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Phytochemical assessment of common *Punica granatum* L. varieties grown in district Gilgit, Pakistan

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Abstract

Regular consumption of fruits, vegetables and their byproducts lessen the chances of many health hazardous ailments due to existence of numerous valuable nutrients. To evaluate some physcio-chemical characteristics of three pomegranate varieties (sweet, sour and doom) juices from three different localities (Danyore, Nomal and Sakwar) of district Gilgit were collected. The parameters studied were pH, total soluble solids, reducing sugars, titratable acidity, flavonoids, carotenoids, anthocynins. The pH content found among the sweet cultivars varied from 2.99-3.9, anthocynins ranged from 294.99-295.33mgC3G, flavonoids differed from 232.21-234.83mgQC/g, reducing sugars fluctuated from 10.99-12.24%, carotenoids varied from 12.12-12.76 mg ß-carotene/g, TSS ranged from15.9-17.3 brix^{o} and titratable acidity fluctuated from 0.362-0.403%. Among doom variety pH differed from 3.19-3.33 anthocynins ranged from 289.9-290.29mgC3G, flavanoid varied from 233.31-235.09mgQC/g, reducing sugars range from 9.09-10.2%, carotenoids fluctuated from 13.09-13.3 mg ß-carotene/g, TSS varied from 15.8-16.66 brix^{o} and Ttitratable acidity range from 0.588-0.645%. Among the sour cultivars pH varied from 2.35-2.66, anthocynins ranged from 292.82-293.7mgC3G, flavanoid ranged from 230.37-232.14mgQC/g, reducing sugars varied from 7.27-8.03%, carotenoids differed from 11.7-11.9mg ß-carotene/g, TSS ranged from 14.0-15.2 brix^{o} and titratable acidity range from 0.719-0.817%.

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Introduction

Fruits and vegetables are full of nutritional and non nutritional component which are indispensable for continuation of a healthy life. Pomegranate (*Punica granatum L.*) is a member of *Punicacea* family. It is non-climacteric and deciduous fruit (Jurenka, 2008). It is enlisted among important commercial horticultural fruits which are indigenous to central Asia and widely grown in different zones of Asia, North Africa, the Mediterranean, and the Middle East (Biale, 1981; Harde *et al.,* 1970; Sarkhosh *et al.*, 2006)..

Studies on pomegranate has demonstrated that it is a rich source of energy, protein, carbohydrate, sodium, potassium, calcium, magnesium, iron, copper, niacin, vitamin C, anthocynins, flavonids, organic acids and numerous other components compulsory for metabolic activities (Grove and Grove, 2008). Due to its valuable phenolic concentration the pomegranate is considered as a "healthy" fruit. The high content of polyphenols particularly, of ellagitannins, the major ones being punicalagins. Other phenolic includes anthocyanins, condensed tannins, and flavonoids (quercetin, kaempferol and luteolin glycosides) are also present in pomegranates (Lansky and Newman, 2007).

The important nutritional composition, functional characteristics and nutraceutical benefits of pomegranate makes it valuable ingredient for food and pharma industry. The epidemiological studies have revealed that the pomegranate polyphenols are remedy for the prevention of cardiovascular stroke, cancer diseases and neurological damage in consumers (Aviram *et al.*,2008; Cerda *et al.*,2003).

The pomegranate juice is an excellent source of anthocyanins, being the 3-glucosides and 3,5-diglucosides derivatives of delphinidin, cyanidin and pelargonidine, which are the principal health promoting ingredients (Gil *et al.*,2000). Reported to reduce blood pressure, reduces prostate cancer chances arthritis, remedy during diarrhea, (Malik *et al.*,2005) protecting phagocyte cells from oxidative damages, and keeps blood glucose level in normal range (Aviram *et al.*,2005). Numerous studies have confirmed their good results against AIDS and inflammation (Seeram *et al.*,2008).

The widely grown fruit is not only preferred as fresh but also processed in numerous valuable nutritive food products. Pomegranate fruit juice is among most prominent healthy fruit juices liked and consumed around the globe.

