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NUTRITIONAL ASSESSMENT OF POMEGRANATE, THE FRUIT OF REAL TASTE AND HEALTH COMPANION

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ABSTRACT

The pomegranate (*Punica granatum* L.) is popular for its high medicinal value. It is a common table fruit and whole fruit contains important vitamins, minerals and antioxidants. Four pomegranate varieties (Pearl, Golden, Sultan, and Qandhari) were analyzed in the Biochemistry laboratory of Post Harvest Research Centre, Ayub Agricultural Research Institute (AARI), Faisalabad Pakistan during the year 2021-2022. The results showed that juice carried a remarkably higher value of TSS (total soluble solids) ranging from 12.4% (Pearl) to 17.2 % (Sultan), total invert sugars were minimum (11.8%) in Golden and maximum (14.3%) in Sultan. The value of vitamin-C varies from 10.6 mg/100 mL (Qandhari) to 11.5 mg/100 mL (Pearl) and acidity ranges from 0.8% (Sultan) to 1.9% (Pearl). Sultan showed a higher value for total phenols and antioxidants (1111µg GAE L-1 and 43.5% DPPH inhibition respectively) and lowest in Pearl (1022µg GAE L-1 and 27.7% respectively). Peel powder of different varieties carry a different percentage of crude fat and crude fiber but Sultan is statistically at par with Qandhari but significantly higher than pearl. Ash/mineral matter was significantly different in different varieties. Sultan was better than other varieties having ash contents of 3.67%. The lowest value was observed in Golden (2.7%). Keeping in view the current results Sultan was better than Pearl and Golden and comparable to Qandhari.

KEYWORDS: Antioxidants, phenols; physico-chemical properties; *punica granatum*

1. INTRODUCTION

The juice of pomegranate carries higher value of multiple phenol and antioxidants. Previous studies have shown that pomegranate juice can exert antioxidant, anti-inflammatory and antihypertensive effects. Among all juices, pomegranate juice (PJ) is superior due to its dietary polyphenols and antioxidants as fortified source attributed. The phenolic contents enhance the quality of pomegranate juice making it beneficial for health.

According to Seeram *et al.* (2008), among all the fruit juices, pomegranate is considered the superlative source of antioxidants due to its plant based flavonoid and vitamin C. It lowers the risk of cardiac diseases and prevents different types of cancer. Minerals are also present in it.

Viuda-Martos *et al*. (2010 a,b) have narrated that the use of food products for the prevention and treatments of diseases has gained prime importance and is being warmly accepted by researchers, the food industry and consumers. Useful foods are assigned basic importance due to their role as a preventive measure for diseases, in physiological benefits and in the development of chronic diseases. Due to its clinical and nourishing benefits, pomegranate is placed at leading position in functional foods (Rowayshed *et al*., 2013).

Pomegranate plays a great significance in human nutrition as it has many ingredients that decrease disease risks (Jaiswal *et al*., 2010). These phytochemicals can be extracted from different components of pomegranate tree, fruit, fruit juice seeds and peel. Pomegranates may be consumed in the form of fresh juice, fresh fruit and beverages. Its use is beneficial as a constitutional ingredient in many food supplements and herbal medicines (Elfalleh *et al*., 2011). Since ancient times, bioactive compounds have been an integral component for herbal medicines that are preferably present in pomegranate. Its juice effectively prevents many diseases due to its high antioxidant activity, lipoprotein, atherosclerosis, platelet clusters and cardio-vascular illness (Adhami and Mukhtar, 2006).

Each component of this super fruit i.e. pomegranate

(Punica granatum L.) including the peel, juice, husk and even leaves of this tree, has attracted scientist to conduct research on its role in various bioactivities (Lansky and Newman, 2007). Pomegranate seeds provide a variety of medicinal or disease preventive substances like benzoic and/or hydroxyl benzoic acids, γ-tocopherol, pumice acid and sterols etc. (Liu *et al*., 2009).

Due to its strong anti-oxidizing properties, pomegranate juice by-product plays a great role as a nutraceutical or functional food (Rowayshed *et al*., 2013).

