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EFFECT OF DIFFERENT EXTRACTION METHODS ON SENSORY AND PHYSICO-CHEMICAL PROPERTIES OF POMEGRANATE (*PUNICA GRANATUM* L.) JUICE OBTAINED FROM CV. *BHAGWA*

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ABSTRACT

The pomegranate juice (cv. *Bhagwa*) was extracted using blending the arils in domestic mixer and crushing the whole fruit halves in pilot scale juice extractor. The household mixer extracted juice had higher yield of 54.62% as well as more sensory acceptability 8.55 than other juice where yield was 41.38% and organoleptic evaluation score was 5.65. The total soluble solids, titratable acidity (% citric acid) and pH, 13°Bx, 0.324% and 3.528, respectively, were higher in domestic mixer juice than mechanically extracted juice. The juice from arils also had higher glucose and fructose levels, 61.00 and 63.54 g/L than the other containing 59.00 and 59.37 g/L, respectively. Further, 13.49% of total sugars, 12.64% of reducing sugars and 9.60 mg/100g of ascorbic acid were found to be higher in domestic mixer juice than obtained using juice extractor. However, the whole fruit juice contained greater amount of non-reducing sugars, 0.89 %, total polyphenol content, 295.38 mg/100mL and tannin content, 0.190%, as compared to aril juice showing more anti-oxidant properties. Hence, it can be concluded that domestic mixer juice had higher yield, acceptability and physico-chemical components (except non-reducing sugars, phenols and tannins) than mechanically extracted juice.

INTRODUCTION

The pomegranate (*Punica granatum* L.), known as super fruit of next generation, has been regarded as a food medicine of great importance for therapeutic purposes like colic, colitis-diarrhea, dysentery, leucorrhea, paralysis and headache (Sadeghi *et al.,* 2009). It contains a significant proportion of organic acids, soluble solids, polysaccharides, vitamins, fatty acids and minerals (Fadavi *et al.,* 2006). The composition of pomegranate juice depends on cultivar type, environmental and postharvest factors and storage and processing factors (Gil *et al.,* 2000).

The edible portion of fruit is about 55-60% of the total fruit weight and consists of about 75-85% juice and 15-25 % seeds (Cadze *et al.,* 2012). The foremost challenge in juice extraction is the peeling of the fruit as it is time consuming and irritating. Pomegranate rind is rich in polyphenols including ellagitannins, gallotannins, ellagic acids, gallic acids, catechins, anthocyanins, ferulic acids, and quercetins. These polyphenols exhibit various biological activities, such as eliminating free radicals, inhibiting oxidation and microbial growth, and decreasing the risk of cardio- and cerebrovascular diseases and some cancers (Sangeetha and Vijayalakshmi, 2011). In addition to this, the phenolic constituents are responsible of astringency and bitterness as well as the formation of cloudy appearance of fruit juices during concentration and storage (Gil *et al.,* 2000). Nutritionist, however, recommend in contrast to preserve these compounds during the fruit juice processing because of their health protective effects.

Recently, Faria and Calhau (2010) noticed some differences in phenolic composition between commercial juices and experimental ones of pomegranate as the use of the arils alone or the whole fruit to make juice has an enormous impact on polyphenols content and consequently on antioxidant capacity of the juice. Miguel *et al.* (2004) evaluated the fresh juice, 20, 40, 60 and 72 hours stored pomegranate juice (cv. *Assaria*) extracted using centrifugation of seeds and squeezing fruit halves revealing that the anthocyanins content, the juice colour, the organic acids and sugars composition, as well as the pH values were not affected much during storage in both the cases and juice obtained by squeezing was more stable over time.

Due to increasing interest in the pomegranate consumption, anti-oxidant activity, health benefits and knowledge that composition varies according to the extraction method, the present study was designed to evaluate the effect of different extraction methods on total phenolics, tannins, ascorbic acid and other physico-chemical properties of pomegranate juice.

MATERIALS AND METHODS

Freshly harvested pomegranate fruits (cv. *Bhagwa*) were procured from local market of Parbhani. All reagents, solvents and chemicals were of analytical reagent grade and were procured from Department of Food and Industrial Microbiology and Department of Food Chemistry and Nutrition, College of Food Technology, VNMKV, Parbhani.