Its juice contains 85.4% moisture and carries substantial concentration of organic acids, vitamins, polysaccharides, polyphenols and important minerals total soluble solids, total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins and exhibits antioxidant characteristics (Al-Maiman and Ahmad, 2002).

In addition to the pomegranate juice, fruit is also processed in numerous food products in food industry. A good quality of jellies, wine, jam, paste concentrates, seed oil, salad dressing and flavoring and coloring agents are widely prepared (Sumner *et al.* 2005) . it raw fruit products are used as herbal to cure wound and skin diseases since ancient civilization due to these attributes it's got title of super food (Akpinar-Bayizit *et al.*,2012). The aim objective of the study was to evaluate nutritional significance of the commonly grown verities in the selected region.

Materials and methods

To examine some physcio-chemical attributes of selected varieties of pomegranate from different localities of district Gilgit, an investigation was conducted in the departmental lab of Agriculture and Food Technology, Karakorum International University, Pakistan.

Sample collection

Three fully ripened varieties (Sweet, Sour, Doom) of pomegranate were collected from three different valleys Danyore, Nomal and Sakwar of district Gilgit,

Juice extraction

After thorough washing manually seeds were extracted using knife. The pulp was extracted from all varieties by using the electric blender and juice was filtered by using muslin cloths.

Physico-chemical and phytochemical analysis of pomegranate juice

pH of the samples were measured with the help of digital pH meter (AD 1020) by standard method described by AOAC (2006). Juices of each variety were poured in 100 ml beakers separately then electrode was inserted after calibration of pH meter with pH 4.0, pH 7.0 and pH 9 buffer solutions**.**

The electrode was washed with distilled water and wiped with tissue paper before taking each reading. Total soluble solids were measured according to procedure of AOAC (2006) using digital refractometer at room temperature. Lenses of refractometer were calibrated with distilled water and readings were taken directly.

To determine reducing sugars the AOAC (2000) method was followed. For this purpose 10ml of juice was taken in a beaker and mixed with 100ml hot distilled water to dissolve the sample completely and filtered with filter paper. 10 ml of 1N HCl was also added to this 100ml solution and boiled for 5 minutes and when cooled down that solution was neutralized with 10ml of 1N NaOH. Finally volume of that mixture was made up to 250ml with distilled water. That solution was filled in burette. 5ml fehling solution A, 5ml fehling solution B and 10ml distilled water was taken in a conical flask, heated and titrated against burette solution and in this way three readings were taken results were calculated with the following formulas:

$$\text{Reducing sugar}(\%) = \frac{\text{Factor (4.95)} \times \text{dilution (250)}}{\text{Titre} \times \text{weight of sample} \times 10} \times 100$$

Total titratable acidity was measured by standard method given by AOAC (2006). For determination of titratable acidity about 10ml juice from each variety was taken separately in 100ml volumetric flasks and volumes were made up to 100ml with distilled water. (10ml) of diluted sample was taken in a conical flask separately and 2-3 drops of phenolphthalein was added as an indicator and diluted samples were titrated against 0.1N NaOH solution until or unless a light pink color achieved which remained for 15-20 seconds. The ml of 0.1N NaOH used was noted for the entire samples and acidity was calculated by following formula:

$$\text{Acidity}\% = \frac{\text{F} \times \text{T} \times 0.1\text{N NaOH}}{\text{L} \times \text{M}} \times 100$$

F =Factor of acid (citric acid) **T** = ml of 0.1 N NaOH solution used. **M** = diluted sample taken for titration **L** = sample taken for dilution.

For determination of total flavonoid content Kale *et al.*(2010) prescribed method was used. 1ml (mg/ml) of the juice was mixed separately with 4ml distilled water, 0.3ml 10% $AlCl_3$ and 2ml 1M NaOH in a volumetric flask and finally volume was made up to 10ml with distilled water. Absorbance of the mixture was measured 510nm with the help of UV spectrophotometer by using standard curve of quercitine.

