < 0.05$) (Table 1).

Table 1. Chemical properties, total phenolic compounds and antioxidant activity of pomegranate juice samples.

Samples	Soluble Solids (°B)	pH	TA (% citric acid)	TPC (GAE mg/L)	AA (TEAC mM/L)
SPJ ¹	15.2 ± 0.2 ^{bc}	3.00 ± 0.02 ^{de}	1.58 ± 0.07 ^{ab}	2460 ± 164 ^{ab}	11.06 ± 0.91 ^{bc}
LPJ ²	16.8 ± 0.2 ^a	3.25 ± 0.01 ^b	1.15 ± 0.09 ^{cd}	2545 ± 97 ^{ab}	11.36 ± 0.94 ^{ab}
IPJ (TT)	14.1 ± 0.1 ^{cd}	3.34 ± 0.01 ^a	1.01 ± 0.06 ^{de}	1923 ± 177 ^{bc}	9.59 ± 0.93 ^{bc}
IPJ (TB)	14.5 ± 0.1 ^c	3.02 ± 0.01 ^d	1.61 ± 0.08 ^{ab}	1293 ± 113 ^{cd}	6.91 ± 0.97 ^{cd}
IPJ (UP)	16.1 ± 0.1 ^{ab}	3.32 ± 0.01 ^{ab}	1.09 ± 0.05 ^{de}	2630 ± 245 ^{ab}	13.43 ± 0.99 ^{ab}
IPJ (IT)	13.3 ± 0.1 ^d	3.15 ± 0.01 ^c	1.27 ± 0.07 ^{cd}	1193 ± 171 ^d	6.48 ± 0.90 ^{bc}

¹. Juice produced from small size fruit's arils by electric juicer in two stages;

². Juice produced from large size fruit's arils by electric juicer in two stages.

IPJ: Imported pomegranate juices.

The data represent the means ± SD of triplicate analyses from each trial. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters in the same column.

The colour evaluation results are presented in Table 2. The values of L*, a*, C, H and colour index were not significantly different ($P < 0.05$) between SPJ and LPJ, however, SPJ showed higher b* value (yellowness) than LPJ. Compared to the fresh juices (SPJ & LPJ), the colour values were significantly different ($P < 0.05$) for commercial products (IPJs) that were all produced from concentrated PJ. The commercial products showed significantly higher ($P < 0.05$) L* values (i.e. brighter), a* value (more red except for IT) and b* value (yellow) than the fresh juices. The IPJ coded IT showed a significantly higher ($P < 0.05$) L* and b* values but the lowest a* value (Table 2). According to H° formula [$H^\circ = \tan^{-1}(b^*/a^*)$] any increase in the redness of a sample (a*+) or drop in its yellowness (b*+) results in low H° value. The H° values of IPJs were significantly higher ($P < 0.05$) than SPJ and LPJ, while their colour index values were significantly lower ($P < 0.05$) than SPJ and LPJ (Table 2). These results were correlated with visual appearance of samples and indicated that SPJ and LPJ's colour were more appealing while the commercial products showed inferior colour compared to fresh juices [37, 42, 43].

Table 2. Colour evaluation in pomegranate juice samples.

Samples	L*	a*	b*	c	H°	Colour index
SPJ ¹	17.50 ± 0.08 ^e	15.83 ± 0.70 ^{cd}	7.89 ± 0.46 ^d	17.69 ± 0.83 ^{de}	26.50 ± 0.36 ^e	4.36 ± 0.10 ^b
LPJ ²	17.45 ± 0.32 ^e	15.43 ± 1.50 ^{cd}	6.24 ± 0.58 ^{cd}	16.64 ± 1.60 ^{de}	22.05 ± 0.44 ^e	4.64 ± 0.25 ^b
IPJ (TT)	26.67 ± 0.18 ^d	20.38 ± 0.96 ^{bc}	21.33 ± 0.80 ^{ab}	29.36 ± 1.48 ^{bc}	46.32 ± 0.30 ^c	2.39 ± 0.07 ^d
IPJ (TB)	27.16 ± 0.55 ^{dc}	23.22 ± 2.06 ^b	17.86 ± 1.90 ^b	29.29 ± 2.79 ^{bc}	37.54 ± 0.54 ^d	2.53 ± 0.16 ^d
IPJ (UP)	25.37 ± 0.99 ^d	29.07 ± 1.22 ^{ab}	19.56 ± 1.20 ^{ab}	35.04 ± 1.68 ^{ab}	33.92 ± 0.52 ^d	2.42 ± 0.10 ^d
IPJ (IT)	43.18 ± 0.89 ^a	6.67 ± 0.02 ^{cd}	23.46 ± 0.74 ^a	24.40 ± 0.72 ^{cd}	74.11 ± 0.42 ^{ab}	1.57 ± 0.04 ^e

