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Standardization of 'Bhagwa' Pomegranate (*Punica granatum* L.) Nectar

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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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 %.

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Keywords: Beverage; bhagwa; nectar; pomegranate.

LIST OF ABBREVIATIONS AND SYMBOLS

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)*
et al	:	*And others*
fig	:	*Figure*
g	:	*Gram*
i.e	:	*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*

1. 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 [7]. So, standardization of unfermented beverage (pomegranate nectar) with optimum proportion of juice, TSS and acidity for better nutritional and keeping quality had been studied.

2. 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*. J_1 – 20 ml, J_2 – 22 ml and J_3 – 24 ml (factor 1) for 100 ml, levels of sugar *viz*. S_1 - 15° Brix and S_2 - 17° Brix (Factor 2) and levels of acidity *viz*. A_1 – 0.25 %, A_2 – 0.30 % and A_3 – 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±1° C) for 0 to 24 hours without any clarifying agent [7]. The pomegranate nectar beverage was processed with different concentration of ingredients such as juice, sugar and citric acid [8]. 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 (° 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, 2^{nd}, 4^{th} and 6^{th} month of storage. The data pertaining to biochemical parameters and organoleptic parameters of nectar were analyzed statistically as per Panse and Shukhatme [10].

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

Fig. 1. Process flow chart for preparation of pomegranate nectar

3.1 Biochemical Parameters

TSS (° Brix): It is evident from the data that the significantly maximum TSS was recorded when nectar prepared with S_2 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.

Acidity (%): The data revealed that the changes varied according to different acidity levels used for preparation of pomegranate nectar. The acidity at A_3 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.

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 J_3 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.

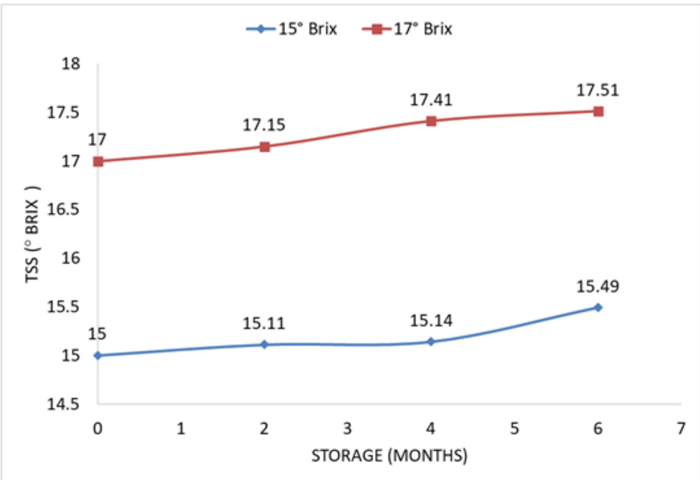

Fig. 2. Influence of TSS levels on the TSS of pomegranate nectar during storage

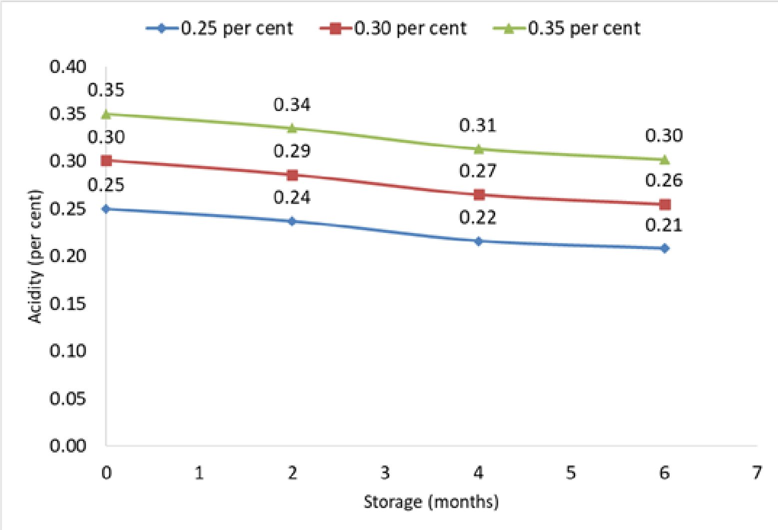

Fig. 3. Influence of acidity levels on the acidity of pomegranate nectar during storage

