

Standardization of 'Bhagwa' pomegranate (*Punica granatum* L.) nectar

ABSTRACT

The experiment of standardization of different combination of juice (20 ml, 22 ml and 24 ml), Total soluble solids (TSS 15° Brix and 17° Brix) and acidity (0.25 %, 0.30 % and 0.35 %) levels were used for preparation of pomegranate nectar and evaluated for changes in biochemical, sensory and qualities parameters during storage period of six months at room temperature. The experiment was laid out in complete randomize design (Factorial) with 18 treatments and 3 replications for 4 storage interval which, carried out in the year 2013 to 2014 at the Department of Post Harvest Technology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari. The increasing trend in TSS (17 to 17.51° brix), reducing sugars (4.24 to 7.64 %) and total sugars (16.37 to 16.67%) whereas decreasing trend in titratable acidity (0.35 to 0.30 %) and ascorbic acid (3.13 to 1.80 mg/100ml) was noticed irrespective of treatments during storage. A decline score in sensory parameters of nectar in terms of colour (8.34 to 7.78), flavour (8.72 to 8.09) and overall acceptability (8.56 to 7.94) were observed during entire period of storage. In quality parameters, there was no sedimentation and absence of colony (microbial growth) during Total Plate Count (TPC) observed in the pomegranate nectar during storage period. The highest ascorbic acid and organoleptic acceptance were found with the recipe prepared with higher concentration of juice at 24 ml with optimum TSS 15° Brix as well as acidity 0.30 %.

Key words: Beverage, 'Bhagwa', Nectar, Pomegranate

Introduction

The pomegranate (*Punica granatum* L.) is also commonly known as *The Multi Seeded Apple*, *Chinese Apple*, *Apple of Carthage* and *Super fruit* [1]. India secures first rank in production of pomegranate (28.44 million ton) by producing around 50 % of world's production [2]. Gujarat is second largest pomegranate producing state after Maharashtra where the pomegranate is being cultivated over area of an about 0.31 million ha with production of 4.62 million ton with productivity of 15.13 tha^{-1} [3]. The 'Bhagwa' is most popular Indian cultivar of pomegranate, that released from The Mahatma Phule Krishi Vidyapeeth Rahuri in the year 2003, which is also known by *Shendri*, *Asthagandha*, *Mastani*, *Jai Maharashtra*, *Red Diana* and *Kesar* [4]. It has been better adaptation nationally and internationally in recent time due to its high yielding and most desirable fruit characters like nutrition, attractive fruit in a glossy saffron red colour, maximum weight as well as soft seeded [5].

Pomegranate with excellent flavour, nutritional value and medicinal property indicate high potential for processing into value added products [6]. There is increasing demand for pomegranate products such as pomegranate juice, functional beverages, pomegranate powder, as well as other pomegranate-derived products is major factor projected to drive the global pomegranate market growth. The global pomegranate and pomegranate arils market was valued at 8.2 billion USD in 2018 and is expected to reach 23.14 Billion USD by year 2026, at a compound annual growth rate (CAGR) of 14.0 % [2]. However, in India, due to poor storages, undeveloped markets, very limiting in the value addition and restricted export facilities, it could be difficult to utilize surplus produce which is perishable in nature. Therefore, the value added product with good keeping quality could be solution for the glut in the markets and uneconomic & distress sell of produce by the farmer. Therefore, nectar, *Drink of the God* and *Ambrosia* can be a refreshing beverage that is free of carbonation, contains no preservatives, and provides an excellent source of several important vitamins and minerals. However, a little attention has been given for utilizing the pomegranate fruit for making nectar, which has immense use especially in hot summer [8]. So, standardization of unfermented beverage (pomegranate nectar) with optimum proportion of juice, TSS and acidity for better nutritional and keeping quality had been studied.