For determination of total carotenoid content Rodriguez Amaya, (1999) method was used. 1ml of juice (mg/ml) was added with 2ml methanol and 18 ml petroleum ether in a ratio of (1:9) in a separating funnel. When two layers were formed the bottom layer was drained out and the mixture was transferred to the volumetric flask and volume was made up to 20 ml with petroleum ether. Absorbance of the mixture was measured at 450nm with the help of UV spectrophotometer by using standard curve of beta carotene.

The total anthocyanin was estimated with some modification Cintra 5 UV–vis Spectrophotometer by the pH-differential method (Sellappan and Akoh, 2002) using two buffer systems – potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M).Briefly, 0.4 ml of the extract was

mixed with 3.6 ml of corresponding buffers and read against a blank at 510 and 700 nm. Absorbance (A) was calculated as

A = (A510 _ A700)pH 1.0 _ (A510 _ A700)pH 4. Monomeric anthocyanin pigment concentration in the extract was calculated as

cyanidin-3-glucoside (mg/l) = A · MW · DF · 1000 /(MA · 1)

where A: absorbance; MW: molecular weight (449.2); DF: dilution factor; MA: molar absorptivity (26,900).

Result and discussion

The pH results determined from three different pomegranate varieties juices from three various localities are presented in Figure. 1. The lowest values of pH among the sour varieties were obtained from Nomal cultivars **(**2.3) and highest pH result was found from Sakwar (2.66) samples.

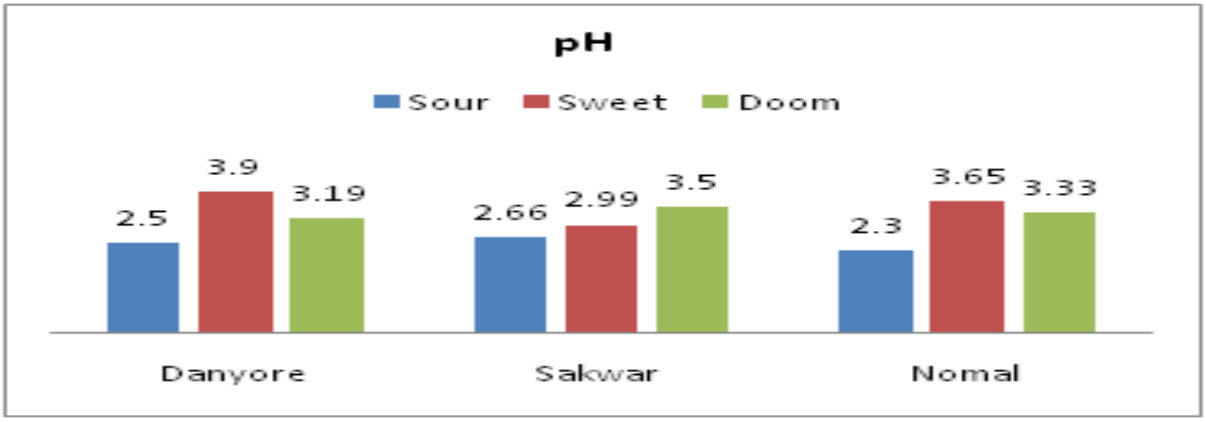

Fig. 1. pH of pomegranates juices from three different locality fruit.

Among the sweet type lowest value was estimated in the variety collected from Sakwar (2.99) and highest value of pH was assessed in cultivar collected from Danyore (3.9). Among the doom variety the highest value of pH was obtained from the variety collected from Sakwar (3.5) while the lowest was evaluated from Danyore (3.19) valley samples. The variation in PH values among different varieties may be due difference in organic acid content in the pomegranate fruit. The findings of the research resemble with the study conducted by Cam *et al.*,2009 and Radunic *et al.*,2015.

