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Production and Assessment of Probiotic Fruit Juice from Punjab Pink Variety of Guava

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Probiotics are considered as live microbial formulations exhibiting health benefits related to their ability to alter the gut microbiota and thereby preventing diseases. The present study was conducted to produce probiotic guava juice in a pilot plant (batch type, 50L capacity) through the application of *Lactobacillus acidophilus* (MTCC no. 10307). The probiotic cells of *Lactobacillus acidophilus* were added aseptically into the guava juice at 15% (v/v) to reach a concentration of 10^8–10^9 CFU/ml and bottling was done using pneumatic bottling unit. The physiochemical (pH, TSS, total sugars [reducing and non-reducing], titratable acidity, antioxidant activity, content of lactic acid and ascorbic acid), microbiological and sensory parameters were analyzed. Among the physiochemical parameters a significant decrease was observed in pH 5.62 to 3.99, TSS from 15.11°brix to 13.45°brix, total sugars from 6.78% to 3.23%, reducing sugar content from 3.46% to 1.62%, non-reducing sugars from 3.32% to 1.60%, content of ascorbic acid from 167.25 mg/100ml to 160.01 mg/100 ml and antioxidant activity from 43.82% to 33.95% except for titratable acidity and lactic acid content which increased significantly during 8 weeks storage under refrigeration. *Lactobacillus* viability was found to remain well above 8 log CFU/ml for four weeks. Total plate count remained within acceptable limits during the first 5 weeks and yeast and mould count was within satisfactory limits throughout the storage period of 8 weeks. Presence of coliforms was not detected during the storage of 8 weeks. Overall acceptability of probiotic guava juice was 8.56.

Keywords: Antioxidant, *Lactobacillus acidophilus*, Microorganisms, Shelf life, Vitamin C

Introduction

Foods play crucial role in growth as well as functional activities of all the living organisms. Especially, the foods that are rich in phytonutrients act as defensive mechanisms against diseases improving the immunity and preventing the outbreak of chronic ailments.[1] Probiotics are considered as live microbial formulations exhibiting health benefits related to their ability to alter the gut microbiota and thereby preventing diseases.[2] Fruit juices exemplify as a suitable matrix for growth of probiotics owing to they being a rich source of sugars and nutrition. The production of fruit based probiotic permits the intake of these microbes by lactose intolerant, casein allergic, hyper cholesterolemic and strict vegans.[3] The suitability of various vegetable and fruit juices has been reported by various researchers as raw material for the production of beverages and probiotic juices.[4]

Diverse bacterial strains belonging to genera *Lactobacillus, Streptococcus, Enterococcus,* and *Bifidobacterium* etc. are used for production of probiotic food products. *Lactobacillus* species is commonly utilized for the production of probiotic products. Guavas are a rich source of dietary fiber and vitamin C equivalent to an orange in case of *Psidium guajava.* It has a generally broad, low-calorie profile of essential nutrients and moderate levels of folic acid which is an important requirement for mothers to be. Punjab pink, Sardar, Allahabad Sufeda, and Arka Amulya are among popular varieties grown in Punjab with total area under fruit crop being 86673 ha and total production of 1850260 MT. The post-harvest losses incurred during various storage and field operations is about 18.05%. The value addition of guava by converting it to a probiotic juice can go a long way to decrease the quantum of post-harvest losses. Assessment of probiotic fruit juice produced from Punjab Pink variety of guava at pilot scale has not been done. Hence the present study was conducted to produce probiotic guava juice in the pilot plant using *Lactobacillus acidophilus* and to analyze it for various physiochemical, microbiological and sensory parameters throughout storage period of 8 weeks.

