



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 physicochemical (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 physicochemical 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 physicochemical, 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 AOAC⁵ 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 spectrophotometrically (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 0th 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.

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 Mahmoodi¹⁹ 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 $43.82 \pm 0.0029\%$ which decreased to $33.95 \pm 0.0037\%$ 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 FSSAI²² standards, probiotic viability should be >8 log CFU/ml. Silva and Ferrari²³ 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 FSSAI²² 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 FSSAI²² 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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