Materials and methods

The experiment was conducted at the Department of Post Harvest Technology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari in completely randomized design with factorial concept comprising different combinations of levels of juice *viz.* J₁ – 20 ml, J₂ – 22 ml and J₃ – 24 ml (factor 1) for 100 ml, levels of sugar *viz.* S₁ - 15° Brix and S₂ - 17° Brix (Factor 2) and levels of acidity *viz.* A₁ – 0.25 %, A₂ – 0.30 % and A₃ – 0.35 % (Factor 3). The treatments were replicated thrice. Mature fully ripen and uniform fresh fruits of pomegranate cv. 'Bhagwa' were collected from Agricultural Produce Marketing Committee, Navsari, and the fruits were analyzed for physio-chemical parameters such as average weight of fruits (g), number of fruits per kg, peel weight of fruits per kg (g), aril weight of fruits per kg (g), average fruit weight (g) and specific gravity of fruit.

The fruits were washed, and surface sterilized with 100 parts per million chlorine solution for five minutes with palatable water in the laboratory. The juice of pomegranate extraction was carried out manually by peeling and arils separation followed by extraction with the help of electrically operated screw type juicer to avoid crushing of seed that reduce bitterness of tannin and haze of juice. After that, a **filtration** was done with two layers of muslin cloth. A collected juice was used after clarification by natural sedimentation at refrigeration temperature ($5\pm 1^\circ\text{C}$) for 0 to 24 hours without any clarifying agent [8]. The pomegranate nectar beverage was processed with different concentration of ingredients such as juice, sugar and citric acid [7]. A mixture of ingredients was heated on low flame without further addition of any preservative and aseptic hot filling carried out with 2 to 3 cm of head space in to the clean and sterilized 200 ml of glass bottles and sealing was done with crown corks. Pasteurization was done for 20 to 30 minutes in the boiling water, then air cooling at room temperature which followed by six months of room storage at ambient temperature (Fig.1).

Each treatment was subsequently analyzed for biochemical parameters *viz.* TSS ($^\circ$ Brix), titrable acidity (%), ascorbic acid (mg/100 ml), total sugars (%) and reducing sugars (%); sensory parameters *viz.* colour, flavour and overall acceptability (based on 9-point hedonic scale); and quality parameters *viz.* sedimentation (%) and microbial growth (TPC) according to the procedure reported by **Rangana** [9] at a period of initial, 2nd, 4th and 6th month of storage. The data pertaining to biochemical parameters and organoleptic parameters of nectar were analyzed statistically as per **Panse and Shukhatme** [10].

Result and discussion

The physical parameters of fresh pomegranate fruit were recorded such as average weight of fruits (293.6 g), number of fruits per kg (3.50), peel weight of fruits per kg (345.33 g), aril weight of fruits per kg (654.67 g) and specific gravity (0.98) whereas biochemical parameters such as TSS (14.9° Brix), acidity (0.38 %), ascorbic acid (13.89 mg/100 ml), total sugars

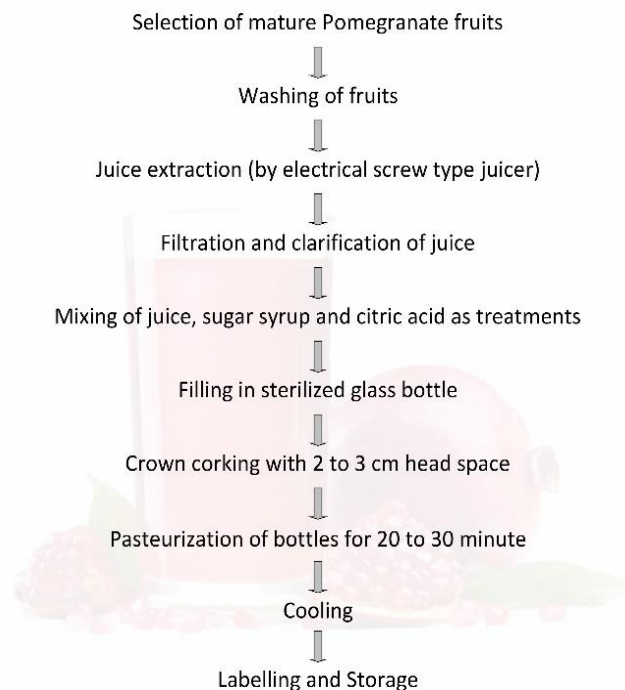


Fig. 1 Process flow chart for preparation of pomegranate nectar

(14.23 %) and reducing sugars (13.17 %) were analyzed from a fresh juice.