¹. Juice produced from small size fruit's arils by electric juicer in two stages;

². Juice produced from large size fruit's arils by electric juicer in two stages.

IPJ: Imported pomegranate juices.

The data represent the means \pm SD of triplicate analyses from each trial. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters in the same column.

Effects of extraction method on pomegranate juice properties

The yields of PJ1, 2, 3 and 5 were not significantly ($P < 0.05$) different. Using the same raw materials, double extraction with electric juicer (PJ1) and manual pressing (PJ2) methods resulted in the same yields, however combining the two methods increased the yield significantly ($P < 0.05$). Thus, PJ4 had the highest yield followed by PJ6 (Table 3). While using the same extraction method on peeled fruits (PJ3) and unpeeled whole fruits (PJ5) resulted in the same yields.

The type of fruit fraction and the extraction methods used showed direct effects on the soluble solids content of all samples. Accordingly, soluble solids in PJs 1 and 2 (15.2 ± 0.2 °B) produced from arils with similar yields were not significantly ($P < 0.05$) different. Due to differences in the fruit parts used for extraction, the soluble solids in PJ5 was significantly ($P < 0.05$) higher (16.4 ± 0.2) than that in PJ3 (15.9 ± 0.1), whereas PJ6 had significantly ($P < 0.05$) more soluble solids (16.9 ± 0.1) than PJ4 (16.1 ± 0.1). While, based on extraction method employed, soluble solids in PJ6 was significantly ($P < 0.05$) higher than that in PJ5, which in turn was significantly ($P < 0.05$) higher than that in PJ4 (Table 3).

Table 3. Yield, chemical properties and effect of heat treatment on TPC and AA of pomegranate juice extracted with different methods.

Sample	Yield ¹	Soluble Solids (° B)	pH	TA (% citric acid)	TPC (GAE mg/L)		AA (TEAC mM/L)	
					BHT ²	AHT ³	BHT ²	AHT ³
PJ1	45.58 \pm 1.96 ^{bc}	15.2 \pm 0.2 ^d	3.00 \pm 0.02 ^{de}	1.58 \pm 0.07 ^{abc}	2460 \pm 164 ^{de}	2293 \pm 248 ^e	11.06 \pm 0.91 ^d	10.24 \pm 0.81 ^d
PJ2	45.04 \pm 2.06 ^{bc}	15.2 \pm 0.2 ^d	3.00 \pm 0.01 ^{de}	1.64 \pm 0.10 ^{ab}	2071 \pm 62 ^e	2064 \pm 123 ^e	9.16 \pm 0.97 ^d	9.03 \pm 0.95 ^d
PJ3	45.23 \pm 2.07 ^{bc}	15.9 \pm 0.1 ^c	3.08 \pm 0.01 ^{bc}	1.32 \pm 0.07 ^{cd}	5760 \pm 609 ^{cd}	5297 \pm 746 ^d	23.13 \pm 2.88 ^c	21.36 \pm 2.63 ^c
PJ4	56.87 \pm 1.64 ^a	16.1 \pm 0.1 ^{bc}	3.10 \pm 0.01 ^b	1.42 \pm 0.09 ^{bcd}	7293 \pm 605 ^c	6930 \pm 555 ^c	30.25 \pm 2.10 ^{bc}	27.70 \pm 1.40 ^{bc}
PJ5	40.04 \pm 2.04 ^{bc}	16.4 \pm 0.2 ^{bc}	3.10 \pm 0.01 ^{ab}	1.36 \pm 0.07 ^{cd}	11545 \pm 503 ^{ab}	10456 \pm 472 ^b	50.65 \pm 1.60 ^a	48.54 \pm 2.14 ^a
PJ6	49.78 \pm 2.12 ^{cd}	16.9 \pm 0.1 ^{ab}	3.10 \pm 0.02 ^b	1.38 \pm 0.09 ^{cd}	12516 \pm 167 ^{ab}	11619 \pm 394 ^{ab}	56.91 \pm 2.79 ^a	53.20 \pm 2.03 ^a

