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INFLUENCE OF STORAGE CONDITION ON THE ANTIOXIDANT ACTIVITY OF POMEGRANATE SQUASH

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Abstract

Pomegranate (*Punica granatum* L.) has been used as a natural source of medicine since ancient time. The fruit exhibit very high antioxidant activity due to the presence of several nutraceutical compounds *viz.* anthocyanins, phenolics, flavonoids, alkaloids, minerals and antioxidant vitamins. The present work was carried out to study the influence of storage condition on the antioxidant properties of pomegranate squash. Pomegranate squash was prepared by FPO specification and stored under room and refrigeration condition for 180 days. The changes in physico chemical characteristics and antioxidant properties such as total poly phenols and total flavonoids were analyzed during storage. The total antioxidant activity was analyzed by using 2, 2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) assay. The results showed that the TSS, acidity and reducing sugar increased, while pH, total sugar and ascorbic acid decreased in squash with increase in storage period. Total poly phenols, total flavonoids and antioxidant activity were decreased during storage. The retention of antioxidant components and activity was high in refrigeration temperature.

Key words : Pomegranate, squash, antioxidant properties, storage condition.

Introduction

Plants are the potential source of natural phytochemical antioxidants, which work as secondary metabolites of plants (Walton and Brown, 1999). They are carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols, tocotrienols etc. The phenolic compounds and antioxidants from fruits play a pivotal role in human health and even pertinence of life is more or less dependent on them (Cao *et al*., 1993). These constituents when included in food, reduces the risk of human diseases (Cilla *et al*., 2008). Antioxidants in lower concentrations delay and prevent the oxidation of substrate in a living system and help to sustain the fruit and product for longer periods (Halliwell and Gutteridge, 2007). Antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature (Hras *et al*., 2000). Various researchers have already reported that consumption of fruits and vegetables to lower the risk for chronic diseases such as cancer, cardiovascular diseases and stroke (Yeum *et al*., 2003).

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Pomegranate (*Punica granatum* L.) is a tropical and sub tropical fruit. One of the oldest known edible fruit and is popularly consumed as fresh fruit, beverage, as a food product and colouring agents as well as jellies (Sumner *et al*., 2005). Pomegranate fruit maturity status is commonly assessed based on external (skin) colour, juice colour and acidity of juice (Cristosto, 2000). Polyphenols are a main group of pomegranate phytochemicals, which act as a phytochemical antioxidant with potent health benefits. Pomegranate juice contains the highest concentration of total polyphenols compared to other fruit juices. The red colour of pomegranate juice is primarily associated and important sources of anthocyanins (cyaniding, delphinidin and pelargonidin) pigment and peel is rich in polyphenols including ellagitannins (such as punicalin, pedunculagin, punicalagin and ellagic acid) (Kulkarni and Aradhya, 2005). These polyphenols exhibits various biological activities such as eliminating free radicals, inhibiting oxidation and microbial growth (Reddy *et al*., 2007). Some clinical research studies suggest that pomegranate juice changes the blood parameters such as low density lipoprotein (LDL), high density lipoprotein (HDL) and cholesterol (Aviram *et al*., 2004) and may be helpful against heart disease (Sumner

et al., 2005), Alzheimer's disease (Singh *et al*., 2008) and cancer (Seeram *et al*., 2007).

A wide variety of processed fruit products are available in the market, fruit beverages have been increasingly gaining popularity throughout the country due to their health and nutritional benefits apart from providing pleasant flavour and taste. They are nutritionally rich and also well-liked by the consumers due to their refreshing flavours and taste. Recognition of the nutritional benefits of the fruit based beverages has led to a gradual but distinct shift of the customer's preference from aerated drinks to fruit beverages. The focus of the present investigation was to prepare squash from pomegranate fruit and to evaluate the influence of storage condition on antioxidant properties.

Materials and Methods

Pomegranate, cv Mridula was used for making squash. Other raw materials like sugar, citric acid and preservative were also procured from the local market. The procured pomegranate fruit was stored at refrigeration temperature until they were used for the product development.