Extraction of Juice

The damaged fruits were removed and healthy fruits of uniform size and appearance were washed with water and wiped completely dry. To obtain juice, two different extraction methods viz. domestic mixer and juice extractor were employed. The first method consisted of manually peeling the fruits, separating the juicy arils and extracting the juice by a household mixer (blending of seeds). While in the second method, fruits were cut in four parts and the juice was immediately extracted using juice extractor. The raw juice obtained was strained through muslin cloth. Then, filtered pomegranate juice was then clarified using commercially available fining agent bentonite (0.5%) and juice was held for 4 hr (Patil *et al.,* 2013). The treated juice was then filtered through muslin cloth. This juice was pasteurized at 82ºC for 15 min to get clarified juice.

Chemical characteristics of pomegranate juice

Following chemical properties of fresh pomegranate juice were determined:

Total soluble solids (T.S.S.), Titratable acidity and pH

Total soluble solids, titratable acidity and pH were measured using the standard methods given by Ranganna (1991). TSS was obtained using hand refractometer (ERMA make). Titratable acidity, expressed as per cent citric acid, was determined by titration against 0.1N NaOH using phenolphthalein as an end point indicator. The pH value was obtained by using a digital pH meter (ELICO LI612) after standardizing it with buffers of pH 4.0 and 9.0.

Glucose and fructose

The glucose and fructose content were determined in juice by phenol sulfuric acid method (Nielsen, 2010).

Total Sugars, Reducing Sugar and Non-reducing Sugars

Total carbohydrate/sugars was estimated by standard procedure using phenol sulphuric acid (Nielsen, 2010). The amount of reducing sugar of fresh juice was calculated by Nelson – Somogyi method (Syed *et al.,* 2007) and non-reducing sugar was obtained by subtracting reducing sugars from total sugars.

Ascorbic acid (vitamin C)

Ascorbic acid contents of samples were determined according to the titration method using 2, 6-dichlorophenol indophenols (Ranganna, 1991).

Total phenolic content

The concentration of phenolic compounds was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965) where 5g of sample was homogenized in 25 mL of 50% (v/v) ethanol/water solution. The sample (100 mL) was mixed with 5 mL of the 0.2N Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate. The mixture was kept for 2 h at room temperature in the dark before the absorbance was measured at 765 nm spectrophotometrically. Gallic acid standards at eight different concentrations ranging from 100 to 2000 mg/L were prepared. The total phenolic content was calculated from this curve and expressed as mg gallic acid equivalents (mg GAE/100 mL).

Tannin content

Total tannin content of sample was measured by Folin Denis method (Saxena *et al.,* 2013) which is based on the measurement of blue color formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution. 5g of the sample was boiled for 30 minutes with 400 mL of water, then cooled and final volume was made to 500 mL with distilled water. After filtration, 0-10 mL aliquots of prepared sample were taken in volumetric flask containing 75 mL of water. Then, it was mixed with 5 mL of Folin – Denis reagent and 10 mL sodium carbonate and final volume was made to 100 mL. It was mixed well and measured the color after 30 minutes at 760 nm. Tannic acid was used to calculate the standard curve (20 – 100 mg/mL).

Organoleptic evaluation of pomegranate juice

Freshly prepared samples of juice were evaluated for sensory characteristics like appearance, colour, taste, flavour, viscosity and overall acceptability by 10 semi-trained panel members comprised of faculty of College of Food Technology, Parbhani on 9- point Hedonic scale (Meilgaard *et al.,* 1999).

Statistical analysis

All processing equipments and analysis of samples were run in triplicate. Analysis of variance was calculated using standard ANOVA procedure. The data obtained for various treatments was recorded and statistically analyzed by complete randomized design (CRD) to find out the level of significance as per the method proposed by Panse and Sukhatme (1957). The analysis of variance revealed at significance at $P < 0.05$ level. The standard error (SE) and critical difference (CD) at 5 % level were mentioned where required.