¹. Yield calculated on whole fruit base;

². Before heat treatment;

³. After heat treatment (90°C for 15 sec).

Data shown represents the means \pm SD of triplicate analyses from each trial. ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters in the same column.

PJ1 and 2: Juice extracted from manually separated arils with an electrical juicer in two stages (PJ1) or by manual pressing (PJ2).

PJ3 and 4: Juice extracted from peeled, segmented fruit with an electrical juicer in single stages (PJ3) or in two stages followed by manual pressing (PJ4).

The pH of PJs 1 and 2 extracted from arils were not significantly different ($P < 0.05$) but when non-edible piths or peels of the fruit were included in PJ extraction the level of pH increased significantly ($P < 0.05$) to ca. 3.10. This was also evident in the level of titratable acidity in PJs 1 and 2 (1.58 ± 0.07 and 1.64 ± 0.10 respectively) that were more acidic than PJs 3 to 6 which were not significantly different ($P < 0.05$) to each other (Table 3).

Difference in raw materials and extraction methods affected the colour parameters of extracted juices (Table 4). No significant differences ($P < 0.05$) were found between L^* values of PJ1 and 5, PJ2 and 3, and PJ4 and 6; between a^* values of PJ1 and 3, PJ5 and 6; and between b^* values of PJ4 and 5. PJ1 showed the highest H° value (26.50 ± 0.36) that gradually declined as the extraction method became more extensive, the lowest value was found in PJ6 (14.13 ± 0.96). The colour index was not affected by the type of raw materials used, and in contrast with H° values, the highest and lowest colour index was observed in PJ6 (4.68 ± 0.19) and PJ1 (4.36 ± 0.10) respectively. These results were correlated with visual appearances of samples and in agreement with Shwartz *et al.* [42, 43] and Tzulker *et al.* [37] who indicated that the samples with higher colour indices had strong red appearances.

Table 4. Colour evaluation in pomegranate juice extracted with different methods.

Sample	L^*	a^*	b^*	c	H°	Colour index
PJ1	17.50 ± 0.08^{cd}	15.83 ± 0.70^{bc}	7.89 ± 0.46^a	17.69 ± 0.83^{abc}	26.50 ± 0.36^a	4.36 ± 0.10^{bcd}
PJ2	18.50 ± 0.15^{ab}	15.44 ± 1.92^{bcd}	7.47 ± 0.21^{ab}	16.97 ± 2.08^{abc}	24.54 ± 0.37^{ab}	4.39 ± 0.27^{bcd}
PJ3	18.42 ± 0.32^{ab}	15.97 ± 0.77^{bc}	5.86 ± 0.23^{bc}	17.01 ± 0.79^{abc}	20.17 ± 0.34^{bc}	4.51 ± 0.14^{bc}
PJ4	18.09 ± 0.04^{bc}	13.37 ± 1.74^{cd}	4.76 ± 0.52^{cd}	17.20 ± 1.34^{abc}	19.61 ± 0.41^c	4.55 ± 0.18^{bc}
PJ5	17.75 ± 0.20^{cd}	17.45 ± 1.14^{abc}	4.86 ± 0.35^{cd}	18.12 ± 1.19^{ab}	15.56 ± 0.48^d	4.59 ± 0.15^{abc}
PJ6	17.88 ± 0.03^{bc}	17.06 ± 1.68^{abc}	4.28 ± 0.12^d	17.59 ± 1.65^{abc}	14.13 ± 0.96^{de}	4.68 ± 0.19^{abc}

The data represent the means \pm SD of triplicate analyses from each trial.