Biochemical parameters

TSS (° Brix):

It is evident from the data that the significantly maximum TSS was recorded when nectar prepared with S₂ 17° Brix. Initially, no changes in TSS were noted due to the levels of TSS maintained in the pomegranate nectar. The TSS of all the treatments showed increasing trend *i.e.* 17.00 to 17.51° Brix during ambient storage (Fig. 2), whereas independent effect of juice, acidity; interaction effect of juice and TSS, juice and acidity, TSS and acidity; and juice, TSS and acidity on a TSS found nonsignificant. This might be due to the hydrolysis or conversion of insoluble polysaccharides such as starch, cellulose and pectic substances and organic compound (antioxidants, organic acids and anthocyanins) into simple sugars. However, there was no influence of juice and acidity on the changes in TSS and it is in conformity with the results obtained by Shrivastava *et al.* [11] in custard apple and Relekar *et al.* [12] in sapota nectar.

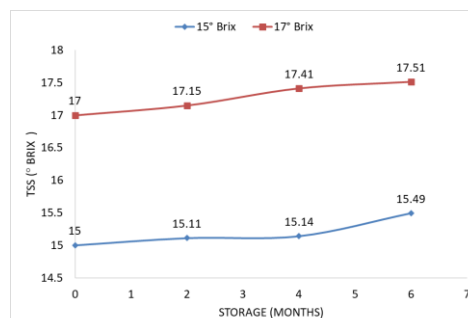


Fig. 2. Influence of TSS levels on the TSS of pomegranate nectar during storage

Acidity (%):

The data revealed that the changes varied according to different acidity levels used for preparation of pomegranate nectar. The acidity at A₃ 0.35 % level found significantly maximum in the independent effect of acidity. However, there were no influences in acidity reported initially due to acidity % maintained in the pomegranate nectar (Fig. 3). But the acidity was decrease continuously with respect to increasing storage period *i.e.* 0.35 to 0.30 %, whereas independent effect of juice, TSS; interaction effect of juice and TSS, juice and acidity, TSS and acidity; and juice, TSS and acidity on acidity analyzed nonsignificant. The possible cause of change might be due to the cumulative effect of chemical interaction of photo and thermal sensitive organic constituents of the beverage particularly organic acids (antioxidants and ascorbic acid), anthocyanins, astringents, polyphenolic compounds, pectic substances and polysaccharides into simple sugar by oxidation. This finding of present study is in consonance with Chavan *et al.* [13] in pomegranate RTS and Relekar *et al.* [12] in sapota nectar.

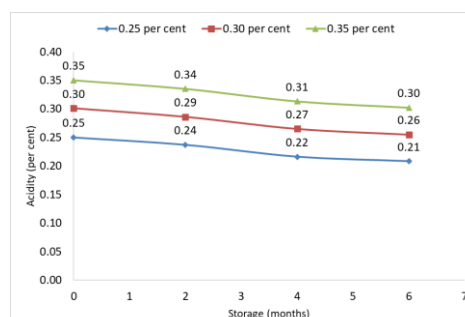


Fig. 3. Influence of acidity levels on the acidity of pomegranate nectar during storage

Ascorbic acid (mg/100ml):

The changes in ascorbic acid varied according to juice content used for preparation of pomegranate nectar. The significantly

maximum ascorbic acid was observed with J₃ 24 ml juice content *i.e.* 3.13 mg/100ml (Fig. 4)

whereas the nonsignificant effect on ascorbic acid analyzed in an independent effect of TSS, acidity; interaction effect of juice and TSS, juice and acidity, TSS and acidity; and juice, TSS and acidity. There was continuous reduction in an ascorbic acid content (3.13 to 1.80 mg/100ml) of

pomegranate nectar with respect to increasing storage period. Ascorbic acid is an important nutrient but is also very sensitive to heat, light and oxidized quickly in presence of oxygen. This continuous decline trend might be due to thermal degradation during processing and oxidation or irreversible conversion of L-ascorbic acid in to dehydro ascorbic acid during storage in presence of heat, light and trapped or residual oxygen in glass bottle. However, no effect as observed due to different levels of TSS and acidity % on ascorbic acid of pomegranate nectar during storage. This result is in line with observations of Choudhary *et al.* [14] in aonla nectar as well as Chavan *et al.* [13] in pomegranate RTS.

