



### RESEARCH ARTICLE

#### DEVELOPMENT OF PROBIOTIC BEVERAGE FROM PINEAPPLE AND ROSE APPLE JUICE

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#### Abstract

Probiotics are microorganism that provide health benefits when consumed. The term probiotics is currently used to indicate the ingested microorganisms associated with beneficial effects to humans and animals. The major probiotics foods available in market are milk based products. Pineapple and Rose apple is mostly grown in tropical and subtropical regions of the world. These fruits are very crispy and tasty kind of fruit that can serve as a raw material in wine and juice production. The overall goal of the research work was to develop a probiotic beverage from Pineapple and Rose apple juice with probiotic bacteria " Lactobacillus plantarum" strain to examine the potential. Probiotic drink was prepared at pH 3.6 and optimum fermentation temperature of 37°C. There was a gradual decline in pH and sugar content in time. In this study to determine the physicochemical properties and to check the shelf-life stability of the juice. Total phenols, flavanoids and antioxidants activity were enhanced in probiotic drink is compared to fresh juice sample. This study revealed that probiotic drink is a good approach for non-dairy products, with high nutritional and health promoting components.

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#### Introduction:-

Today, the concept using foods to promote a state of well-being, improving health and reducing the risk of diseases has become the new frontier in nutrition sciences and related fields. In this context, the development and contribution of functional foods -prebiotics, probiotics and synbiotics must receive attention should be key pillars of health care system. Probiotics are live microorganisms promoted with claims that they provide health benefits when consumed, generally by improving or restoring the gut flora. Probiotics are traditionally found in dairy products, such as cheese, yogurt, etc. fortunately for those dealing with lactose intolerance, suffering from a milk allergy, or choosing a dairy free diet, a non-dairy probiotic is now an option. India is leading producer of fruits and vegetables but unfortunately a major proportion is spoiled due to improper processing and poor post harvesting management. Fruits and vegetables are inherently healthy refreshing, pleasing taste profiles and bundle of beneficial nutrients, so these can be used as suitable substrate for non-dairy probiotics. Fermentation can enhance nutritional, digestibility, shelf life, safety and sensory attributes of vegetables.

#### Pineapple:

Pineapple (*Ananas comosus*, Bromeliaceae) is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavour and immense health benefits. Pineapple contains considerable calcium, potassium, fibre, and

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vitamin C. It is low in fat and cholesterol. Vitamin C is the body's primary water soluble antioxidant, against free radicals that attack and damage normal cells. It is also a good source of vitamin B1, vitamin B6, copper and dietary fibre. Pineapple is a digestive aid and a natural Anti-Inflammatory fruit. A group of sulfur-containing proteolytic (protein digesting) enzymes (bromelain) in pineapple aid digestion (Dr. P Joy,2010).

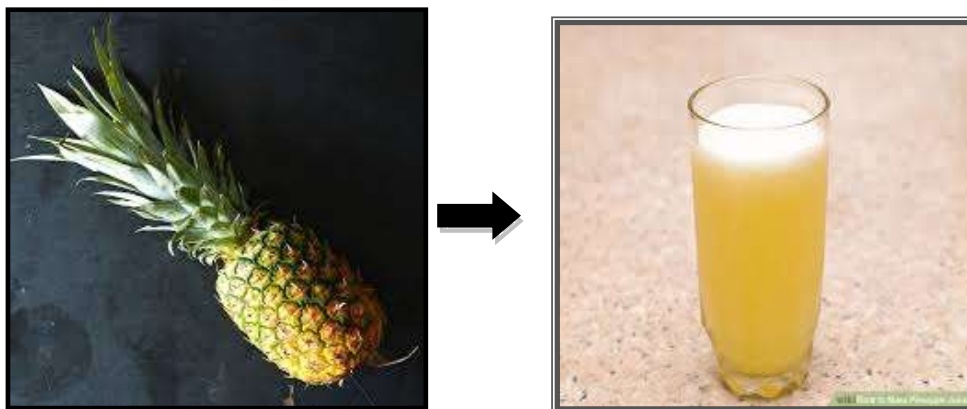


Fig 1:- Pineapple juice.

#### Rose Apple:

Rose Apple possess rich amount of iron, calcium, fiber, vitamin C, protein and vitamin A .100gm of Rose Apple contains calcium (29mg), potassium (123mg), sulphur (13mg), vitamin C (24.78%), Total fat (0.86%), Iron (0.88%), and phosphorus (1.14%). Since which is rich in vitamin C, it prevents the damage of free radicals, pollutants and toxic chemicals, reduces the risk of stroke, boost good HDL cholesterol, prevents diabetes, constipation, muscle cramping, skin health, antimicrobial activity, etc (Manjeshwar Shrinath Baliga et al., 2017).



Fig 2:- Rose Apple Juice.