Therefore, several pomegranate varieties were evaluated for their nutritional and quality parameters with the main objective was to compare these varieties based on their nutritional status.

2. MATERIALS AND METHODS

The nutritional parameters of different varieties of pomegranate were analyzed at the field of Ayub Agricultural Research Institute, Faisalabad in Biochemistry Section during the years 2021 and 2022. The experiment aimed to compare the nutritional status of four pomegranate varieties namely Pearl, Golden, Sultan and Qandhari. Pearl and Golden were obtained from AARI (Horticultural Research Institute) Faisalabad while Sultan and Qandhari were collected from Hill Fruit Research Station Murree at maturity. The surface of pomegranate fruits were completely dried after thorough washing with water and the fruit weight was noted. Fruits were manually peeled and the seeds were separated. Juice was extracted using a mechanical juicer and muslin cloth was used for juice filtration. For further analysis, the fruit juices were immediately stored in the refrigerator at 4°C. Peel was dried, ground and stored in a dry place. Peel was analyzed for mineral matter, crude fat, crude fiber and crude protein. Beta-carotene, total antioxidant, total phenols, pH, acidity, TSS, vitamin C, reducing, non-reducing and total invert sugars were determined in juice.

2.1 Scientific apparatus

The laboratory utilized several essential equipment for the analysis including a pH meter, colori meter, centrifuge, muffle furnace, electric shaker, electric balance, electric juicer, microwave oven, electric blender, and electric drying oven. Additionally, various glass apparatus were employed such as a mortar and pestle, Buchner funnel, burette, test tube holder, volumetric flasks, beakers, conical flasks, desiccators, filter papers, glass rod, measuring cylinder, muslin cloth, pipette, platinum dish, stand with clamps and weighing boat.

2.2 Chemicals

The main reagents and chemicals utilized in the research process included Fehling's solutions, acetone, potassium oxalate, ascorbic acid, citric acid, copper sulphate, distilled water, glucose, hydrochloric acid, methylene blue indicator, nitric acid, oxalic acid, phenolphthalein indicator, sodium bicarbonate, sulphuric acid, sodium carbonate, sodium hydroxide, sodium thiosulphate, sodium potassium tartrate, 2,2-diphenylpicrylhydrazyl (DPPH) and potassium chloride.

2.3 Peels powder preparation

The pomegranate fruits were washed and the peels were removed from the seeds using a sharp knife. Small pieces of peels were dried in a circulating air oven at 65 ºC for 4 hours until the moisture content reached 12-14%. The oven-dried peel pieces were cooled, ground into a powder, and sieved to a size of 20 mesh. The prepared samples were stored in high-density polyethylene bags at room temperature (25±5 ºC) to be used when needed (Devatkal and Naveena, 2010).

2.4 Mineral matter

The ash content/mineral matter analysis of the peel powder was performed by ashing the samples in a furnace at 550°C, using Ranganna's method (Ranganna, 2001). Crucibles made of china clay were weighed, and 4g of the sample was taken and heated at 550°C for 5-7 hours. After cooling the samples in a desiccator, the weight was recorded again, and the process was repeated until a constant weight was obtained.

2.5 Crude fat

The crude fat content of the pomegranate peel was determined using the method described by AOAC (2005). The Soxtec apparatus was used for ether extraction, where 2 grams of dry peel were weighed on a filter paper and placed in a plugged thimble. Petroleum ether (150 ml) was used for the ether extraction in the cylinder. The cylinder was then detached and the ether was allowed to evaporate. The samples were dried in the oven at 65 °C for half an hour, cooled using a desiccator, and weighed. The percentage of crude fat was calculated by determining the difference in weight of the samples before and after extraction.