Material and Methods

Production of Guava Juice

The present work was conducted at the Department of Processing and Food Engineering as well as at the

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Department of Microbiology, PAU, Ludhiana. The guavas (var. Punjab Pink) were procured from Fruit farm through the Department of Fruit Science, PAU, Ludhiana. The fruits were selected after manual sorting and cleaned by washing with tap water. These were then wiped dried and stored at 4°C, before their extraction for juice. Pulping of peeled guavas was carried out in Pulper machine (Brand name "Kalsi"). The pulp was collected and diluted in the ratio of 1:1 with double distilled water. This mixture was then homogenized by passing the collected juice through a multi-purpose strainer and muslin cloth to remove seeds. The brix of the juice was adjusted to 15.2°brix using cane sugar. The resultant juice was pasteurized at 90°C for 1 min.

Inoculum Preparation

Procurement of the probiotic organism *Lactobacillus acidophilus* (MTCC No. 10307) was carried out from Institute of Microbial Technology (IMTECH), Chandigarh in the freeze dried form. It was revived on de Man Rogosa and Sharpe (MRS) media by incubating at 37°C under aerobic conditions for 1 day and followed by regular sub culturing. Probiotic culture was inoculated in MRS broth (1 litre) followed by incubation under aerobic conditions for 48 hours at an optimum temperature i.e. 37°C. After inoculum preparation, *Lactobacillus* cells were inoculated aseptically into de MRS broth (5 litres) and incubated at 37°C for 48 hours. The cells were harvested by centrifugation at 4000 rpm for 20 min at 4°C. *L. acidophilus* cells thus obtained were again transferred to MRS both (5 litres) followed by incubation at 37°C for sufficient time (18 h) to reach the cell count of about 10^8–10^9 CFU/ml. The probiotic cells were again harvested by centrifugation at 4,000 rpm for 30 min at 4°C. The probiotic cells of *Lactobacillus acidophilus* were aseptically inoculated into guava juice at 15% of the volume of juice, to have a concentration of 10^8–10^9 CFU/ml. The bottles were refrigerated at 4°C for eight weeks. The developed probiotic guava juice was taken for physiochemical (pH, TSS, total sugars [reducing and non-reducing], titratable acidity, antioxidant activity, content of lactic acid and ascorbic acid) microbiological and sensory analysis.

Physiochemical Evaluation of Probiotic Guava Juice

pH was determined using digital pH meter. Total Soluble Solids (TSS) content was determined using Erma hand refractometer (0–32°Brix). The titratable acidity was measured in percentage using the method given by AOAC[5] and lactic acid content using method given by AOAC.[6] To 1 ml of juice, 10 ml of 90% ethyl alcohol was added and shaken vigorously followed by titration against 0.1 N NaOH containing five drops of phenolphthalein indicator, till pink color was formed. The method given by Dubois *et al.*[7] was used for determination of total sugars by taking 0.1 ml of juice sample diluted to one ml by addition of distilled water. One ml of 5% phenol was added to the test tube and mixed followed by addition of 5 ml conc. sulphuric acid. Optical density was measured using spectrophotometer at 490 nm against the blank.

Nelson-Somogyi method was used for the estimation of reducing sugars of the juice.[8] In a test tube, 0.5 ml of juice was diluted to 1 ml by addition of distilled water followed by addition of 1 ml of copper solution C. Test tube was then kept for 20 min in a boiling water bath and allowed to cool. After the addition of 1 ml of Nelson's arsenomolybdate reagent, the optical density was read at 510 nm. Non-reducing sugars were calculated using subtraction of reducing sugars from total sugars. The radical scavenging property of the juice was assayed by using the DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) as control as suggested by Blois.[9] The change of color from violet to yellow was recorded spectrophotometerically (517 nm). Ascorbic acid content was estimated by the method of Ranganna using 2, 6-dichlorophenol-indophenol indicator.[10]

Microbiological Analysis

Viable count of the developed probiotic guava juice was analyzed on 0^{th} day and then weekly till eight weeks. The serial dilution of the juice sample was plated using de Man Rogosa and Sharpe (MRS) agar medium by pour plate method. After incubation at 37°C for a period of 48 h, the probiotic count was noted. Total plate count, coliform count and yeast/mould count were determined similarly using pour plate method.

