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RESEARCH ARTICLE

Changes in quality characteristics of pomegranate juice concentrate during refrigerated storage

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ABSTRACT

The present study was carried out to determine the changes in various quality characteristics of pomegranate juice concentrate at refrigerated storage (4°C). Effect of refrigerated storage on anthocyanin, ascorbic acid, total phenolic content and antioxidant activity of pomegranate juice concentrate was determined for 42 days of storage period after every 7 days of time interval. During the refrigerated storage, anthocyanin content, ascorbic acid content, total phenolic content and antioxidant activity decreased as storage time increased. Ascorbic acid showed higher reduction percentage (90.29%) during the storage. For lower storage period, reduction in anthocyanin content, total phenolic content and antioxidant activity was non-significant ($p < 0.05$), however significant reduction was observed for prolonged storage period.

Key words: Pomegranate juice concentrate; Storage; Anthocyanin, Ascorbic acid; Antioxidant activity.

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INTRODUCTION

The Pomegranate (*Punica granatum* L.) is an important commercial fruit crop that is widely cultivated in parts of Asia, North Africa, the Mediterranean, and the Middle East (Sarkhosh et al., 2006). Pomegranate is one of the oldest known edible fruits and is popularly consumed as fresh fruit, juice, and as a food product. Commercial pomegranate juice is obtained by a hydrostatic pressing process of whole fruits. The juice can be processed possible into the squash, syrup, nectar, jelly, concentrates and such other products. Pomegranate juice can be used as an ingredient providing colour to the other products. (Maskan, 2006; Rosenblat and Aviram, 2006). The pomegranate juice is a rich source of polyphenols. The phenolic constituents of pomegranate such as the anthocyanins give the colour and other polyphenols such as flavonoids and some non-flavonoids are responsible of antioxidant properties, astringency and bitterness to juice (Gil et al., 2000). The antioxidant qualities of pomegranate juice, makes it appealing for the production of health supplements and nutraceuticals (Singh et al., 2002).

Juices are produced from various fruits that are not readily available round the whole year. Before shipping to its final destination the extracted juice is concentrated to ensure longer storage life (because of its low water activity) and easier

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transportation. Various quality changes occur during storage of fruit juice concentrate, depending upon the storage conditions. Anaerobic degradation of ascorbic acid mainly appears during storage (Johnson et al., 1995; Solomon et al., 1995). During storage of fruit juices and juice concentrates, there is reduction in polyphenolic content and thus antioxidant activity also decreases (Turkmen et al., 2005; Szajdek and Borowski 2005; Dietrich et al., 2004). The present study was undertaken to determine the extent of quality changes of pomegranate juice concentrate during refrigerated storage.

MATERIALS AND METHODS

Procurement of fruits

Fully ripened fruits of pomegranate were procured from local market and stored at refrigeration temperature until they were used for the juice extraction. All the chemicals and reagents used were of analytical grade (M/s. Loba Chemie Pvt. Ltd, Mumbai, India).

Preparation of pomegranate juice concentrate

Pomegranate fruits were cleaned and washed in running water. The fruits were then cut into 4 pieces and the arils were separated. The juice was extracted from the arils by laboratory type juicer. The extracted juice was filtered through a muslin cloth and concentrated to a predefined concentration of 70 °Brix at a temperature of 60 °C with the help of rotary vacuum evaporator. The juice concentrate was then transferred to transparent glass bottles and stored in laboratory refrigerator at 4 °C temperature for 42 days of storage period and evaluated for changes in various quality parameters after every 7 days of time interval.

Total anthocyanin content measurement

Total anthocyanin content was determined using the pH-differential method described by Lee, Durst, and Wrolstad (2005), using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M), and sodium acetate buffer, pH 4.5 (0.4 M). The concentrate sample was diluted to 8.90 Brix juice. A 0.1 mL aliquot of the juice was transferred to a 10 mL volumetric flask and made up to 10 mL with corresponding buffer and the absorbance was measured at 510 and 700 nm for solutions at pH 1.0 and pH 4.5, respectively. Total anthocyanins were calculated as cyanindin-3-glucoside according to the following equation:

$$Total\ anthocayanin\ content\ (mg/L) = \frac{A \times MW \times DF \times 1000}{(\varepsilon \times 1)} \qquad (1)$$

where A = $(A_{510} - A_{700})_{pH1.0} - (A510 - A700)_{pH4.5}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; 1 = pathlength in cm; ε = 26,900 molar extinction coefficient in L/mol/cm for cyanidin-3- glucoside; 1000 = conversion from g to mg.

Determination of ascorbic acid

Ascorbic acid content of pomegranate squash was estimated by volumetric method of Sadasivam and Manicam (2008). 5 ml of standard ascorbic acid (100µg/ 100ml) was taken in a conical flask containing 10 ml 4% oxalic acid and was titrated against the 2, 6-dichlorophenol indophenols dye. The appearance and persistence of pink colour was taken as end point. The amount of dye consumed (V_1 ml) is equivalent to the amount of ascorbic acid. 5 ml of sample (prepared by taking 5g of squash in 100 ml 4% oxalic acid) was taken in a conical flask having 10 ml of 4% oxalic acid and titrated against the dye (V_2 ml). The amount of ascorbic acid was calculated using the formula,

Ascorbic acid (mg/100 g) = (0.5 mg/V_1ml) × (V_2/15 ml) × (100 ml/Wt. of sample) × 100.

Where, V_1 and V_2 = Volume of dye consumed during titration of standard ascorbic acid solution and solution of sample, respectively and W_s = Weight of the sample, g.