2.6 Crude fiber

The crude fiber content of the sample was determined using the method described by AOAC (2005). Initially, two grams of defatted sample was taken into a 750 ml Erlenmeyer flask, and 0.5 g of Octanol was added as a

defoaming agent. Then, 200 milliliters of boiling 1.25% H_2SO_4 was added to the sample, and the system was attached to a cold water source for condensation. The mixture was boiled on a hot plate for 30 minutes. The contents of the flask were filtered through a muslin cloth, and boiling water was used to wash the material until all the acid was removed. The remaining material on the muslin cloth was transferred back into the same flask, and 200 mL of boiling 1.25% NaOH was added. The same boiling and filtration process was repeated, and the residue was carefully washed with boiling water. The residue was then transferred into a weighed crucible, rinsed with 15 mL of 95% ethanol, and placed in an oven at 100 °C for 60 minutes. The crucible was cooled in a desiccator, weighed, and placed in a Gallenhamp muffle furnace (England) for 30 minutes at 600 °C. The crucible was then cooled and reweighed. The difference in weight was used to calculate the percentage of crude fiber in the sample.

2.7 Crude protein

The Kjeldahl method was used to determine the crude protein in pomegranate peel powder, following the process described in AOAC (2005). One gram of peel powder was weighed into a Kjeldahl digestion flask, and a digestion mixture of K_2SO_4, $CuSO_4.5H_2O$, and Se (100:10:1) was added, followed by 10 ml of 98% H_2SO_4. A clear solution was obtained by digestion, and the digest was subjected to distillation. Twenty mL of 40% NaOH and distilled water were taken up by the apparatus, and the ammonia produced was collected into a beaker containing 20 mL of 4% boric acid solution. As the ammonia mixed with boric acid, the color changed from pink to green. Distillation was stopped after 5 minutes, and the mixture was neutralized against 0.1N HCl until a pink endpoint was reached. The percentage of protein was then calculated.

2.8 Beta-carotene

A modified method based on Ahmad *et al*. (2010) was used for the determination of beta-carotene. Firstly, beta-carotene salt was used to prepare the standard stock solution in acetone. Working standard solutions were prepared on a daily basis with concentrations of 0.03, 0.062, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 µg/ml, using the same solvent. Pomegranate and blank samples were also prepared and analyzed alongside the standards. After preparation, all the solutions were kept in the dark by wrapping the sample containers in aluminum foil. The samples were stored at 4°C for a period not exceeding thirty days, as storage beyond one month may cause errors in the results due to degradation or other processes (AOAC, 1965).

2.9 pH

A standardized and calibrated pH meter was used to determine the pH of the fresh juice. The pH meter was calibrated using standards of pH 4 and 9.

2.10 Total soluble solids

The TSS (total soluble solids) content was determined using a calibrated refractometer. The digital refractometer was calibrated using distilled water. The compensated mode was used to compensate for the temperature factor during the TSS determination.

2.11 Titratable acidity

To determine titratable acidity (TA), a 0.1 M NaOH solution was used until the pH value reached 8.1. The result was expressed as g citric acid/100 g juice (AOAC, 2005). The readings were repeated three times, and their mean values were reported.

2.12 Total antioxidants

A mixture of methanol, acetone, and HCl in a ratio of 90:8:2, respectively, was used to extract pomegranate juice. The extraction process involved mixing 1 mL of pomegranate juice with 5 ml of the extraction mixture, shaking well at the vortex, and centrifuging for 5 minutes at 400 rpm. The supernatant was collected and kept at a cool, dry place for the determination of phenols and antioxidants. Following the method described by Sanchez-Moreno *et al.* (1998), methanol was used as a solvent to prepare a 0.004% DPPH solution. The mixture was shaken well and incubated in the dark for half an hour. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer. The DPPH (0.004%) was used as a blank, and the results were expressed as the percentage inhibition of DPPH.

2.13 Total phenols

The Folin-Ciocalteu colorimetric method was used for the estimation of total phenols and expressed as GAE/100 ml (Gallic acid equivalents). The sample was extracted with an extraction mixture of methanol: acetone: HCl (90:8:2). The upper layer was separated and 0.2 ml was taken. Folin-Ciocalteu reagent was diluted 10 times and mixed with 0.1 ml of the sample and 0.8 ml of 7.5% sodium carbonate solution. The mixture was allowed to stand for half an hour at 25°C and the absorbance was measured on a spectrophotometer at a wavelength of 765 nm (Singleton and Rossi, 1999). A graph was prepared with a standard and total phenols were calculated.