Sensory Analysis

The developed probiotic guava juice was evaluated for sensory parameters by a panel of five semi-trained judges on a nine-point hedonic scale.[11] Sensory properties were assessed on the basis of taste, appearance, color, texture, aroma, mouth feel and overall acceptability.

Statistical Analysis

All the parameters were tested in triplicate and data was analyzed statistically by using One-way ANOVA IBM SPSS Statistics version 22.0.

Results and Discussion

Physiochemical Analysis of the Developed Probiotic Guava Juice

Decrease in pH was observed during the storage period of eight weeks that was significant. In case of probiotic guava juice, the pH was found to decrease from 5.62 ± 0.0023 in the first week to 4.15 ± 0.0029 in the sixth week and 3.99 ± 0.0023 in the eighth week (Table 1). The major factors that contribute to decline in pH can be attributed to the carbohydrate utilization by probiotic bacteria leading to production of organic acids in small amounts causing lowering of pH of the fruit juice. The above mentioned observations are in agreement with the earlier results reported by Zhu *et al.*[12] According to which, a decline in pH of probiotic apple, orange and tomato juice inoculated with *Lactobacillus sanfranciscensis,* during the refrigeration for 4 weeks was observed. Do and Fan[13] in a similar study on vegetable juice mixture inoculated with *Lactobacillus* strains, found a drop in pH over a period of 4 weeks of refrigeration and cited the reason to be accumulation of organic acids. A significant decrease in total soluble solids content (TSS) was observed during the eight weeks of storage. The TSS value of probiotic guava juice decreased from 15.11 ± 0.0035°brix in the first week to 13.45 ± 0.0051°brix in the eighth week (Table 1). This may be due to sugar utilization by bacteria. Mostafa *et al.*[14] also reported a decrease in the TSS (%) of the date juice from 18.45% to 17.8% and from 18.6% to 18% in juice samples inoculated by *Lactobacillus acidophilus* and *Lactobacillus sakei,* respectively during the three weeks storage.

Total sugars were found to decrease from 6.78 ± 0.0052% to 5.73 ± 0.0075% in 4th week which further decreased to 3.23 ± 0.0130% in 8th week (Table 1). Similarly, Zandi *et al.*[15] also reported a reduction in total sugar content of probiotic carrot, beet and apple juices during 4 weeks of refrigerated storage. Do and Fan[13] also found the total sugar content of probiotic vegetable juice mixture to decrease from 48.76 mg/mL to 34.66 mg/mL during storage period of 28 days at 4°C. Similarly, the reducing sugar content, changed after first week during storage. It decreased from 3.46 ± 0.0040% to 2.71 ± 0.0046% in 4th week and subsequently to 1.62 ± 0.0046% in 8th week (Table 1). Non-reducing sugars decreased from 3.32 ± 0.0060% in 1st week to 1.60 ± 0.0060% in 8th week (Table 1). In an earlier study, Thakur *et al.*[16] detected a reduction in reducing sugar content from 12.32% to 9.93% of probiotic pomegranate juice during 4 weeks of refrigeration. The observed decrease in sugars can be attributed to the utilization of sugars as carbon source by inoculated cultures for their development. The titratable acidity was found to increase significantly after 8 week storage from 0.39% to 0.70% (Table 2). Similar trend was observed in lactic acid content (Table 2). Utilization of sugars by lactic acid bacteria leading to organic acid production could have resulted in increase in titratable acidity. The results of the above study are found to be in coherence with the previous studies done by Tenea and Suárez[17] who found an increase in the titratable acidity of a milk beverage inoculated with culture isolated from native Amazonian fruit over a storage period of 28 days from 0.65% to 0.84% and 0.86% to 1.04% in the UTNGt28-based and LacAT-based formulations, respectively. Mantzourani *et al.*[18] also observed significant increase in lactic acid content weekly, reaching 97.8 mg/100 mL, in the case of FC (free) and 148.5 mg/100 mL in the case of IC (immobilized culture) in probiotic cornelian cherry juice beverage.