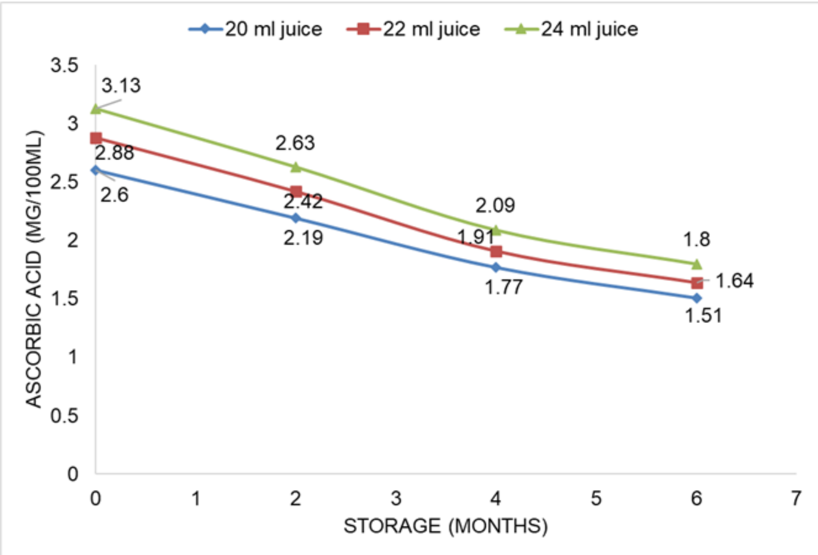

Fig. 4. Influence of juice levels on the ascorbic acid of pomegranate nectar during storage

Total sugars (%): Independent effect of juice, TSS analyzed significantly maximum total sugars % when the pomegranate nectar prepared with J_3 24 ml juice, S_2 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 A_3 0.35 % acidity, which was statistically at par with A_2 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 et al. [15] in guava nectar and Shrivastava et al. [11] in custard apple nectar.

Reducing sugars (%): Significantly, the highest reducing sugars was recorded when pomegranate nectar was prepared with J_3 24 ml juice (4.23 to 8.25 %), S_2 17° Brix TSS (4.24 to 7.64 %)and A_3 0.35 % acidity (4.12 to 7.75 %); and their possible interactions such as J_3 24 ml juice x S_2 17° Brix TSS; J_3 24 ml juice x A_3 0.35 % acidity; S_2 17° Brix TSS x A_3 0.35 % acidity and J_3 24 ml juice x S_2 17° Brix TSS x A_3 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.

3.2 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 J_3 24 ml juice *i.e.* 8.34 that was at par with J_2 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.

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 (%)			
Storage (months)	0	2	4	6	0	2	4	6
				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			

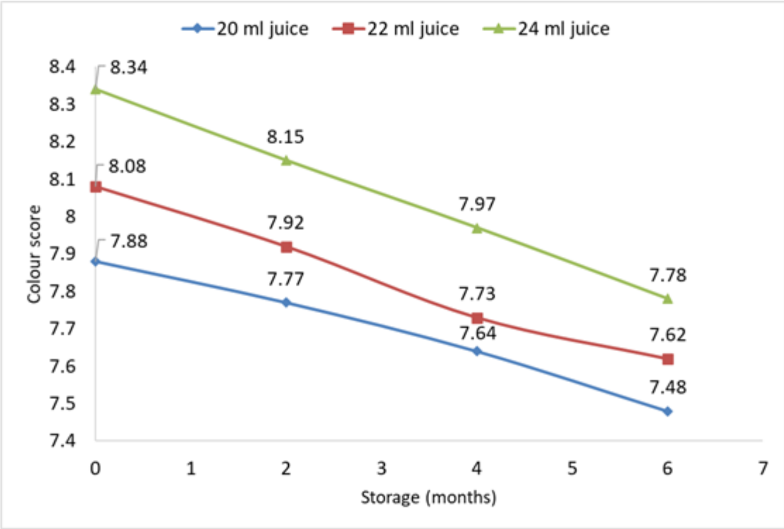

Fig. 5. Influence of juice levels on the colour score of pomegranate nectar during storage

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

Treatments	Flavor score				Overall score			
Storage (months)	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

Flavour: The highest flavour score was observed 7.68 in J_3 24 ml juice, which was at par with J_2 22 ml juice *i.e.* 7.56. Significantly maximum flavour score was recorded 7.67 in S_1 15° Brix TSS while significantly the highest flavour score was noted 8.50 with A_2 0.30 % acidity, the significantly maximum flavour score was found in an interaction effect of S_1 15° Brix TSS and A_2 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.

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 J_3 24 ml juice, which was at par with J_2 22 % juice *i.e.* 7.86. The significantly highest overall acceptability was recorded 7.94 in S_1 15° Brix TSS. The significantly maximum overall acceptability in treatment A_2 0.30 % acidity *i.e.* 8.41. Significantly the highest overall acceptability was found in an interaction effect of S_1 15° Brix TSS and A_2 0.30 % acidity *i.e.* 8.56 (Tables 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.

4. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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