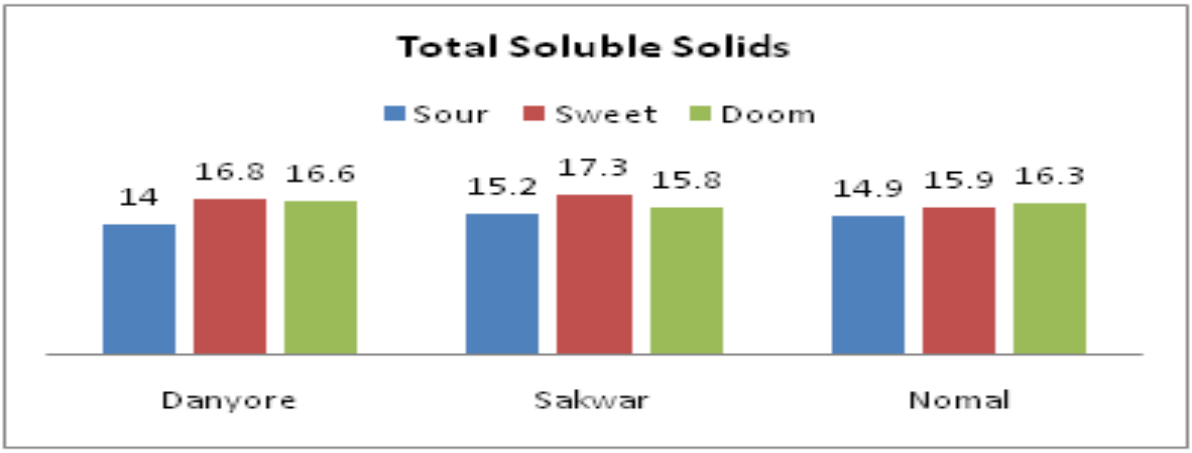

Fig. 2. Total Soluble Solid of Pomegranate from three different locality fruit.

The total soluble solids (TSS) content from various pomegranate varieties juices tested from three different vicinities are presented in Figure 2. Maximum TSS result among sweet cultivars was 17.3 brix° recorded from Sakwar while lowest was 15.8 brix° estimated from Danyore region. Among the doom growers peak calculations were 16.6 brix° investigated from Danyore and least result was 15.8 brix° observed from Sakwar valley. The highest TSS results were 15.2 brix° calculated from Sakwar and least was recorded 14.0 brix° from Danyore zone among the sour cultivars. While our results were in conformity with values reported by Fadavi *et al.*(2005).

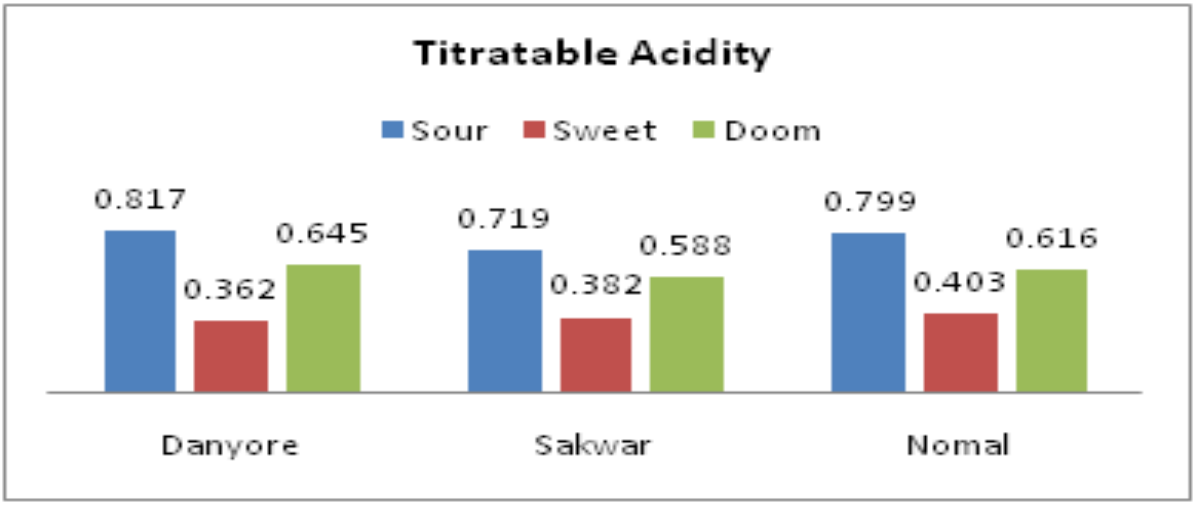

Fig. 3. Titratable Acidity in pomegranate from three different locality fruit.