Guavas are immensely a rich source of vitamin C. Ascorbic acid content was found to decrease during storage of 8 weeks from 167.25 ± 0.0110 mg/100 ml to 160.01 ± 0.0040 mg/100 ml (Table 2)

Table 1 — Changes in physicochemical parameters of probiotic guava juice w.r.t. control guava juice during storage

Storage Period (Days)	pH		TSS (°brix)		Reducing Sugars (%)		Non-reducing sugars (%)		Total Sugars (%)	
	Probiotic guava juice	Control guava juice	Probiotic guava juice	Control guava juice	Probiotic guava juice	Control guava juice	Probiotic guava juice	Control guava juice	Probiotic guava juice	Control guava juice
0	5.62^{a} ± 0.0023	5.62^{a} ± 0.0017	15.11^{a} ± 0.0035	15.11^{a} ± 0.0023	3.46^{a} ± 0.0040	3.46^{a} ± 0.0023	3.32^{a} ± 0.0035	3.32^{a} ± 0.0046	6.78^{a} ± 0.0052	6.79^{a} ± 0.0029
7	5.38^{b} ± 0.0029	5.53^{b} ± 0.0017	14.98^{b} ± 0.0023	15.10^{ab} ± 0.0029	3.09^{b} ± 0.0029	3.24^{b} ± 0.0023	3.10^{b} ± 0.0040	3.23^{b} ± 0.0029	6.20^{b} ± 0.0052	6.47^{b} ± 0.0023
14	5.12^{c} ± 0.0017	5.31^{c} ± 0.0029	14.73^{c} ± 0.0280	15.09^{b} ± 0.0035	2.96^{c} ± 0.0052	3.12^{c} ± 0.0012	2.95^{c} ± 0.0040	3.11^{c} ± 0.0023	5.91^{c} ± 0.0035	6.23^{c} ± 0.0023
21	4.98^{d} ± 0.0028	5.10^{d} ± 0.0023	14.50^{d} ± 0.0034	15.02^{c} ± 0.0038	2.86^{d} ± 0.0032	3.00^{d} ± 0.0030	2.87^{d} ± 0.0035	2.96^{d} ± 0.0017	5.73^{d} ± 0.0029	5.96^{d}±0.0029
28	4.70^{e} ± 0.0029	4.97^{e} ± 0.0040	14.32^{e} ± 0.0035	14.99^{d} ± 0.0023	2.71^{e} ± 0.0046	2.92^{e} ± 0.0029	2.76^{e} ± 0.0046	2.81^{e} ± 0.0023	5.47^{e} ± 0.0046	5.74^{e} ± 0.0040
35	4.18^{f} ± 0.0369	4.89^{f} ± 0.0035	14.23^{f} ± 0.0017	14.93^{e} ± 0.0032	2.40^{f} ± 0.0058	2.71^{f} ± 0.0029	2.37^{f} ± 0.0040	2.63^{f} ± 0.0029	4.77^{f} ± 0.0035	5.34^{f} ± 0.0023
42	4.15^{f} ± 0.0029	4.67^{g} ± 0.0023	13.97^{g} ± 0.0044	14.87^{f} ± 0.0029	2.13^{g} ± 0.0046	2.49^{g} ± 0.0017	1.92^{g} ± 0.0046	2.43^{g} ± 0.0023	4.05^{g} ± 0.0035	4.93^{g} ± 0.0023
49	4.09^{g} ± 0.0017	4.51^{h} ± 0.0017	13.72^{h} ± 0.0028	14.66^{g} ± 0.0049	1.91^{h} ± 0.0063	2.32^{h} ± 0.0046	1.77^{h} ± 0.0092	2.29^{h} ± 0.0051	3.69^{h} ± 0.0075	4.61^{h} ± 0.0069
56	3.99^{h} ± 0.0023	4.49^{i} ± 0.0028	13.45^{i} ± 0.0051	14.57^{h} ± 0.0034	1.62^{i} ± 0.0046	2.28^{i} ± 0.0096	1.60^{i} ± 0.0034	1.99^{i} ± 0.0167	3.23^{i} ± 0.0075	4.28^{i} ± 0.0040
C.D (5%)	0.037	0.008	0.0029	0.0103	0.014	0.012	0.014	0.019	0.015	0.011