Preparation of squash

Fully ripened fruits of pomegranates were cleaned, washed in running water. The fruits were cut into 4 pieces and the arils were separated. The juice was extracted from the arils in the food processor. The extracted juice was filtered through a muslin cloth. Squash was prepared as per the FPO (Fruit Product Order) specification (fruit juice - 25 per cent, TSS - 45º brix, acidity -1.0 per cent and preservative - 600 ppm of Sodium benzoate). Required amount of sugar, water, citric acid were taken in a vessel and heated till the ingredients were dissolved completely. The prepared syrup was filtered and the fruit juice was mixed to the sugar syrup after cooling. Preservative was mixed with the squash at the end of the squash preparation and mixed well. After that the squash was filled immediately in sterilized squash bottles of 750 ml capacity leaving a head space of 2 cm and stored in room and refrigeration condition for 180 days.

Physico-chemical analysis

The pomegranate was analysed for various physico-chemical parameters. The total soluble solids of the squash were determined using a hand refractometer (0 to 32º brix). Titratable acidity was determined by titration method (Kadam *et al*., 2010). The pH of the squash was estimated by the method described by Saini *et al*. (2000). The total and reducing sugars were estimated by Shaffer Somoyogi method as described by McDonald and Foley (1960).

Estimation of ascorbic acid content

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.

Preparation of the methanol extracts

Methanol was used to extract antioxidant phytochemicals from samples. Fifty grams of sample was ground in a domestic blender and 1 g of the ground sample was crushed using a pestle and mortar with 30 ml of methanol. The homogenate was centrifuged at 4,500 rpm for 10 min to obtain a clear supernatant liquid. The residue was re-extracted with methanol (15 ml) and centrifuged. The supernatants were pooled together and filtered using Whatman No. 1 filter paper and made up to a known volume (Gupta and Prakash, 2009).

Determination of total polyphenols

Total phenolic contents were determined by the spectrophotometric method of Sadasivam and Manicam (2008). Aliquots of (0.2 - 2 ml) of extracts were taken into test tubes and made up the volume to 3 ml with distilled water. To this 0.5 ml of Folin – ciocalteau reagent was added and mixed well. After 3 min, 2 ml of 20% sodium carbonate was added and mixed well again. The test tubes were placed in boiling water bath for exactly one minute cooled and measured the absorbance at 650 nm against a reagent blank. 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 g on fresh weight basis (FWB).

Determination of total flavonoids

Total flavonoids were measured using aluminium chloride colorimetric assay, as described by Marinova *et al*. (2005). One ml of methanolic extract or standard solution of quercetin prepared using distilled water (10 - 100 µg/ ml) was added to test tubes containing 4 ml of double - distilled water. To the mixture was added 0.3 ml

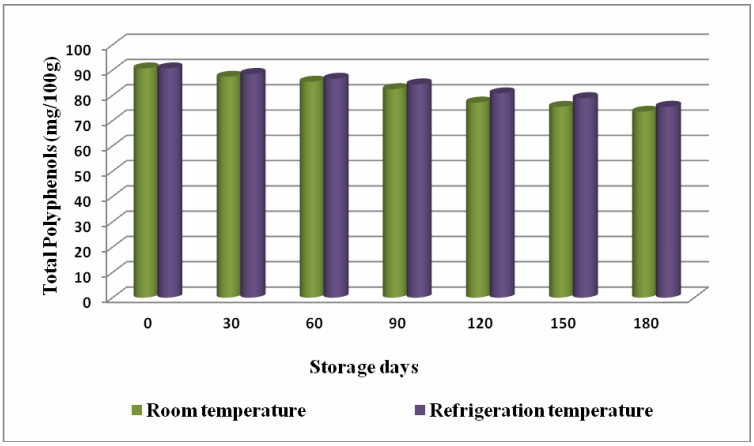

Fig. 1 : Total polyphenol content in pomegranate squash during storage.

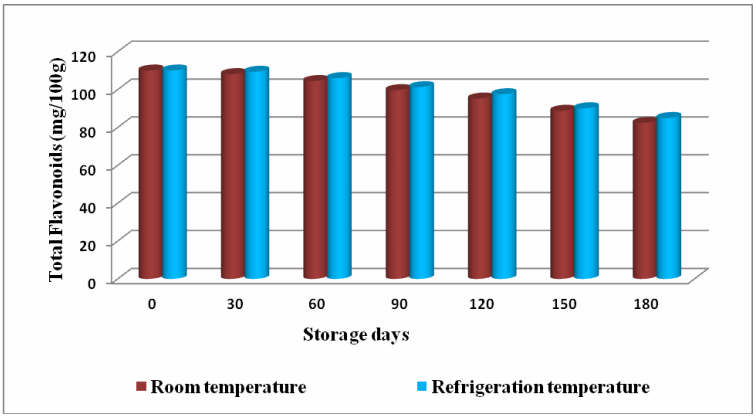

Fig. 2 : Total flavonoid content in pomegranate squash during storage.