Total sugars (%):

Independent effect of juice, TSS analyzed significantly maximum total sugars % when the pomegranate nectar prepared with J₃ 24 ml juice, S₂ 17° Brix TSS (Table 1). Whereas different acidity % did not influence significantly until second month of storage period. But there was a significant increase in total sugars by acidity % during fourth and sixth month of storage. The highest total sugars were recorded 15.40 % with A₃ 0.35 % acidity, which was statistically at par with A₂ 0.30 % acidity *i.e.* 15.37 %. However, interaction effect of juice and TSS, juice and acidity, TSS and acidity; and juice, TSS and acidity on total sugar evaluated nonsignificant. The total sugars of pomegranate nectar showed increasing trend with respect to the juice content (15.56 to 15.89 %), TSS level (16.37 to 16.67 %), acidity % (15.40 to 15.70 %) with respect to storage period, which might be due to juice and acids itself is good source of organic compounds that degraded or hydrolyzed into simple sugar while TSS in nectar contained major portion of total sugars as sucrose, which might be due to hydrolysis or conversion of insoluble polysaccharides such as starch, cellulose and pectic substances. These findings of present study are in conformity with the results obtained by Bal

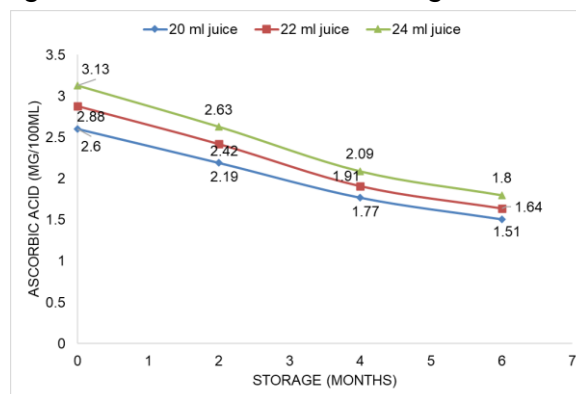


Fig. 4. Influence of juice levels on the ascorbic acid of pomegranate nectar during storage

et al. [15] in guava nectar and Shrivastava *et al.* [11] in custard apple nectar.

Table 1. Effect of juice, TSS and acidity levels on the total and reducing sugar % of pomegranate nectar during storage

Treatments	Total sugar (%)				Reducing sugar (%)			
	0	2	4	6	0	2	4	6
Storage (months)								
Juice ml (J)								
J ₁ 20 ml	15.16	15.27	15.34	15.40	3.82	5.10	5.97	6.39
J ₂ 22 ml	15.36	15.49	15.58	15.65	3.96	5.53	6.84	7.50
J ₃ 24 ml	15.56	15.69	15.82	15.89	4.23	5.94	7.47	8.25
CD _{0.05}	0.089	0.098	0.106	0.086	0.019	0.028	0.037	0.049
TSS level (S)								
S ₁ 15° Brix	14.35	14.46	14.56	14.62	3.76	5.27	6.50	7.12
S ₂ 17° Brix	16.37	16.50	16.60	16.67	4.24	5.78	7.02	7.64
CD _{0.05}	0.073	0.080	0.086	0.070	0.015	0.022	0.030	0.040
Acidity % (A)								
A ₁ 0.25 %	15.31	15.43	15.50	15.57	3.87	5.24	6.44	7.04
A ₂ 0.30 %	15.37	15.49	15.59	15.66	4.01	5.52	6.75	7.35
A ₃ 0.35 %	15.40	15.53	15.64	15.70	4.12	5.81	7.10	7.75
CD _{0.05}	NS	NS	0.106	0.086	0.019	0.028	0.036	0.049
J X S	NS	NS	NS	NS	0.027	0.039	0.051	0.069
J X A	NS	NS	NS	NS	0.033	0.048	0.063	0.084
S X A	NS	NS	NS	NS	0.027	0.039	0.051	0.069
J X S X A	NS	NS	NS	NS	0.046	0.068	0.089	0.119