The total titratable acid assessed from three pomegranate varieties juices from three different areas is displayed in Figure. 3. Among the sour cultivars highest result (0.817%) was calculated from Danyore while lowest (0.719) was assessed from Sakwar. The peak outcomes (0.403%) among sweet pomegranate growers were obtained from Nomal and least (0.362%) were estimated from Danyore valley, while among the doom samples peak (0.645%) values were calculated from Danyore and lowest (0.588%) was obtained from Sakwar zone. The minimum acidity of pomegranate juice is 0.35% which was reported by Mustafa *et al.* (2008); Fadavi *et al.*,(2005). According to Melgarejo, (1993), the predominant acid of this fruit is malic acid.

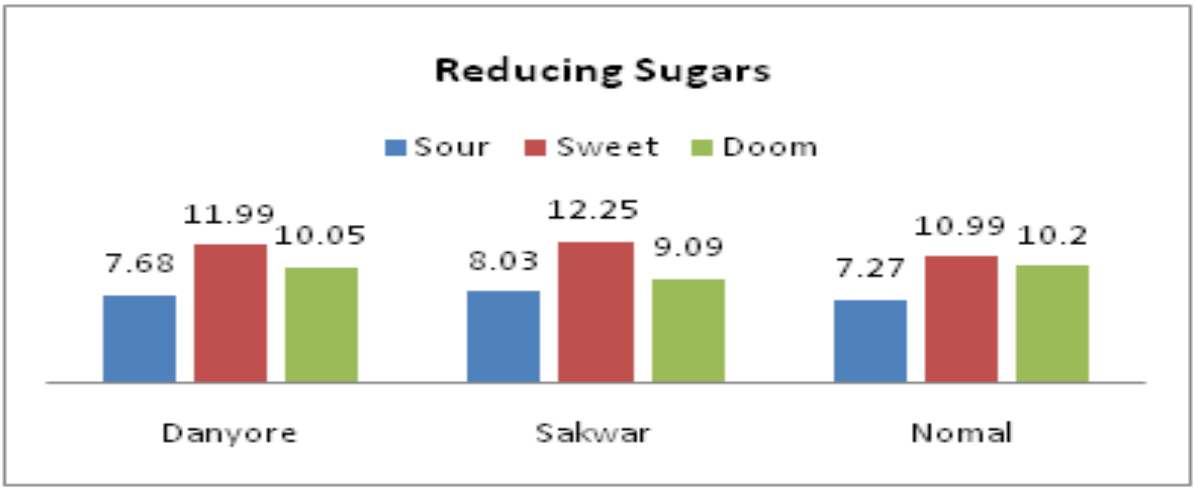

Fig. 4. Reducing sugar in Pomegranate juices from three different locality fruits.

Reducing sugar

The reducing sugar results obtained from different pomegranate varieties collected from three various vicinities has been documented in Figure 4. According to the table highest reducing sugar results among doom cultivars was 10.2% obtained from Nomal and least was 9.09 observed from Sakwar. Among the sweet variety peak value was 12.25% recorded from Sakwar and minimum was 10.99% evaluated from Nomal, while among the sour variety highest result was 8.03% investigated from Sakwar and lowest was 7.27% obtained from Nomal valley. The findings of total reducing sugars is in agreement with the findings of Al-Kahtani, (1992) and Saxena *et al.*(1987).

