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# Development of Probiotic Beetroot Drink

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**Abstract**

The study was planned to prepare non- dairy probiotic drink using beetroot juice. Probiotic potential was examined on the basis of viability of *Lactobacillus rhamnnosus, Lactobacillus plantarum* and *Lactobacillus delbrueckii sb.* Probiotic drink was prepared at pH 6.5 and optimum fermentation temperature of 37 °C. There was gradual decline in pH and sugar content with time. Total phenols, flavonoids and antioxidant activity were enhanced in probiotic drink as compared to fresh juice sample. Study revealed that beetroot drink is a good approach for non-dairy probiotic, free from cholesterol and with health promoting components.

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**Keywords**

*Lactobacillus rhamnnosus, Lactobacillus plantarum*, Sensory Evaluation.

## Introduction

As economic status has improved, growing interest in health and well-being has resulted in increased intake of natural and health foods[1] eveloping value added health promoting foods as well as phytochemical rich new raw material sources. Health professionals are motivating the consumers to avail beneficial effects of probiotic food for good health. The probiotic foods currently produced are dairy products and has limitations due to milk allergies and cholesterol content[2]. So non- dairy alternatives may be used to substitute dairy probiotics and can provide other health benefits also. Probiotic food formulations are leading to development of dietary supplementation with nutraceuticals and prebiotics[3]. Fruits and vegetables are highly valued food for their high carbohydrate, minerals, vitamins, dietary fibers, and antioxidants which will help in buildup, repair and maintain alkaline reserve of body[4] and also do not possess any dairy allergens[2]. India is leading producer of fruits and vegetables but unfortunately a major proportion is spoiled due to improper processing and poor postharvest management[5]. Fruits and vegetable are inherently healthy, refreshing, pleasing taste profiles and bundle of beneficial nutrients, so these can be used as suitable substrate for nondairy probiotics. Fermentation can enhance nutritional, digestibility, shelf life, safety and sensory attributes of vegetables and also nutrients are preserved which are otherwise destroyed by thermal food processing.

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Beetroot contains numerous nutrients including sodium, magnesium, potassium, vitamin C, betanin and antioxidants[6,7].The beet root contained phenolic compound, carotenoids, betalain, vitamins and minerals which are major important bio compound and micronutrients and it is the 10th most powerful vegetables with antioxidant properties[8]. Betalins are present mainly in two forms i.e. betacyanin (red-violet Colour) and betaxanthin (yellow-orange colour). Betalain is water-soluble and nitrogen containing natural pigments which have a high coloring capacity[9] along with antiviral, antioxidant, anti-inflammatory properties[10], anti-cancerous[11] with no side effect[12]. Both sub classes of betalins: betacyanin (red-violet Colour) and betaxanthin (yellow-orange colour), are present in red beet root in high concentrations. An effort was made to utilize all these health potential of beetroot in the form of a drink. The present study was planned to prepare a beetroot based probiotic drink

## Materials and Methods

### Sample Procurement and Preparation

The fresh raw material beetroot was sorted from local market, cleaned and stored at 4 $^0$C for further use. Juice was extracted by juicer in a food processor. The extracted filtered juice was pasteurized at 80 $^0$C for 10 min. Pasteurized juice was further cooled to room temperature for microbial inoculation..

### Inoculum Preparation

Probiotic microorganisms *Lactobacillus rhamnnosus, Lactobacillus plantarum* and *Lactobacillus delbrueckii sb* were obtained from IMTECH, Chandhigarh. For inoculum, glycerol stock culture tube of *Lactobacillus plantarum, Lactobacillus rhamnnosus* and *Lactobacillus delbrueckii sb.* was transferred in 250 ml Erlenmeyer flask having 100 ml MRS broth. Broth was incubated for cell growth at 37 $^0$C. Growth was observed using spectrophotometer (590 nm). Incubation was carried out till the cell density reached 0.600 value corresponding to 9.00 Log CFU/ml, scale designed by McFarland[13].