Values are mean ± SE, where no. of replications = 3, Presence of different superscripts indicate statistically significant difference

Table 2 — Changes in nutritional constituents of probiotic guava juice w.r.t control guava juice during storage

Storage Period (days)	Antioxidant activity (%)		Titratable acidity (%)		Lactic acid content (mg/ml)		Ascorbic acid content (mg/100ml)	
	Probiotic guava juice	Control guava juice	Probiotic guava juice	Control guava juice	Probiotic guava juice	Control guava juice	Probiotic guava juice	Control guava juice
0	$43.82^{a} \pm 0.0029$	$43.95^{a} \pm 0.0046$	$0.39^{a} \pm 0.0020$	$0.39^{a} \pm 0.0012$	$35.64^{a} \pm 0.0404$	$35.19^{a} \pm 0.0231$	$167.25^{a} \pm 0.0110$	$167.21^{a} \pm 0.0046$
7	$39.37^{b} \pm 0.0052$	$42.77^{b} \pm 0.0023$	$0.43^{b} \pm 0.0040$	$0.39^{a} \pm 0.0029$	$38.79^{b} \pm 0.0173$	$35.55^{b} \pm 0.0173$	$165.24^{b} \pm 0.0069$	$167.14^{b} \pm 0.0069$
14	$38.42^{c} \pm 0.0052$	$41.10^{c} \pm 0.0035$	$0.48^{c} \pm 0.0035$	$0.40^{a} \pm 0.0009$	$43.47^{c} \pm 0.0173$	$36.42^{c} \pm 0.0346$	$164.06^{c} \pm 0.0087$	$166.78^{c} \pm 0.0034$
21	$37.83^{d} \pm 0.0046$	$40.63^{d} \pm 0.0035$	$0.56^{d} \pm 0.0029$	$0.41^{b} \pm 0.0023$	$50.57^{d} \pm 0.0291$	$37.53^{d} \pm 0.0462$	$162.67^{d} \pm 0.0040$	$166.22^{d} \pm 0.0064$
28	$37.21^{e} \pm 0.0040$	$39.02^{e} \pm 0.0046$	$0.59^{e} \pm 0.0029$	$0.42^{bc} \pm 0.0040$	$53.19^{e} \pm 0.0289$	$38.61^{e} \pm 0.0404$	$162.03^{e} \pm 0.0052$	$165.83^{e} \pm 0.0046$
35	$35.23^{f} \pm 0.0040$	$38.78^{f} \pm 0.0046$	$0.61^{f} \pm 0.0040$	$0.45^{bc} \pm 0.0017$	$55.26^{f} \pm 0.0289$	$41.13^{f} \pm 0.0346$	$161.68^{f} \pm 0.0040$	$165.64^{f} \pm 0.0075$
42	$34.69^{g} \pm 0.0064$	$37.26^{g} \pm 0.0046$	$0.63^{g} \pm 0.0012$	$0.48^{cd} \pm 0.0017$	$57.33^{g} \pm 0.0291$	$44.01^{g} \pm 0.0404$	$160.47^{g} \pm 0.0052$	$164.96^{g} \pm 0.0052$
49	$34.29^{h} \pm 0.0017$	$36.99^{h} \pm 0.0023$	$0.66^{h} \pm 0.0026$	$0.49^{d} \pm 0.0028$	$59.54^{h} \pm 0.0346$	$44.68^{h} \pm 0.0346$	$160.19^{h} \pm 0.0034$	$164.59^{h} \pm 0.0051$
56	$33.95^{i} \pm 0.0037$	$36.58^{i} \pm 0.0046$	$0.70^{i} \pm 0.0023$	$0.50^{d} \pm 0.0034$	$63.23^{i} \pm 0.0404$	$45.67^{i} \pm 0.0230$	$160.01^{i} \pm 0.0040$	$164.11^{i} \pm 0.0063$
C.D (5%)	0.013	0.012	0.0094	0.031	0.091	0.101	0.019	0.017