ANOVA was used to determine the significance of differences at a confidence level of 0.05 as identified by different letters in the same column. PJ1 and 2: Juice extracted from manually separated arils with an electrical juicer in two stages (PJ1) or by manual pressing (PJ2).

PJ3 and 4: Juice extracted from peeled, segmented fruit with an electrical juicer in single stages (PJ3) or in two stages followed by manual pressing (PJ4).

PJ5 and 6: Juice extracted from chopped whole fruit with an electrical juicer in single stages (PJ5) or in two stages followed by manual pressing (PJ6).

Phytochemical content of pomegranate juice

The health benefits attributed to pomegranate fruit consumption are related, at least in part, to their antioxidant activity (AA) [42, 43, 62]. The AA of SPJ, LPJ as determined by ABTS method (section 2.2.3) were found to be 11.06 and 11.36 mM/L TEAC respectively, while IPJs coded UP, TT, TB and IT showed 13.43, 9.59, 6.91 and 6.48 mM/L TEAC, respectively (Table 1).

In pomegranates like many other fruits such as blueberry, black cherry, cranberry or red wine the level of AA can be attributed to the level of total phenolic compounds [26, 37, 42, 43, 63]. Therefore, TPC in these juices were also measured by F-C method (section 2.2.2) and the results were expressed as mg/L GAE (Table 1).

Freshly extracted juices SPJ and LPJ contained 2,460 and 2,545 mg/L GAE, respectively; while the IPJs coded UP, TT, TB and IT contained 2,630, 1,923, 1,293 and 1,193 mg/L GAE, respectively (Table 1). Gil *et al.* [26] suggested that the industrial extraction process either increases the amount of TPC or enhances the activity of the antioxidants. Industrially, the whole fruit is pressed hydrostatically which results in the extraction of a large amounts of polyphenols from the peels [37]. The juice is then filtered, concentrated, stored and sold to juice packaging companies where it is diluted with water and packed. It is this level of dilution that determines the TPC and the AA of the commercial juice. Among the IPJs tested in this project, only sample coded UP showed a comparable TPC level to fresh juice obtained from arils while other

commercial samples failed in this regard. These results are in agreement with the soluble solids of samples tested although soluble solids alone could not be a good guide to AA activity, since some IPJs may have added sugar to adjust their soluble solids to an acceptable level.

These results support the hypothesis that the AA of PJ samples was directly related to the levels of TPC in those samples. Thus, any process that increases the level of TPC in a sample could result in an increased antioxidant activity.

Effects of raw materials and extraction procedures on phytochemical content of pomegranate juice

To improve the TPC level in PJ samples different parts of pomegranate fruits were used in different extraction procedures (PJ1 to PJ6). In juices extracted from arils, the TPC level in PJ1 (2460 ± 164 mg/L GAE) was marginally higher than PJ2 (2071 ± 62 mg/L GAE) due to difference in the extraction method employed but the AA of both samples were not statistically different ($P < 0.05$) (Table 2). The TPC level and AA of PJs from peeled fruits were higher than those from arils. On the other hand, due to the intensity of extraction method PJ4 showed significantly ($P < 0.05$) higher TPC and AA (7293 ± 605 mg/L GAE and 30.25 ± 2.10 mM/L TEAC) than PJ3 (5760 ± 609 mg/L GAE and 23.13 ± 2.88 mM/L TEAC) (Table 2).

Upon using unpeeled chopped whole fruit pieces for juice extraction the TPC level and AA of PJs 5 and 6 were further improved compared to all other samples (Table 2), however, the difference between PJs 5 and 6 in terms of the TPC content (11545 ± 503 vs. 12516 ± 167 mg/L GAE) or the antioxidant activity (50.65 ± 1.60 vs. 56.91 ± 2.79 mM/L TEAC) was not statistically significant ($P < 0.05$).