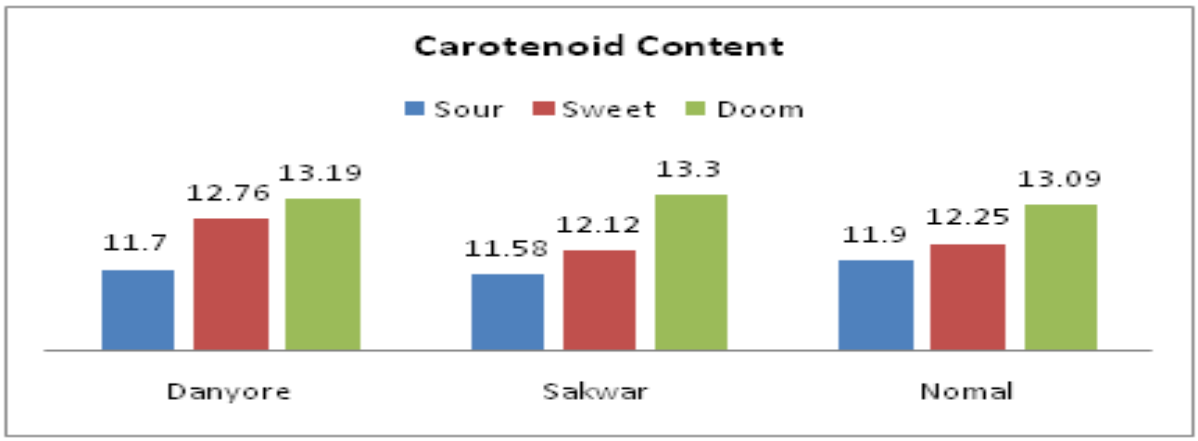

Fig. 5. Carotenoid content of pomegranate juice from three different locality fruits.

Carotenoids are pigments vital for human and animal nutritional prospectus due to conversion of some of pigment in vitamin A, which is essential micronutrient for vision and numerous metabolic functions in the human body (Bhaskarachari *et al.,* 1995). The total carotenoids result of current research work is displayed in Figure. 5. According to the table highest result (12.76 mg ß carotene/g) among sweet fruits was estimated from Danyore and lowest (12.12 mg ß carotene/g) was analyzed from Sakwar. Among the doom cultivars peak findings (13.3 mg ß carotene/g) were assessed from Sakwar and minimum (13.09 mg ß carotene/g) was determined from Nomal while among the sour type high results (11.9 mg ß carotene/g) were observed from Nomal and low (11.58 mg ß carotene/g) were calculated from Sakwar valley.

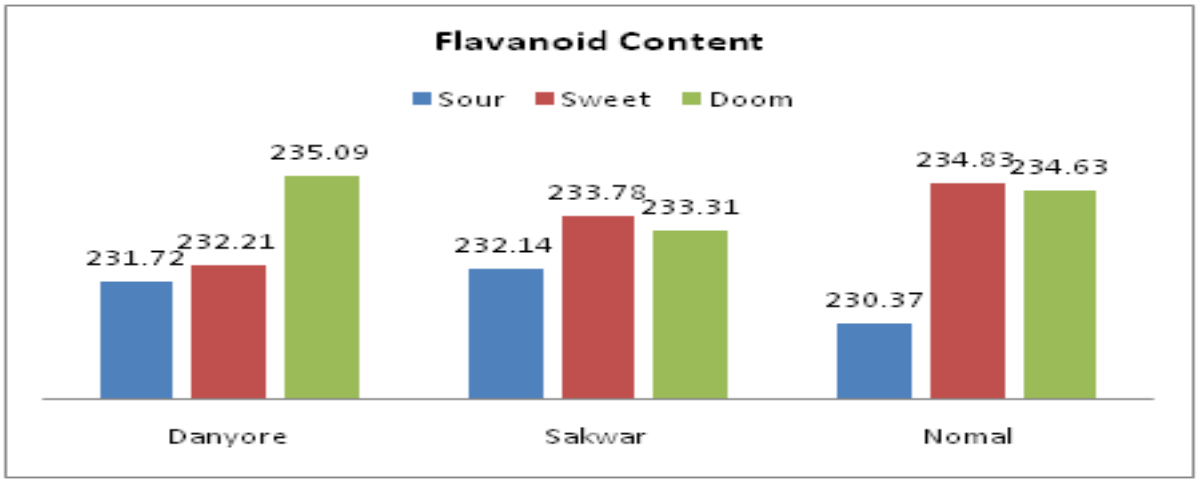

Fig. 6. Flavonoids content of pomegranate juice from three different locality fruits.