Values are mean ± SE, where no. of replications = 3, Presence of different superscripts indicate statistically significant difference

Table 3 — Probiotic viability and microbiological assessment of probiotic guava juice

Storage Period (days)	Probiotic viability and microbiological assessment (log CFU/ml)							
	Probiotic viability	FSSAI Specification (2017)	Total Plate Count	FSSAI Specification (2017)	Yeast and Mould count	FSSAI Specification (2017)	Coliform Count	FSSAI Specification (2017)
0	$9.23^{a} \pm 0.0046$		$1.06^{a} \pm 0.0075$	Satisfactory below 2 log CFU/ml	Ab*	Satisfactory below 2 log CFU/ml	Ab	
7	$9.11^{b} \pm 0.0012$		$1.47^{b} \pm 0.0029$		Ab		Ab	
14	$8.91^{c} \pm 0.0081$		$2.11^{c} \pm 0.0092$	Acceptable between 2-4 log CFU/ml	Ab		Ab	
21	$8.54^{d} \pm 0.0081$		$2.37^{d} \pm 0.0139$		$0.69^{b} \pm 0.0005$		Ab	
28	$8.25^{e} \pm 0.0035$	Probiotic viability Should be above 8 log CFU/ml	$3.01^{e} \pm 0.0139$		$0.90^{c} \pm 0.0052$		Ab	Coliforms should be absent in the given food sample
35	$7.47^{f} \pm 0.0069$		$3.39^{f} \pm 0.0040$		$1.07^{d} \pm 0.0121$		Ab	
42	$6.39^{g} \pm 0.0092$		$4.00^{g} \pm 0.0156$	Unsatisfactory above 4 log CFU/ml	$1.17^{e} \pm 0.0109$		Ab	
49	$6.18^{h} \pm 0.0034$		$4.19^{h} \pm 0.0034$		$1.27^{f} \pm 0.0046$		Ab	
56	$5.82^{i} \pm 0.0028$		$4.36^{i} \pm 0.0040$		$1.82^{g} \pm 0.0057$		Ab	
C.D (5%)	0.018		0.028		0.019			

*Absent, Values are mean ± SE, where no. of replications = 3, Presence of different superscripts indicate statistically significant difference

owing to the reason because of the sensitivity of ascorbic acid to oxygen, water solubility, enzymatic oxidation by ascorbate peroxidase (APX) and photo-oxidation. Hashemi and Mahmoodi[19] also reported ascorbic acid content to be reduced in probiotic barberry juice during refrigerated storage of 28 days. The antioxidant activity showed a reduction throughout 8 week refrigeration. The initial antioxidant activity was 43.82 ± 0.0029% which decreased to 33.95 ± 0.0037% during storage at 4°C (Table 2). The decrease in antioxidant activity can be correlated to the reminiscent activity of inoculated culture during refrigeration or due to the formation and accumulation of toxic metabolites in cells owing to the presence of dissolved oxygen in samples. This can lead to cell death by oxidative damage.[20] Nguyen *et al.*[21] also reported significant decline in antioxidant activity of probiotic pineapple juice.

Microbiological Analysis

Viable count was found to reduce after 4 weeks of storage under refrigeration. It decreased from 9.23 ± 0.0046 log CFU/ml at the beginning of storage to 5.82 ± 0.0028 log CFU/ml after 8 weeks of storage (Table 3). It may be due to decline in pH, rise in titratable acidity and decreased content of sugars. According to FSSAI[22] standards, probiotic viability should be >8 log CFU/ml. Silva and Ferrari[23] reported a reduction in cell count (log CFU/mL) from 9.0 to 5.9 and 6.1 log for probiotic grape juices. The study was in coherence with the observations by Nematollahi *et al.*[24] that reported decrease in viability of *Lactobacillus casei* in cornelian cherry juice with pH adjusted at 3.5, during refrigeration.