Determination of total phenolic content

The total phenolic content of pomegranate juice concentrate was determined by Folin-Ciocalteu method, described by Singleton and Rossi, 1965. To 8 mL of diluted solution (1:9) of pomegranate juice concentrate, 500 µL of Folin-Ciocalteu reagent were added. After 3 to 8 min, 1.5 mL of saturated sodium carbonate solution was added. After 2 h the absorbance value was read at 765 nm. The total polyphenol content was calculated from the standard calibration curve obtained from gallic acid (10 - 100µg/ml) and expressed as mg of gallic acid equivalent (GAE) per 100 mL of juice. The measurements were done in duplicate.

Determination of antioxidant activity

Antioxidant activity was determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) as free radical reagent according to the procedure described by Delgado et al. (2010) with some modifications. A mass of 0.0024 g of DPPH was dissolved in 100 mL of methanol to obtain a solution of 6.09·10–5 mol/L. The juice concentrate was diluted in methanol/water in a ratio of 4:1 (by volume) and 300 µL of the solution was added to 2.7 mL of DPPH methanolic solution. After 1 h in the dark at room temperature, the absorbance was read at 517 nm. Methanol was used as the control. Antioxidant activity was expressed as the percentage of scavenging activity according to the following equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100 \qquad (2)$$

where Abs control is the absorbance of control and Abs sample is the absorbance of sample.

Statistical analysis

All the analyses were carried out in triplicates and the results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Total anthocyanin content

Anthocayanin pigments are considered the most important elements of the quality of pomegranate juice and the red color of pomegranate juice is primarily associated with anthocyanin pigment (Alighourchi and Barzegar, 2009). The anthocyanin content of fresh prepared pomegranate juice concentrate was 823 mg/100g. With increase in storage period, total anthocyanin content of pomegranate juice concentrate decreased from 823 to 342 mg/100g (Table 1). However, significant reduction was observed for prolonged storage period. Decrease in anthocyanin content could be related to low stability and degradation of anthocyanin pigments during storage

Ascorbic acid content

Vitamin C is often used as an estimate for the overall nutrient retention of food products because it is by far the least stable nutrient. It is highly sensitive to oxidation and begins to degrade immediately after harvest and also continues to degrade even during prolonged storage of frozen products (Franke et al., 2004). Results related to changes in ascorbic acid content of pomegranate juice concentrate during storage at refrigeration temperature are presented in Table 1. Results indicated that

ascorbic acid content of pomegranate juice concentrate decreased significantly during the entire storage period. The reduction may be due to oxidation of ascorbic Acid into dehydroascorbic acid. Similar results were also reported by Mandal and Nath (2013), worked on storage stability of aonla (*Emblica officinalis)* squash.

Table 1. Effect of refrigerated storage on total anthocyanin content and total ascorbic acid content of pomegranate juice concentrate

Storage time (days)	Total anthocyanin content (mg cyanindin-3-glucoside /100ml)	Ascorbic acid content (mg/100g)
0	823±2.67^{a}	9.89±0.18^{a}
7	811±5.69^{ab}	8.15±0.13^{b}
14	793±3.24^{b}	6.67±0.23^{c}
21	659±2.78^{c}	4.96±0.55^{d}
28	555±3.87^{d}	2.56±0.49^{e}
35	478±2.45^{e}	1.89±0.12^{f}
42	342±2.91^{f}	0.96±15^{g}

Table 2. Effect of refrigerated storage on total Phenolic content and antioxidant activity of pomegranate juice concentrate

Storage time (days)	Total phenolic content (mg GA/100ml)	Antioxidant activity (%)
0	4795±2.67^{a}	19.23±0.72^{a}
7	4781±1.69^{a}	18.14±0.83^{a}
14	4769±5.24^{a}	16.01±0.57^{b}
21	4671±2.78^{b}	14.23±0.23^{c}
28	4422±3.87^{c}	12.19±0.55^{d}
35	4150±2.45^{d}	9.33±0.49^{e}
42	3728±2.91^{e}	7.29±0.80^{f}

Total phenolic content

Phenolic compounds play an important role in determining the colour and flavor of a product. They are highly volatile and easily oxidized (Karpagavalli and Amutha, 2015). There was a gradual decrease in total polyphenols in pomegranate juice concentrate during storage (Table 2). Prolonged storage period of above 14 days showed significant effect on total phenolic content. A gradual loss of total phenols during storage might be due to their condensation into brown pigments. Similar results were also reported by Kannan and Thirumaran (2001) for jamun products and Sharma et al. (2012) for guava-jamun blended RTS drink and squash.

Antioxidant activity

The changes in the total antioxidant activity of pomegranate juice concentrate during storage are presented in Table 2. Total antioxidant activity of freshly prepared pomegranate juice concentrate was 19.23%. For a storage period of 7 days there was

no significant decrease in total antioxidant activity. However, after prolonged storage, there was a significant reduction in antioxidant activity. The decrease in antioxidant activity could be related to decrease in ascorbic acid, total poly phenols and total flavonoid content. Tomczak (2007) also observed a decrease in antioxidant activity during storage of black chokeberry juice concentrate. Yang et al. (2007) reported that storing the juice for 30 days at the temperature of 4°C or 24°C, free radical scavenging capacity was found to decrease by 36 and 83 per cent, respectively.

CONCLUSION

This investigation demonstrates that ascorbic acid showed higher reduction percentage during the storage of pomegranate juice concentrate. Besides this, changes in anthocyanin content, total phenolic content and antioxidant activity of pomegranate juice concentrate during refrigerated storage were not significant for low storage period. However the reduction was significant for higher storage period.

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