2.14 Vitamin C

A fresh juice sample of 10 ml was homogenized using a homogenizer with the addition of 0.4% oxalic acid solution. The mixture was then filtered with a cloth

and made up to a volume of 100 ml in a volumetric flask with 0.4% oxalic acid. A 5 ml aliquot was pipetted and distilled water was added to make the endpoint clear. The sample was then titrated against a standard dye until a pink color appeared (Pegg, 2010). The titration method was used to determine the ascorbic acid content using 2, 6-dichlorophenol indophenol as reported in the AOAC methods (AOAC, 2005).

2.15 Sugars

The determination of sugar was carried out using a modified method based on Ranganna (2001). First, 10 ml of juice was taken in a 250 ml volumetric flask and mixed with 100 ml of distilled water, 25 ml of lead acetate, and 10 ml of potassium oxalate. The volume was made up to 250 ml with distilled water. Fehling's solution (10 ml) was diluted with water and heated until boiling. The Fehling solution was then taken in a beaker, and the sample was added drop by drop to the beaker while continuously boiling slowly. The end point was reached when a brick red color appeared. To make the end point clear, 3-4 drops of methylene blue indicator were added.

2.16 Statistical analysis

The data was arranged using an MS Excel matrix. The two-year data was organized by replications and means, and then analyzed using the Statistix 8.1 software. Standard errors of means were calculated based on the method described by (Steel *et al*. 1997) and means were compared using Duncan's Multiple Range Test (Duncan, 1955).

3. RESULTS AND DISCUSSION

Pomegranate samples were collected from the field area of Horticulture Research Institute AARI, Faisalabad, and Hill Fruit Research Station Murree. The tabulated data represents the results concerning the physical and nutritional parameters of pomegranate juice and peel. Juice parameters are given in Tab. 1, 2, and 3. Fruit weight varied significantly among varieties, with the lowest weight in Golden (131.4 g/fruit) and the highest in Sultan (183.4 g/fruit). Juice percentage ranged from 20.0% (Golden) to 33.4% (Sultan). TSS of pomegranate juice samples ranged from 12.4 to 17.2%, with the higher value of TSS (17.2%) noted in Sultan while the lower percentage was documented in Pearl (12.4 %). The highest percentage of peel was found in Pearl (57.3%), while the lowest percentage of peel was observed in Sultan (54.7%). The range of seed percentage in samples was 41.2% (Pearl) to 45.3% (Sultan). The percentage of reducing sugar varied from 8.3% (Golden) to 12.2% (Sultan). As for non-reducing sugar, Sultan (4.1%) had the highest rank, and Pearl (2.9%) had the lowest. Observations regarding total invert sugar showed that pomegranate juice contained total sugar ranging from 11.8% (Golden) to 14.3% (Sultan). The value of Vitamin C was observed highest in Pearl (11.5 mg/100g) and the lower value was noted in Qandhari (10.6 mg/100g). Titratable acidity varied significantly among the varieties, ranging from 0.8% (Sultan) to 1.9% (Pearl). Juices of all the varieties showed varied pH but pH of all the varieties fall in the acidic range and differed non-significantly. Beta-carotene was observed in red juice of pomegranate but in a very low extent, i.e., in micrograms. It was 60 and 61 in Qandhari and Sultan, respectively, but in traces in Pearl and Golden. Table 3 carries data regarding total antioxidant and total phenols of pomegranate samples. The body of living organisms manages the oxidants in normal conditions, but for excess oxidants, the body needs antioxidants as a supplement. Antioxidants are determined to find out the capability of fruit to meet the body's requirements. The current study showed the variation in antioxidant ability of the pomegranate varieties. Varieties showed a statistically significant range from 27.7 (Pearl) to 43.5% (Sultan). Total phenols were studied greater in Sultan (1111 µg GAE L^{-1}) than in Qandhari (1085 µg GAE L^{-1}) and lowest in Pearl (1022 µg GAE L^{-1}). Data regarding peel analysis was given in Tab.4. In peel analysis, varieties showed a different percentage of crude protein. Peel of Sultan contained higher crude protein (3.67%), which was found significantly better than other varieties. Qandhari showed a lower value for protein (3.17%) than Sultan, but it was significantly better than Golden (2.97%) and Pearl (2.73%).