RESULTS AND DISCUSSION

Effect of different extraction method on juice yield

The yield of pomegranate juice extracted using domestic mixer and pilot scale juice extractor was presented in Table 1. The data revealed that extraction using domestic mixer (54.62%) had significantly higher juice yield as compared to mechanical extraction (41.38%). This may be due to the crushing of whole arils in case of domestic mixer (Patil *et al.,* 2013), while in mechanical extraction, the skin remains intact with the arils and they can't be crushed thoroughly. The results obtained are more or less similar to that of Ismail *et al*. (2014) who

Table 1: Effect of different extraction method on yield of juice

S. No.	Sample(s)	Yield (%)
1	P_m	54.62
2	P_e	41.38
	SE ±	0.291
	CD at 5%	1.14

Table 2: Effect of different extraction method on organoleptic quality of prepared pomegranate juice (on 9 point hedonic scale)

S. No.	Sample(s)	Appearance	Color	Taste	Flavor	Consistency	Overall Acceptability
1	P_m	8.8	8.75	7.8	8	8.9	8.55
2	P_e	6.55	6.55	5.65	5.55	7.5	5.65
	SE ±	0.0952	0.1099	0.2466	0.1267	0.0702	0.1417
	CD at 5%	0.3732	0.4308	0.9666	0.4968	0.2753	0.5554

- Each value is an average of ten determinations;- P_m: Pomegranate juice extracted by domestic mixer, P_e: Pomegranate juice extracted by juice extractor

Table 3: Effect of different extraction method on chemical characteristics of juice

S. No.	Parameter(s)	P_m	P_e	F-value	SE ±	CD at 5%
1	Total Soluble Solids (°Brix)	13.0	11.9	181.50	0.0577	0.2263
2	Titratable acidity (% citric acid)	0.324	0.256	22.08	0.0102	0.0401
3	pH	3.528	3.519	24.30	0.0013	0.0051
4	Glucose (g/L)	61.00	59.00	41.37	0.2198	0.8617
5	Fructose (g/L)	63.54	59.37	278.52	0.1766	0.6925
6	Total sugars (%)	13.49	13.27	35.41	0.0261	0.1025
7	Reducing sugars (%)	12.64	12.38	17.94	0.0433	0.1701
8	Non reducing sugars (%)	0.85	0.89	30.25	0.0094	0.0369
9	Ascorbic Acid (mg/100g)	9.60	8.70	261.23	0.0392	0.1537
10	Total phenolic content (mg/100 mL)	208.29	295.38	9243.6	0.6405	2.5107
11	Tannin (%)	0.116	0.190	483.18	0.0023	0.0093

- Each value is an average of three determinations; - P_m: Pomegranate juice extracted by domestic mixer, P_e: Pomegranate juice extracted by juice extractor

revealed that pomegranate juice obtained by blending seeds was significantly higher than mechanical extraction in case of *Wardey* (73.21±0.58 to 45.02±3.28 per cent) and *Manfalouty* cultivars (72.60±0.92 to 53.01±4.10 per cent). These findings are also in agreement with Zarei *et al*. (2010) who reported that yield of obtained juice using an electric extractor for cultivars grown in Iran varied between 48.02% and 63.52% fruit weight.

Effect of different extraction methods on organoleptic quality of pomegranate juice

The average scores of organoleptic evaluation are depicted in Table 2. The results of sensory assessment showed that the perception of the quality of juice varied significantly with the type of extraction method used. Highest score obtained in overall acceptability (8.55) for P_m sample showed that it was highly accepted by judges. The overall acceptability score for P_e sample was 5.65, which was significantly lower than P_m sample.

The P_m sample also obtained significantly higher scores for appearance (8.8), color (8.75), taste (7.8), flavour (8) and consistency/viscosity (8.9) as compared to P_e sample. The sensory data shows that P_e sample had poorer taste and flavour.

The presence of tannins in sample P_e is the main problem when juices are extracted from whole fruits. As a result, a bitter taste develops that must be corrected by industrial processing (Vardin and Fenercio¢glu, 2003) for making whole fruit juice acceptable.

Effect of different extraction method on chemical characteristics of pomegranate juice

The data in Table 3 revealed that the content of TSS in pomegranate juice prepared by mixer (13°Bx) was significantly higher than that prepared by mechanical extraction (11.9°Bx). The high TSS content is highly desirable in pomegranate fruit juice as it enhances sweetness and flavour especially if accompanied by a decrease in juice acidity and tannin concentration (Shwartz *et al.*, 2009). The obtained results are similar to those reported by Ismail *et al*. (2014) where whole fruit juice had TSS of 14.00°Bx and seed juice had 14.46 °Bx for *Wardey* cultivar and for *Manfalouty* variety, TSS ranged from 14.08 to 15.41°Bx for whole fruit as well as seed juice.