The TPC level in pasteurised PJs 1P (2293 ± 248 mg/L GAE), 3P (5297 ± 764 mg/L GAE) and 5P (10456 ± 472 mg/L GAE) were 6.8, 8.0 and 9.4% lower than unpasteurised PJs 1 (2460 ± 164 mg/L GAE), 3 (5760 ± 609 mg/L GAE) and 5 (11545 ± 503 mg/L GAE) respectively (Table 3), while in PJ2P, PJ4P and PJ6P the TPC level remained unchanged ($P < 0.05$) after heat treatment (90 °C for 15 sec). These results confirmed that the pasteurisation regime employed did not have a serious impact on TPC levels or the AA of all samples.

Probiotic counts in yoghurt containing pomegranate juice

A probiotic yoghurt was produced from skim milk supplemented with different level of PJ. The IPJ coded IT was used in the preliminary supplementation trials. Different supplementation levels (9, 13, 17 and 20%) were trialled before and after heat treatment of milk at 90°C for 10 min. Supplementation before heat treatment was limited to 9%, since above this level the milk curdled, whereas after heat treatment up to 20% PJ could be added without any adverse effect. It appears that heat treatment of standardised milk increased its stability and the buffering capacity of milk proteins. By increasing the level of supplementation to 20% the pH of milk before incubation declined to 5.56 (Figure 2). After inoculating with probiotic culture (ABT-5) the samples were incubated at 43°C to reach pH 4.7. At this pH the time was recorded and yoghurt samples were transferred to refrigerated storage at 4°C. The activity of the starter culture in the preliminary study was estimated from changes in time to reach target pH. Comparing the yoghurt setting times in 17 and 20% (*ca.* 5 h) and in 9 and 13 % (*ca.* 6 h) supplemented samples it was noted that PJ could have an influence on the activity of starter culture.

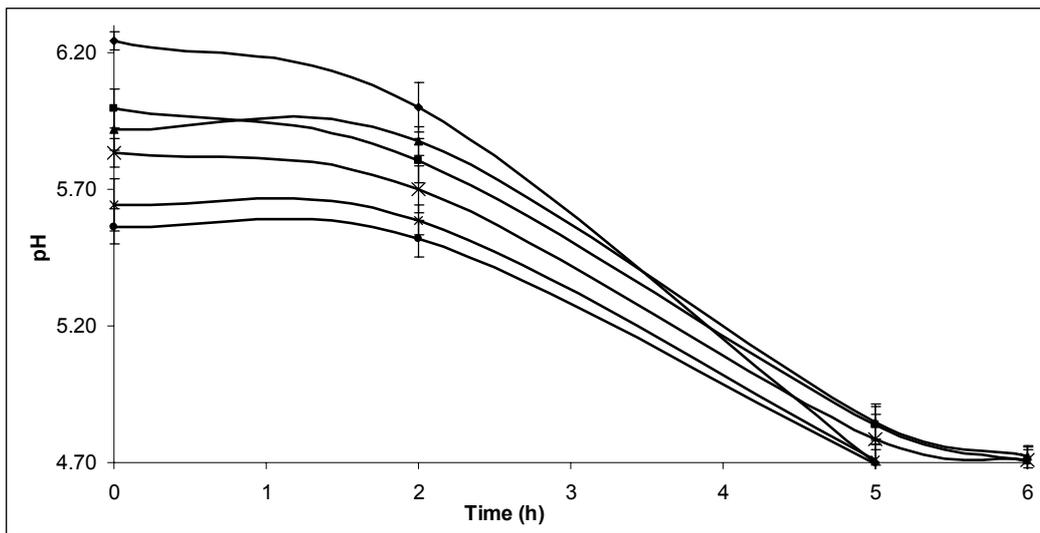


Figure 2. pH range in yoghurt supplemented with PJ.