The data regarding the proximate analysis of the peel are presented in Table 4. The percentage of crude fat in the peel powder of different varieties ranged from 1.50% to 1.69%, but they were statistically non-significant. The crude fiber content was the lowest in Pearl (6.89%) and the highest in Sultan (11.9%), but Sultan was statistically at par with Qandhari (11.07%) and Golden (10.2%). The ash/mineral matter contents varied among the different varieties. Sultan was significantly better than the other varieties, with ash contents of 3.67%. The lowest value was observed in Golden (2.7%), followed by Qandhari (3.3%) and Pearl (2.77%).

Food industries are interested in the nutritional value of fruit products, in addition to their taste, to attract consumers. Therefore, food products should support maintaining health and play a role in preventing and treating many illnesses (Viuda-Martos *et al*., 2010 a,b). Today, significant attention is given to diets that are

Table 1. Analysis result of pomegranate varieties for physical parameters

Pome Var.	Fruit wt. (g/fruit)	Juice (%)	TSS (%)	Peel (%)	Seed (%)
Pearl	135.0 c	25.9 b	12.4 d	57.3 b	41.2 d
Golden	131.4 d	20.0 d	13.2 c	55.6 c	44.4 c
Qandhari	158.2 b	22.6 c	14.9 b	58.8 a	47.2 a
Sultan	183.4 a	33.4 a	17.2 a	54.7 d	45.3 b
LSD	1.005	1.017	0.4037	0.6945	0.5994

Values sharing the same letters are statistically similar

Table 2. Analysis result of pomegranate varieties for quality parameters

Pome Var.	Reducing sugar (%)	Non-reducing sugars (%)	Total invert sugars (%)	Vitamin C (mg/100ml)
Pearl	9.2 c	2.9 b	12.1 b	11.5
Golden	8.3 d	3.5 ab	11.8 b	10.9
Qandhari	10.4 b	3.3 ab	13.7 a	10.6
Sultan	12.2 a	4.1 a	14.3 a	10.7
LSD	0.8258	0.9353	0.6993	NS

Values sharing the same letters are statistically similar

Table 3. Analysis result of pomegranate varieties for phenolic

Pome Var.	Total Antioxidant (%DPPH activity)	Total phenols (µg GAE L^{-1})	Acidity (%)	pH	Beta-carotene (µg/100g)
Pearl	27.7 d	1022 d	1.9 a	3.27 c	0.02 b
Golden	31.2 c	1047 c	1.4 ab	3.20 c	0.04 b
Qandhari	42.4 b	1085b	1.1bc	3.53 b	60.80 a
Sultan	43.5 a	1111 a	0.8 c	3.80 a	61.80 a
LSD	0.6751	13.05	0.5680	0.2492	1.5437

Values sharing the same letters are statistically similar

Table 4. Analysis result of pomegranate varieties for proximate parameters

Pome Var.	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)
Pearl	2.73 c	1.56	6.89	2.77 bc
Golden	2.97 bc	1.50	10.22	2.70 c
Qandhari	3.17 b	1.55	11.07	3.30 ab
Sultan	3.67 a	1.69	11.90	3.67 a
LSD	0.3954	NS	NS	0.5947