#### Probiotic Fermentation:

Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. This study focuses on the fermentation of pineapple and rose apple juice using the single probiotic strain *Lactobacillus plantarum*, which are supplemented by food that beneficially affect the host by improving its intestinal balance. The supplementation of probiotics to food provides several health benefits such as reduction of serum cholesterol, improved gastrointestinal function, enhance immune system, and lower risk of colon cancer. This review provides an overview on the current research prospects of LA fermentation of fruits (Pineapple and Rose Apple) with regard to human nutrition and health.

#### Materials and Methods:-

##### Materials Required:-

1. Pineapple
2. Rose Apple
3. Sugar

4. Culture
5. Preservatives
6. Stabilizer

After a detailed literature review on health benefits of probiotics, the freeze dried culture of *Lactobacillus Plantarum* was obtained from Microbiological Laboratory, Coimbatore, TamilNadu.

## **Methods:-**

### **Sample Procurement and Preparation:**

The fresh raw pineapple and watery rose apple was purchased from local market, cleaned and stored at 4°C for further use. Juice was extracted by juicer in a food processor. The extracted filtered juice was sterilized using Autoclave at 121°C for 15 min. This pasteurized juice was further cooled to room temperature for microbial inoculation.

### **Inoculum Preparation:**

Dried pellets culture were reconstituted in 50 mL MRS (deMan, Rogosa and Sharpe medium) broth and grown over night (14-16 hrs) in an incubator shaker at 110 rpm and 37°C. This was sub cultured and grown overnight again (Priyanka Shukla et al., 2017). The culture thus obtained after the second sub-culture was used for further experiments.

### **Preparation of probiotic beverage:**

The optimum fermentation conditions were optimised using central composite rotated experimental design (CCRD) at pH range 3-6 at temperature of 37°C. These conditions were selected because *Lactobacillus* can grow at this pH and temperature condition. The pH of clarified pineapple and rose apple juice was adjusted with NaOH(0.1N). Then pre-determined concentration of inoculum ( $1 \times 10^5$  CFU/mL) was added to pineapple and rose apple juice as recommended for probiotic foods i.e., 7.00 log CFU/ml (1 ml of MRS broth containing 9.00CFU/ml of *Lactobacillus plantarum*). Fermentation was performed statically in an incubator for 24 hr adjusted at 37°C temperature.

### **Physiochemical analysis:**

Different parameters such as Titratable acidity, Ascorbic acid content, pH, Total Soluble Solids, Protein content, Total Viable count were examined to study the impact of fermentation time and storage period of the developed probiotic beverage.

### **Sensory Evaluation:**

Sensory analysis was carried out by using semi trained panellists using 9point hedonic scale from liked extremely (9) to disliked extremely (1). Sample was evaluated on the basis of appearance, aroma, consistency, taste, mouth feel and overall acceptability.

### **Statistical analysis:**

Statistical procedure were used to analyze the data for the interpretation of results. Mean, standard deviation and analysis of variance (ANOVA) were used to describe the results.

## **Results and Discussion:-**

The results of the study are being presented and discussed in following section.

### **Development of probiotic beverage:**

The probiotics beverage was prepared by blending pineapple and rose apple juice in different proportions i.e. S1 (80:20), S2 (75:25), S3 (70:30), S4 (65:35) and S5 (50:50). All the prepared probiotic beverages were then fermented for 24 hrs with *Lactobacillus plantarum* strain and their viable counts were checked. All prepared samples were then packed in glass bottles and stored at 4°C refrigerated temperature.

### **Effect of fermentation time on developed probiotic beverage:**

The viable counts were assessed after 5, 15, 20 and 24 hrs of incubation at 37°C for the probiotic beverage developed fermented with *Lactobacillus plantarum*. As the amount of Rose apple juice increased in the probiotic beverages there was a decrease in total viable counts. But in each probiotic beverage fermented with *Lactobacillus*

plantarum the total viable count increased significantly with increase in fermentation time from 5 to 24 h (Sasi Kumar R, 2015). The probiotic beverage attained a total viable count of more than  $10^6$  cfu/ml within 24 hrs of fermentation. The results were shown in the Table 1. As the 24 hrs fermentation showed maximum viable counts all the probiotic beverages were fermented for 24 hrs for further analysis.

**Table 1:-** Effect of fermentation time on developed probiotic beverage (colonies in  $10^8$ ).

Fermentation time	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>
5	0.21	0.46	0.41	0.42	0.38	0.34
15	6.89	5.78	5.42	5.31	5.20	5.11
20	9.34	7.65	7.45	6.85	6.42	6.23
24	9.78	8.59	8.43	8.33	8.21	8.04

#### Physicochemical properties of developed probiotic beverages:

The probiotic beverage developed fermented with *Lactobacillus Plantarum* was fermented for 24 hrs. Then the physicochemical properties of each probiotic beverage were determined. The pH of the different probiotic beverages decreased as the amount of pineapple and rose apple increased in the probiotic beverages. The pH of probiotic beverages fermented with *Lactobacillus plantarum* ranged from 3.86 to 3.43. The TSS (Total soluble solids) of the different probiotic beverages fermented with *Lactobacillus plantarum* ranged from 12.7 to 10.1. The TSS of the probiotic beverage decreases as the amount of rose apple juice increases in the beverage. The titratable acidity of the probiotic beverages ranged from 0.897 to 1.25, the ascorbic acid content in mg/100 ml of the probiotics ranged from 38.52 to 52.89, the protein content of the ranged from 0.41 to 0.19. The protein content of the probiotic beverages decreases, when the heat treatment are denatured at temperature of 70°C and above with the reduction in pH. The results were shown in the Table 2.