Fruits are rich in physiological active materials like Flavonoid, which can powerfully combat with biological oxidation. They are widely used in medicines and health care products (Schubert *et al.*, 1999). The current research findings for the total flavonoid content from all the sample has been mentioned in Figure. 6. According to the table peak results among sweet cultivars was 234.83 mg QC/g investigated from Nomal and minimum was 232.21 mg QC/g from Danyore, among the doom varieties highest outcome was estimated 235.09 mg QC/g from Danyore and lowest was 233.31 mg QC/g calculated from Sakwar, while among the sour varieties highest value was 232.14 mg QC/g determined from Sakwar while minimum was 230.37 mg QC/g calculated from Nomal valley.

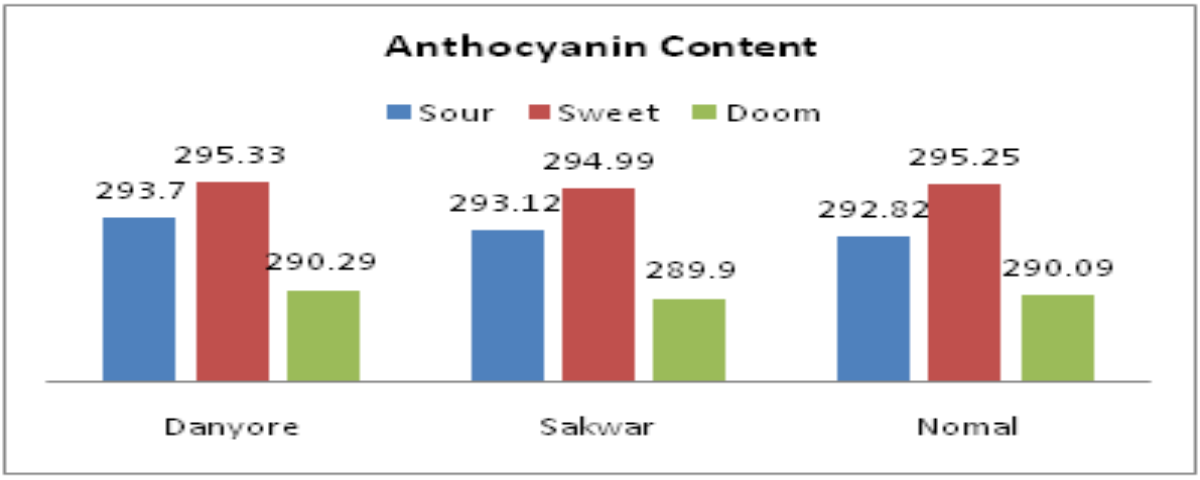

Fig. 7. Anthocyanin content of pomegranate juice from three different locality fruits.

Anthocyanin are the most abundant and important group of water soluble naestural pigments which pose health promoting activities in consumers (Withy *et al.,* 1993). The current research results for total anthocyanin content in different samples has been described in Figure. 7.

The table shows maximum values (295.33 mgC3G) from Danyore while minimum values (294.99 mgC3G) from Sakwar among the sweet cultivars. Among the doom cultivars highest (290.29 mgC3G) anthocyanin values were obtained from Danyore and least (289.9 mgC3G) from Sakwar while among the sour cultivars peak results (293.7 mgC3G) were obtained from Danyore and lowest values (292.82 mgC3G) were recorded from Nomal valley.

Conclusion

The current study revealed the excellent nutritional composition of pomegranate juice which is helpful to keep away serious health ailments from the consumer like heart problems, cancer, HIV, neurological disorder, cellular oxidation, arthritis and many other diseases. Further thorough study can also evaluate the other medicinal and nutritional significance of pomegranate fruit and its byproducts.

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