Table 2. Interaction effect of juice and TSS levels, juice and acidity, TSS and acidity and juice, TSS and acidity on the reducing sugars % of pomegranate nectar during storage

Treatments	Storage period months																
	Initial				2				4				6				
	A ₁	A ₂	A ₃	Mean	A ₁	A ₂	A ₃	Mean	A ₁	A ₂	A ₃	Mean	A ₁	A ₂	A ₃	Mean	
J ₁	S ₁	3.55	3.60	3.67	3.61	4.66	4.83	5.08	4.86	5.49	5.67	6.00	5.72	5.89	6.04	6.46	6.13
	S ₂	3.84	4.07	4.14	4.02	5.04	5.36	5.64	5.35	5.83	6.20	6.60	6.21	6.23	6.63	7.08	6.65
	Mean	3.70	3.84	3.91		4.85	5.10	5.36		5.66	5.94	6.30		6.06	6.34	6.77	
J ₂	S ₁	3.62	3.73	3.88	3.74	5.01	5.28	5.60	5.30	6.26	6.59	7.01	6.62	6.88	7.25	7.73	7.29
	S ₂	4.06	4.19	4.26	4.17	5.48	5.76	6.03	5.76	6.67	7.12	7.40	7.06	7.27	7.80	8.10	7.72
	Mean	3.84	3.96	4.07		5.25	5.52	5.82		6.47	6.86	7.21		7.08	7.53	7.92	
J ₃	S ₁	3.79	3.91	4.07	3.92	5.36	5.67	5.94	5.66	6.92	7.17	7.43	7.17	7.73	7.92	8.18	7.94
	S ₂	4.38	4.56	4.68	4.54	5.90	6.23	6.56	6.23	7.43	7.73	8.15	7.77	8.21	8.48	8.98	8.56
	Mean	4.09	4.24	4.38		5.63	5.95	6.25		7.18	7.45	7.79		7.97	8.20	8.58	
	J X S			0.027				0.039				0.051				0.069	
	J X A			0.033				0.047				0.063				0.084	
	S X A			0.027				0.039				0.051				0.069	
	J X S X A			0.046				0.068				0.089				0.119	

Reducing sugars (%):

Significantly, the highest reducing sugars was recorded when pomegranate nectar was prepared with J₃ 24 ml juice (4.23 to 8.25 %), S₂ 17° Brix TSS (4.24 to 7.64 %) and A₃ 0.35 % acidity (4.12 to 7.75 %); and their possible interactions such as J₃ 24 ml juice x S₂ 17° Brix TSS; J₃ 24 ml juice x A₃ 0.35 % acidity; S₂ 17° Brix TSS x A₃ 0.35 % acidity and J₃ 24 ml juice x S₂ 17° Brix TSS x A₃ 0.35 % acidity *i.e.* 4.54 to 8.56 %, 4.38 to 8.58 %, 4.68 to 8.98 % and 4.68 to 8.98 % during storage investigation respectively (Table 1 & 2). In general, reducing sugars showed increasing trend in all treatments along with the advancement of storage period. However, the variation was mainly due to the initial juice content, level of TSS and acidity % adjusted at the time of the preparation of pomegranate nectar. The highest conversion into reducing sugars were observed in the recipes having the highest juice content, level of TSS and acidity %. This might be attributed to more availability of polysaccharides and non-reducing sugars in these treatments for conversion into reducing sugars. This pattern of increasing of reducing sugars

during storage might be due to enhanced acid hydrolysis of polysaccharides and inversion of non-reducing sugars to invert sugars through break down process of citric acid; and the hydrolysis or conversion of organic compound (antioxidants, organic acids and anthocyanins) of juice into simple sugars when stored in ambient condition. The finding of present studies are in conformity with the results obtained by Bal *et al.* [15] in guava nectar and Shrivastava *et al.* [11] in custard apple nectar.