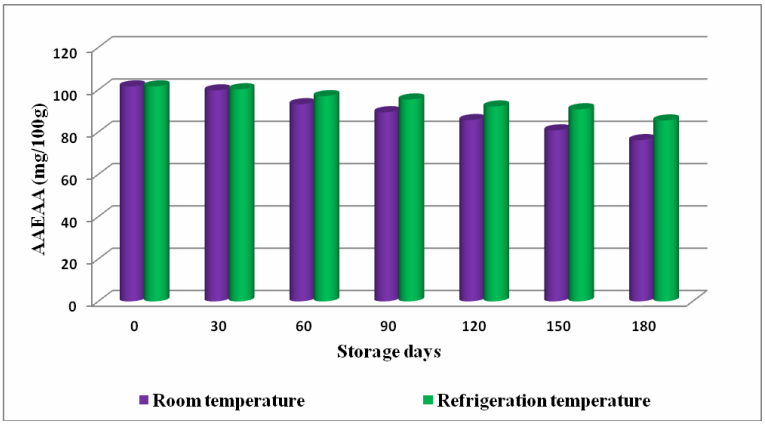

Fig. 3 : Total antioxidant activity of pomegranate squash during storage.

5% $NaNO_2$. After 5 min, 0.3 ml 10 % $AlCl_3$ was added. After 6 min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with double-distilled water. The solution was mixed thoroughly and the absorbance of the samples and standard against reagent blank were read at 510 nm using Double beam UV–VIS spectrophotometer 2201, Systronics. Total flavonoid content was expressed as mg of quercetin equivalent (QE) per 100 g on fresh weight basis (FWB).

Determination of antioxidant activity by DPPH free radical scavenging assay

The radical scavenging ability of pomegranate squash was tested on the basis of the radical scavenging effect on the DPPH free radical by Goupy *et al.* (1999). In clean and labelled test tubes, 2 ml of DPPH solution (0.002% in methanol) was mixed with 2 ml of different concentrations of sample extracts separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-Vis Double beam Spectrophotometer 2201 Model. The absorbance decrease was measured at 517 nm using methanol as a blank in a spectrophotometer. Negative control was prepared by adding 2 ml of DPPH solution to 1 ml of methanol and the absorbance was noted. Ascorbic acid was used as positive control. The calibration curve was plotted using absorbance versus concentrations of ascorbic acid standard and the results were reported as mg ascorbic acid equivalent of antioxidant activity per 100 g of sample on FWB.

Statistical analysis

The results were expressed as mean ± standard deviation. All measurements were replicated three times. The data obtained were analyzed statistically for analysis of variance (ANOVA) to evaluate the significance at $p<0.05$ (Gomez and Gomez, 1984).

Results and Discussion

Physico-chemical analysis

The results of the changes in chemical composition of pomegranate squash (table 1) showed that increase in total soluble solids of squash was noticed during the storage period of 180 days from 45 to 46.14° brix. This might be due to hydrolysis of polysaccharides like starch, cellulose and pectic substances into simple substances. Similar results were obtained in squash from pomegranate cv Ganesh and Mridula (Srinivas *et al.*, 2007). The increase in acidity and decrease in pH during storage of pomegranate squash was noted. The decrease in pH during storage may be attributed to increase in acids due to formation of organic acids by the degradation of ascorbic acid (Tiwari *et al.*, 2010). A significant increase in reducing sugars of pomegranate squash was also noticed during storage. This might be due to inversion of non reducing sugar to

Table 1 : Changes in the chemical composition of Pomegranate squash during storage.