**Table 2:-** Effect of fermentation on Physicochemical properties of developed probiotic beverages.

Parameters	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>
pH	4.53	3.86	3.76	3.64	3.52	3.43
TSS(°Brix)	13.5	12.7	12.4	11.3	10.8	10.1
TA %	1.34	1.25	1.10	0.93	0.90	0.89
Ascorbic acid (mg/100ml)	0	38.52	39.95	40.36	42.89	52.89
Protein (%)	0.6	0.41	0.34	0.26	0.22	0.19

#### Sensory analysis:

In all the probiotic beverages fermented with *Lactobacillus plantarum* the points representing the average score of its different parameters like appearance, color, taste, aroma and overall acceptability have been presented in the Tables 3. The results revealed that colour, taste aroma and overall acceptability of the probiotic beverage 3 (70:30) gets the best results.

The results above declares from physicochemical and sensory evaluation, that only probiotic beverage S3 (70:30) has shown best results from both the bacterial species fermented samples. Therefore for shelf life study only probiotic beverage 3 (70:30) has been taken.

**Table 3:-** Sensory Analysis of developed beverages.

Parameters	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>
Color and Appearance	6.4	6.2	8.9	8.3	8.5
Taste	7.1	7.6	8.8	7.3	6.3
Consistency	6.0	7.2	7.9	7.4	6.1
Flavour	7.3	7.5	8.4	8.2	7.5
Overall acceptability	6.5	7.0	8.5	8.0	7.3

#### Shelf Life study of developed probiotic beverage sample 3 (70:30):

Microbial test was done to check the shelf life of the probiotic beverage sample. The sample was packed in glass bottles and were stored at  $4 \pm 1^\circ\text{C}$ . The juice was evaluated for total viable counts and nutritional composition after

every 15 days (Suguna et al., 2012) The total viable counts in the probiotic beverage fermented with *Lactobacillus plantarum* decreased from  $8.32 \times 10^8$  to  $5.9 \times 10^7$ . The result was showed in Table 4.

**Table 4:-** Shelf life study of probiotic beverage.

Storage period (In days)	Viable count (cfu/ml)	Acidity	Protein	Ascorbic acid(mg/100ml)
0	8.32	0.921	0.25	46.32
15	6.65	0.933	0.23	45.59
30	5.48	0.945	0.20	44.36
45	0.59	0.961	0.18	43.58

There was nominal change found in acidity on storing the samples for 45 days. The decrease in viable count is due to the decrease in the pH of the medium and accumulation of organic acid as a result of growth and fermentation. As the pH of the medium decreased the titratable acidity increased.

#### **Effect of storage period on viable count of yeast and moulds of developed probiotic beverage:**

Some species of microorganisms grow in food products with low pH, but results show that there were no microbial activity including acidophilic bacteria, lactic acid bacteria, yeast and mold over a period of 45 days at 4°C in the samples of fruit juice beverages. The microbial analysis was done by the standard plate count for yeast and moulds. The SPC was done in duplicates and was analyzed for 45 days of storage at every 15 days of interval. The results shown in Table 5 depicts that the growth in the product at  $10^5$  dilutions was nil. Thus it was fit for further storage.

**Table 5:-** Effect of storage period on viable count of yeast and moulds of developed probiotic beverage.

Storage period (In days)	Oct-5	Nov-5	Dec-5	Jan-5
0	0.55	-	-	-
15	0.65	-	-	-
30	1.23	68	12000	-
45	1.56	120	59000	-
60	2.22	135	72000	-

#### **Conclusion:-**

Study revealed satisfactorily good quality probiotic beverage prepared by using a 70:30 blend of Pineapple and rose apple juice inoculated with 1% (v/v) of *Lactobacillus plantarum* incubated for 24 hours with a shelf life of 45 days. The microbial count found was  $8.32 \times 10^8$  cfu/ml in *Lactobacillus plantarum*. The values of pH, TSS, TA, Ascorbic acid content and Protein determined were 3.64, 11.3°Brix, 0.93%, 40.36 mg/100 ml and 0.26% of protein. On the basis of above results revealed in the present study it might be concluded that the formulated probiotic juice beverage was possible to satisfy consumer taste and preferences.

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