As shown in Table 3, the significantly higher content of titratable acidity (0.324 per cent) was observed in P_m than that in P_e (0.256 per cent). The pH of pomegranate juice characterizes its acidic taste (Zarei *et al.*, 2011). The highest pH value reached a maximum of 3.528 for mixer extracted juice, followed by that for extractor extracted juice (3.519). These values are almost in range reported by Ismail *et al*. (2014). The obtained results are also more or less similar to Ghadge and Jadhav (2015) who found that *Arakta* and *Ganesh* variety of pomegranate had titratable acidity within range of 0.35 to 0.32 per cent, respectively and pH value of 3.59 to 3.54, respectively.

The pomegranate juice had large amount of fructose than the glucose which is supported by Ekþi and Özhamamcý (2009) who found that glucose content ranged from 45.8-65.6 g/L and fructose content varied from 48.4-69.9 g/L in the 23 pomegranate juice samples under study**.** Both the glucose and fructose content were significantly higher in pomegranate juice extracted by mixer (61.00 and 63.54 g/L, respectively) as that of obtained by juice extractor (59.00 and 59.37 g/L, respectively). These results are more or less in contrast with those obtained by Miguel *et al*. (2004) who reported that the amounts of glucose and fructose were quite similar in juices obtained through application of centrifuging seeds and squeezing the fruit halves.

The data in the Table 3 demonstrates that the total sugars contents of the two juices varied from 13.27-13.49 per cent. The highest significant content of total and reducing sugars was observed in the domestic mixer extracted juice (13.49

and 12.64 per cent, respectively) while the lowest amount of total and reducing sugars was for juice (13.27 and 12.38 per cent, respectively) obtained by the mechanical extractor. On the other hand, the non-reducing sugars in the juice extracted by the two used methods of extraction ranged between 0.85-0.89 per cent. The lowest significant non-reducing sugar percentage was found in juice extracted by mixer while other contained significant highest amount of non-reducing sugars. These results are in agreement with Ismail *et al.* (2014) who found that total sugars and reducing sugars were highest in the juice obtained by blending of seeds while non-reducing sugars was highest in whole fruit juice for *Wardey* and *Manfalouty* cultivar. The amount of total sugars, reducing sugars and non-reducing sugars for juice extracted using mixer were also more or less similar to those revealed by Ghadge and Jadhav (2015).

The pomegranate juice extracted by mixer had significantly higher ascorbic acid (9.60 mg/100g) than in juice prepared by mechanical extractor (8.70 mg/100g). The above results are in agreement with Ismail *et al.* (2014) where whole fruits' juice contained 5.50 and 4.28 g/100g and blending of seeds had 6.80 and 5.26 g/100g of ascorbic acid for *Wardey* and *Manfalouty* cultivar. The value of ascorbic acid for mixer extracted juice is similar to those obtained by Ghadge and Jadhav (2015) for *Ganesh* (9 mg/100g) and *Arakta* (9.5 mg/100g) cultivar of pomegranate.

Fruits are excellent sources of phenolic compounds, flavonoid and natural antioxidants (Swapana *et al.*, 2012). The total phenolic content expressed as mg of gallic acid equivalent (GAE) per 100 ml juice was in the range of 208.29 -295.38 mg. In the mechanical extracted juice, the phenolic content was significantly higher than that of mixer extracted juice. Such variations might be due to the effect of extraction method on the whole fruits. The pressure used might extract phenolic compounds from the rind, which passes onto the juice because pomegranate peel is rich source of phenolic compounds. The obtained results are similar to those obtained by Ismail *et al.* (2014). They are highly varied in species, cultivars, and fruit tissue (Tomas- Barberan and Espin, 2001).

The main antioxidant compounds in pomegranate juice are hydrolysable tannins (Gil *et al.,* 2000). Like, total phenol content, the juice obtained from the whole fruit had higher content of tannins (0.190%) as compared to those obtained from the arils (0.116 %). These results are in the same line with those obtained by Gil *et al.* (2000), who reported that pomegranate juice content of tannins depending on the extraction pressure and that juice obtained by blending the arils contained less tannins than that obtained from whole fruits. These results are also in agreement with Ismail *et al.* (2014) who showed that tannin content varied from 0.206 to 0.216 per cent for whole fruit juice and from 0.120 to 0.132 per cent for juice obtained by blending for *Wardey* and *Manfalouty* cultivar, respectively.

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