Storage days	Pomegranate Squash											
	TSS (°brix)		Acidity (%)		pH		Reducing sugar (g/100g)		Total sugar (g/100g)		Ascorbic acid (mg/100g)	
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
0	45.00	45.00	1.020	1.020	2.74	2.74	25.17	25.17	43.20	43.20	9.05	9.05
30	45.33	45.18	1.058	1.042	2.71	2.72	25.33	25.28	43.00	43.05	8.75	8.82
60	45.85	45.34	1.095	1.075	2.70	2.71	25.62	25.44	42.45	42.62	8.22	8.43
90	46.17	45.75	1.112	1.104	2.68	2.69	26.87	26.05	42.14	42.28	7.81	8.02
120	46.60	46.41	1.136	1.122	2.67	2.68	26.98	26.78	41.52	41.86	7.25	7.55
150	46.98	46.82	1.164	1.145	2.65	2.67	27.06	26.92	41.04	41.25	6.92	7.14
180	47.25	47.14	1.175	1.160	2.62	2.65	27.22	27.08	40.85	40.35	6.55	6.71
	S.ED	***CD (0.05)***	***S.ED***	***CD (0.05)***	***S.ED***	***CD (0.05)***	***S.ED***	***CD (0.05)***	***S.ED***	***CD (0.05)***	***S.ED***	***CD (0.05)***
R	0.30794	0.63080^{NS}	0.00878	0.01799^{NS}	0.02011	0.04119^{NS}	0.17436	0.35717^{NS}	0.27905	0.57163^{NS}	0.05385	0.110331*
S	0.57610	1.18011*	0.01643	0.03365**	0.03762	0.07706^{NS}	0.32620	0.66820**	0.52206	1.06941*	0.10074	0.20637**
RS	0.81472	1.66893^{NS}	0.02323	0.04759^{NS}	0.05320	0.10898^{NS}	0.46131	0.94498^{NS}	0.73830	1.51238^{NS}	0.14247	0.29185^{NS}

R_1 - Room Temperature; R_2 - Refrigeration Temperature.

reducing sugars under acidic conditions and hydrolysis of polysaccharides. These results are also in accordance with guava-jamun blended squash developed by Sharma *et al*. (2012). The pomegranate squash showed a decline in the total sugar content from 43.20 to 41.95 per cent after storing for six months at room temperature. A remarkable increase in the reducing sugar and decrease in total sugar throughout the storage period was observed in tomato and orange blended squash (Shivakumar *et al*., 2009). Results indicated that ascorbic acid content of squash decreased continuously during the entire storage period. The reduction may be due to oxidation into dehydroascorbic acid by oxygen. Similar results also noted by Mandal and Nath (2013).

Total polyphenols

Phenolic compounds play an important role in determining the colour and flavor of a product. They are highly volatile and easily oxidized. There was a gradual decrease in total polyphenols in pomegranate squash during storage (fig. 1). 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) in total phenols of jamun squash and in guava-jamun blended squash developed by Sharma *et al*. (2012).

Total flavonoids

A significant reduction in flavonoids content was observed in pomegranate squash during 180 days of storage (fig. 2). Plaza *et al*. (2011) stated that the flavanone content of orange juice decreased significantly (around 50%) during the first 20 days of storage at 4ºC. Processing of fresh fruits and vegetables may decrease flavonoids content by 50 per cent owing to their leaching into water or by removal of the richest parts of the plant (Polydera *et al*., 2005).

Total antioxidant activity

The changes in the total antioxidant activity of pomegranate squash during storage depicted in fig. 3. Total antioxidant activity in pomegranate squash was 101.64 mg AAEAA/100g, which was decreased to 76.23 mg AAEAA/100g and 85.54 mg AAEAA/100g in room and refrigeration condition respectively during storage of 180 days. The decrease in ascorbic acid, total poly phenols and total flavonoids content led to a decrease of antioxidant activity.

Tomczak (2007) observed a decrease in antioxidant activity during storage of black chokeberry juice concentrate. Under facultative anaerobic conditions after 20 days of storage antioxidant activity decreased by 7-12 per cent at 10°C, 12-15 per cent at 20°C and by 16-35 per cent at 30°C. Under aerobic conditions these changes were much bigger and ranged from 63 to 76 per cent after 10 days and from 64 to 79 per cent after 20 days storage. The decrease of antioxidant activity increased with increasing storage temperature. Yang *et al.* (2007) reported that storing the noni 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

In conclusion, this investigation demonstrates that pomegranate squash is a good source of phenolic compounds and antioxidant activity. Results obtained in this study indicate that the TSS, acidity and reducing sugar increased, while pH, total sugar and ascorbic acid decreased in squash with increase in storage period. Storage of pomegranate squash tends to reduce the content of ascorbic acid, phenols and flavonoids. The retention of antioxidant components and activity was high in refrigeration temperature. It can also be concluded that antioxidant activity of pomegranate squash is significantly affected by temperature and time of storage. The recommended storage temperature for pomegranate squash is refrigeration temperature.

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