- | | | | |
|---|------------------------------|---|------------------------------|
| ◆ | Plain yoghurt | X | +13% PJ after heat treatment |
| ■ | +9% PJ before heat treatment | × | +17% PJ after heat treatment |
| ▲ | +9% PJ after heat treatment | ● | +20% PJ after heat treatment |

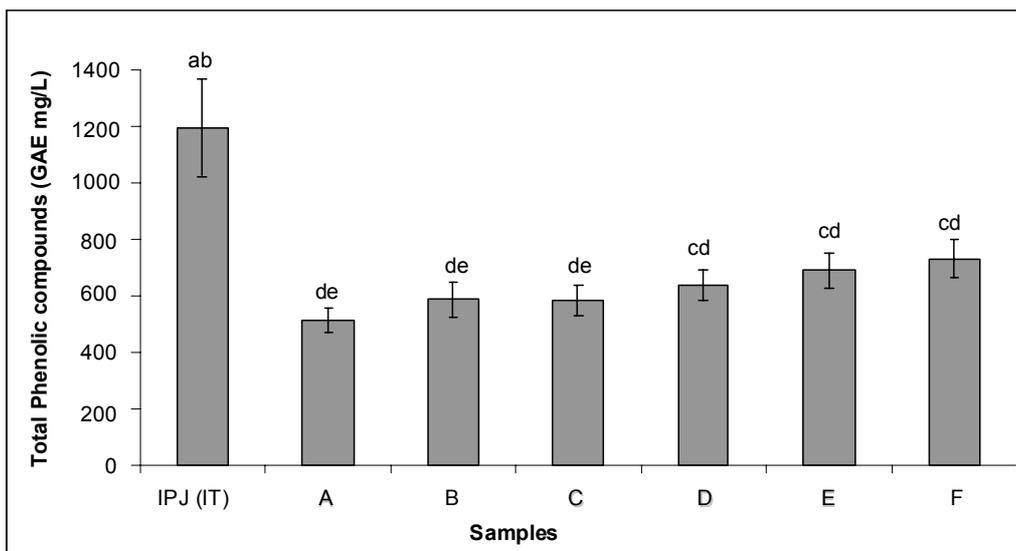


Figure 3. Total phenolic compounds (mg/L GAE) in IPJ (coded IT) and in yoghurt supplemented with IPJ (IT).

The data represent the means ± SD of triplicate analyses from each trial.

ANOVA was used to determine significance of difference at a confidence level of 0.05, identified by different letters.

- | | | | |
|----|------------------------------|----|------------------------------|
| A: | Plain yoghurt | D: | +13% PJ after heat treatment |
| B: | +9% PJ before heat treatment | E: | +17% PJ after heat treatment |
| C: | +9% PJ after heat treatment | F: | +20% PJ after heat treatment |

The TPC content of probiotic yoghurts containing 9% (B) pomegranate juice (before heat treatment) and 9 (C), 13 (D), 17 (E) and 20% (F) (after heat treatment) was found to be 585, 583, 637, 688 and 731 mg/L GAE, respectively while the background TPC in plain yoghurt (A) was 514 mg/L GAE and in pure PJ (IT) was 1193 mg/L GAE (Figure 3).

Conclusions

The larger fruit seemed to have lower juice yield, with lower acid and higher sugar content (higher soluble solids) and contained over 3.3% more TPC and nearly 2.7% higher AA than juice from smaller fruit. Freshly extracted juice showed higher TPC and AA than three of the imported commercial PJs, except the sample coded UP which had comparable levels of TPC and AA.

Up to six fold increase in TPC level could be achieved in PJs by increasing the intensity of the extraction method and incorporation of the inedible parts of fruit in the extraction feed material. While, strong astringency of these juices could limit their applications for direct human consumption, they may however find uses in nutraceuticals formulation as AA supplements. Pasteurisation (90°C for 15 sec) did not significantly ($P<0.05$) affect the AA of the extracted juices, although TPC levels of PJ1, PJ3 and PJ5 slightly declined after heat treatment.

In the production of probiotic yoghurt up to 20% single strength PJ could be added to heat treated milk with no apparent antagonism between the cultures growth and activity and the PJ phytochemicals. Considering the recommended polyphenols intake of *ca.* 1 g/day [64] this probiotic product offers a pleasant and effective route to increasing the antioxidant intake in our daily diet.

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