Sensory parameters

Colour:

The individual effect of juice content showed significant effect on the colour score of pomegranate nectar. The highest colour score was revealed when pomegranate nectar prepared with J₃ 24 ml juice *i.e.* 8.34 that was at par with J₂ 22 ml juice *i.e.* 8.08 (Fig. 5). whereas independent effect of TSS, acidity; interaction effect of juice and TSS, juice and acidity, TSS and acidity; and juice, TSS and acidity on a colour score found nonsignificant. The highest score was recorded initially and it declined subsequently with increasing storage period *i.e.* 8.34 to 7.78. The decline in colour acceptability score might be due to unstable anthocyanin natural pigments, which degraded by the catalytic effect of light and temperature, when nectar was packed in glass bottles and stored in ambient condition that directly affect on the colour of product. Similar result and trend were also noticed by Mehtre *et al.* [16] in pomegranate RTS and Byanna and Gowada [17] in sweet orange nectar.

Flavour:

The highest flavour score was observed 7.68 in J₃ 24 ml juice, which was at par with J₂ 22 ml juice *i.e.* 7.56. Significantly maximum flavour score was recorded 7.67 in S₁ 15° Brix TSS while significantly the highest flavour score was noted 8.50 with A₂ 0.30 % acidity, the significantly maximum flavour score was found in an interaction effect of S₁ 15° Brix TSS and A₂ 0.30 % acidity *i.e.* 8.72 (Table 3 & 4). However, nonsignificant interaction effect of juice and TSS, juice and acidity; and juice, TSS and acidity observed on a flavour score. A continuous decline in flavour acceptability score has been observed with the advancement of storage period. This might be due to loss of typical aroma owing to the reactions of acids with other constituents especially the polyphenols and the acid deteriorates the volatile compounds like flavonoids by oxidation and polymerization. The finding is in accordance with Mehtre *et al.* [16] in pomegranate RTS and Byanna & Gowada [17] in sweet orange nectar.

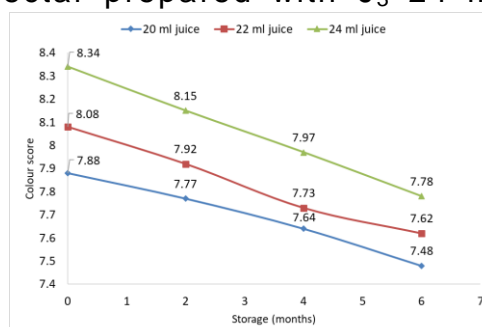


Fig. 5. Influence of juice levels on the colour score of pomegranate nectar during storage

Table 3. Effect of juice, TSS and acidity levels on the flavour and overall score of pomegranate nectar during storage

Treatments Storage (months)	Flavor score				Overall score			
	0	2	4	6	0	2	4	6
Juice ml (J)								
J ₁ 20 ml	7.51	7.27	7.07	7.00	7.76	7.60	7.42	7.29
J ₂ 22 ml	7.56	7.33	7.15	7.03	7.86	7.71	7.48	7.34
J ₃ 24 ml	7.68	7.47	7.27	7.18	7.99	7.80	7.58	7.42
CD _{0.05}	0.139	0.159	0.138	0.150	0.169	0.103	0.090	0.075
TSS level (S)								
S ₁ 15° Brix	7.67	7.51	7.29	7.20	7.94	7.79	7.57	7.43
S ₂ 17° Brix	7.49	7.20	7.03	6.94	7.80	7.61	7.42	7.26
CD _{0.05}	0.114	0.129	0.113	0.122	0.138	0.084	0.073	0.061
Acidity % (A)								
A ₁ 0.25 %	7.53	7.22	7.07	7.00	7.85	7.65	7.43	7.32
A ₂ 0.30 %	8.50	8.28	8.00	7.88	8.41	8.23	7.97	7.78
A ₃ 0.35 %	6.73	6.57	6.42	6.33	7.36	7.22	7.08	6.94
CD _{0.05}	0.139	0.159	0.138	0.150	0.169	0.103	0.090	0.075
J X S	NS	NS	NS	NS	NS	NS	NS	NS
J X A	NS	NS	NS	NS	NS	NS	NS	NS
S X A	0.197	0.224	0.195	0.212	0.239	0.146	0.127	0.106
J X S X A	NS	NS	NS	NS	NS	NS	NS	NS