Values sharing the same letters are statistically similar

beneficial, such as pomegranate. Apart from its basic nourishing activities, it provides biological assistance and plays a significant role in suppressing the initiation of lethal diseases. Pomegranate has medicinal and nutritional benefits due to its multifunctionality. Faria and Calhau (2010) reported almost the same findings regarding antioxidants and total phenols as our results. The antioxidant and antibacterial properties of pomegranate have also been previously reported (Al-Zoreky, 2009). Pomegranate is a natural source of vitamins and its antioxidant, antimicrobial, and chemo preventive properties make it an important part of the human diet (Huxley and Neil, 2003). The parameters that were determined, such as vitamin C, antioxidants, and phenols, are supported by previous studies by Huxley and Neil (2003) who explained that these components occur naturally in pomegranate juice. Elfalleh *et al*. (2011) also mentioned that pomegranate contains plant-based antioxidants. One of the important characteristics of pomegranate is that it contains antioxidants, making it a healthier option for consumption. Its antioxidant properties can help to reduce the risk of heart disease and other chronic illnesses (Khan *et al*., 2007). The total soluble solids (TSS) present in the juice of all pomegranate varieties are consistent with the results obtained by Tehranifar *et al*. (2010).

Variations in fruit weight were observed among different varieties under different ecological conditions, such as in Murree and Faisalabad, as reported by Zaouay et al. (2012). The study highlighted that both environmental factors and variety may influence fruit weight. The high juice content observed in some varieties is a promising characteristic for commercial juice and cold drink industries, as reported by Rajasekar *et al*. (2012).

Variations in the phenolic pattern of the varieties affect the pH and acidity of the juice, leading to differences in these parameters among the varieties, as reported by Gil *et al*. (2000). Natural sugar content was found to be desirable in all varieties, and our results for total sugar content were similar to those reported by Aviram et al. (2000) and Poyrazoglu *et al*. (2002). Our findings regarding Vitamin C were consistent with those reported by Youssef et al. (2007) for pomegranate varieties. Furthermore, our study demonstrated significant differences in the biochemical contents of the various varieties, as reported by Miguel *et al*. (2004).

The proximate analysis values of pomegranate peel powder presented in Table 4 are consistent with those reported by Kushwaha et al. (2013). Pomegranate peel is a valuable component, as it is rich in phenolic compounds (Li *et al*., 2006). Our findings indicate that pomegranate peel contains a high-quality protein, which could potentially be used in animal feed. The proximate analysis of pomegranate peel in our study is consistent with that reported by Rowayshed *et al*. (2013), who found that pomegranate peel powder contained high levels of protein and other nutrients that are often lacking in most food items. The ash content results for pomegranate peel powder are in agreement with those reported by Rowayshed et al. (2013), who reported that pomegranate by-products are rich in minerals. Our crude fat results for pomegranate peel powder are supported by Omer *et al.* (2019). The results of our study are consistent with those obtained by Kotsampasi et al. (2014).

5. CONCLUSION

The phytochemical characteristics of pomegranate varieties play a significant role in determining their biologically active capacity. The Wonderful variety stands out with the highest number of bioactives observed. Therefore, pomegranate varieties that are rich in antioxidants, phenols, vitamin C, and other nutritional contents can be beneficial for consumers in terms of their health. All varieties have a substantial amount of bioactive compounds; however, Sultan stands out with better performance in all parameters.

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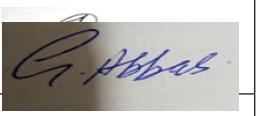
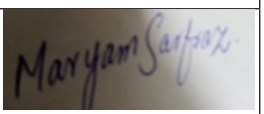
1.	Naseem Akhtar	Conducted the research and wrote the manuscript	
2.	Ghulam Abbas	Check the English grammar and spellings of paper	
3.	Maryam Sarfraz	References check and set according to the journal requirement	
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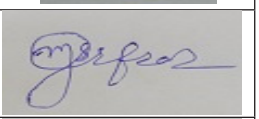
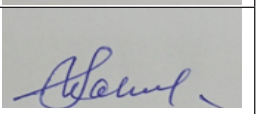
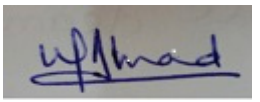
5.	Muhammad sarfraz	Facilitate the research work	
6.	Shakeel Ahmad Anwar	Supervised the Research and analysis work	
7.	Waqar Ahmad	Critically reviewed the manuscript	
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