The total plate count detected till five weeks of probiotic guava juice storage was found to be within the FSSAI[22] specified limits (Table 3). During the first week, the count was within satisfactory limits (< 2 log CFU/ml) and during the second, third, fourth and fifth weeks of storage it was found to be within acceptable limits (between 2 and 4 log CFU/ml). Total plate count of the developed juice started to increase significantly after sixth week onwards till eight week which was above the FSSAI prescribed limits (>4 log CFU/ml). Rathod *et al.*[25] also found an increase in total plate count of probiotic apple and orange juice in the 4 weeks of storage.

Yeast and mould count was not detected in first two weeks of refrigerated storage of probiotic guava juice (Table 3). During third, fourth and fifth weeks, yeast and mould growth was spotted. This increase was statistically significant ($p<0.05$) but count was found to be within the satisfactory limits (< 2 log CFU/ml). There was an increase in yeast and mould count of the developed probiotic guava juice during sixth week, seventh week and eighth week of storage which was satisfactory within FSSAI[22] prescribed specified limits (<2 log CFU/ml). The

Table 4 — Sensory evaluation of probiotic guava juice and control guava juice

Group	Taste	Appearance	Color	Texture	Aroma	Mouth Feel	Overall Acceptability
Probiotic Guava Juice	8.56	8.44	8.53	8.60	8.67	8.54	8.56
Control Guava Juice	8.24	8.19	8.32	8.32	8.40	8.25	8.28
t-value	3.26*	3.20*	1.84^{NS}	5.89**	2.70*	3.22*	1.80^{NS}

** means at 1% level of significance
* means at 5% level of significance NS = non-significant

increase in yeast and mould count can be due to decrease in pH after probiotic culture inoculation. There are a number of psychrotolerant yeast species reported in literature that can grow under refrigeration conditions. Gallina *et al.*[26] also reported similar trend in yeasts and moulds counts ranged from 3 to 6 CFU/mL (0.47-0.77 log CFU/ml) in probiotic smoothie beverages. Coliforms were not detected during the entire refrigerated storage period of eight weeks (Table 3). This is an indication of acceptable quality of the probiotic guava juice and gives assurance that it is safe for consumption. Thakur *et al.*[16] reported no growth of coliform bacteria in the samples over the entire storage period in probiotic pomegranate juice due to the hygienic conditions the double distilled water used for analysis work.

Sensory Analysis

Sensory analysis showed a significant difference in taste, appearance, aroma, mouth feel and texture of guava juice vis-a-vis probiotic guava juice whereas difference in other parameters like color and overall acceptability were found to be statistically insignificant (Table 4). Overall acceptability for probiotic guava juice was 8.56.

Conclusions

Keeping in view, the observations of the present study, it can be inferred that the probiotic guava juice prepared with *Lactobacillus acidophilus* was found to be organoleptically acceptable. Based on microbiological analysis of the probiotic guava juice produced in the pilot plant the probiotic viability remained above 8 log CFU/ml for 4 weeks. The biochemical and sensory evaluation of the developed product revealed its shelf life to be one month under refrigeration (4°C), without any significant alteration in sensory profile. Fruits are an important part of our balanced diet and the fruit juices exemplify as appropriate medium for cultivation of lactic acid bacteria owing to they being a rich source of sugars and nutrition. Probiotics are beneficial for human health because they help in curing diarrheal diseases, prevention of diarrhea caused due to intake of antibiotics and lactose metabolism. The microbial association exploited to make these juices confer long shelf life hence commercial production of such juices is beneficial economically as well as in mitigation of serious health issues. However, attempts can be made to increase the shelf life of the product by using various physicochemical approaches or by trying alternate formulations like reconstituted or freeze dried products. More research work in this field by exploiting a recent technology (microencapsulation) and microorganisms other than *Lactobacillus acidophilus* is the need of the hour.

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Conflict of Interest

It is hereby submitted that both the authors have read and approved the submission. None of the authors have any competing financial and non-financial conflict of interest.

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