Table 4. Interaction effect of TSS and acidity levels on the flavour and overall acceptability during storage

Treatments	Storage (months)											
	0			2			4			6		
	A ₁	A ₂	A ₃	A ₁	A ₂	A ₃	A ₁	A ₂	A ₃	A ₁	A ₂	A ₃
Flavour (S x A)												
S ₁	7.77	8.72	6.53	7.53	8.53	6.47	7.37	8.23	6.27	7.30	8.09	6.2
S ₂	7.28	8.28	6.92	6.90	8.03	6.67	6.77	7.77	6.57	6.70	7.67	6.47
CD _{0.05}	0.197			0.224			0.195			0.212		
Overall (S x A)												
S ₁	8.01	8.56	7.26	7.82	8.38	7.16	7.60	8.12	6.99	7.50	7.94	6.86
S ₂	7.69	8.26	7.46	7.48	8.09	7.28	7.27	7.82	7.17	7.14	7.62	7.02
CD _{0.05}	0.239			0.146			0.127			0.106		

Overall acceptability:

The highest overall acceptability was recorded 7.99 with J₃ 24 ml juice, which was at par with J₂ 22 % juice *i.e.* 7.86. The significantly highest overall acceptability was recorded 7.94 in S₁ 15° Brix TSS. The significantly maximum overall acceptability in treatment A₂ 0.30 % acidity *i.e.* 8.41. Significantly the highest overall acceptability was found in an interaction effect of S₁ 15° Brix TSS and A₂ 0.30 % acidity *i.e.* 8.56 (Table 3 & 4). Whereas interaction effect of juice and TSS, juice and acidity; and juice, TSS and acidity were not evaluated significant on an overall acceptability score. It was also noticed that the overall acceptability scores declined when the juice proportion were affected the colour quality of the nectar with advancement of storage period. This finding of present study is in consonance with Mehtre *et al.* [16] in pomegranate RTS and Byanna & Gowada [17] in sweet orange nectar.

Quality parameters

There was no sedimentation and absence of colony (microbial growth) during Total Plate Count (TPC) observed in the pomegranate nectar during storage period. It might be due to pomegranate juice extraction and clarification method used as well as aseptic condition at the time of nectar preparation.

Conclusion

The colour of pomegranate nectar is the key successor of a processed product in the global beverage market. The colour acceptability score vary according to juice content alone of the recipes due to bright red colour of 'Bhagwa' pomegranate juice. The nectar recipe with 24 ml juice, 15° Brix TSS and 0.30 % acidity could be rich in ascorbic acid, colour, flavour and overall acceptability for the preparation of pomegranate nectar, which could pack in glass bottles and kept under ambient condition for a good palatable form for a period of six months.

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List of Abbreviations and symbols

Abbreviations	Meaning
tha ⁻¹	Ton/hector
USD	United States Dollar
CAGR	compound annual growth rate
/	Per
%	Per cent
° C	Degree Celsius
Anon.	Anonymous
CD	Critical difference
CFU	Colonies forming units
CO ₂	Carbon dioxide
cv.	Cultivar (s)
<i>et al.</i>	And others
fig.	Figure
g	Gram
<i>i.e.</i>	That is
kg	Kilogram
mg	Milligram
ml	Milliliter
NS	Non significant
RTS	Ready-to-serve
TPC	Total plate count
TSS	Total soluble solids

var.	Variety (s)
viz.	Namely

UNDER PEER REVIEW