### Inoculation and Fermentation

The optimum fermentation conditions were optimised using central composite rotated experimental design (CCRD) at pH range 4-7 at temperature of 37 $^0$C. These conditions were selected because *Lactobacillus* can grow at this pH and temperature condition. The pH of clarified beetroot juice was adjusted with HCL (0.1 N). Then, pre-determined concentration of inoculum was added to beetroot juice as recommended for probiotic foods[14] i.e., 7.00 log CFU/ml (1 ml of MRS broth containing 9.00 CFU/ml of *Lactobacillus plantarum, Lactobacillus rhamnnosus* and *Lactobacillus delbrueckii sb*.). Fermentation was performed statically in an incubator for 24 hr adjusted at 37 $^0$C temperature.

### Sample Analysis

The fresh and probiotic beetroot juice analyzed for protein, acidity content, pH, TSS and betalain content by using different methods. The bile and acid tolerance were analyzed before addition of lactic acid bacteria[15].

### Growth of Probiotic Strains

Utilizing the optimized factor value (i.e., temp 37 °C and pH 6.5), culture were developed in pasteurized beetroot juice (700 mL in 1 L plastic flask) adding inoculum of lactic acid bacteria 2.5 %, 5 %, 7 % (V/V) and incubating at temperature 37 °C for 24 hrs. Tests for measuring the growth and viable cells counts[16] were taken each two hour. Microbial growth was assessed on the basis of pH, acidity content and sugar utilization. pH was estimated by pH meter and acidity was measured by titration method. Sugar content was observed by DNS (dinitrosalicylic acid) method.

### Sensory Evaluation

Sensory analysis was carried out by using semi-trained panelists using nine point hedonic scale from liked extremely (9) to disliked extremely (1) as described by Larmond[17]. Sample was evaluated on the basis of appearance, aroma, consistency, taste, mouth feel and overall acceptability.

### Statistical Analysis

The result for physico-chemical, microbiological and sensory attributes of papaya based whey RTS were analysed with the help of Graph Pad Prism (La Jolla, CA, USA) (version 5.01) software. 2 way ANOVA was conducted for statistical significance for mean differences. The significance level was set at 5 % ($P<0.05$) for all calculations[18].

## Results and Discussion

The three species of lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus rhamnnosus* and *Lactobacillus delbrueckii sb.*) were found to be capable of growing well on pasteurized beetroot juice without any specific nutrient requirement. Viability of three strains on different pH level was observed (Table 1) and it was concluded that at pH 6.5, microbial count was more than 300 for all three strains. So, pH 6.5 was selected for further product development at 37 $^0$C.

**Table 1: Viability of lactobacillus strain at variable pH level after 24 hrs**

| pH | *L. rhamnnosus* | *L. plantarum* | *L. delbruecki* |
|---|---|---|---|
| 4 | 100 | 80 | 90 |
| 5 | 200 | 150 | 190 |
| 6 | 250 | 200 | 240 |
| 6.5 | >300 | >300 | >300 |
| 7 | <250 | <200 | <230 |

Fermentation of pasteurized beetroot juice was carried out by inoculating with culture of mixed starins (*Lactobacillus plantarum, Lactobacillus rhamnnosus* and *Lactobacillus delbrueckii sb.*). To evaluate growth kinetics, change in pH due to acid production and sugar utilization was observed. The probiotic culture suitability was observed on the basis of their acid and bile tolerance and study revealed that microorganisms were resistant to such conditions (Fig. 1).

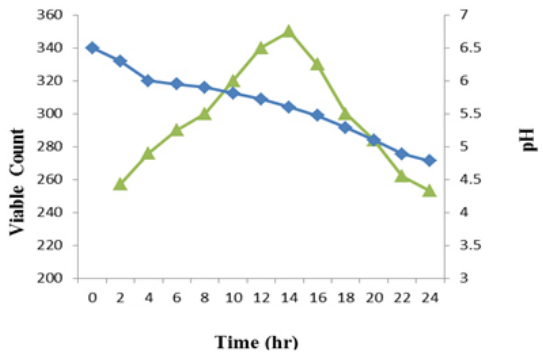


**Fig. 1: Bile tolerance of Lactobacillus strains at different pH level**

The initial pH of inoculated juice was 6.5±0.03 and it gradually decreased to 4.75±0.03 in 24 hr (Fig 1). During fermentation, *lactobacillus* produces lactic acid which accounted for the decline in pH level. The initial acidity of the beetroot juice was 0.49 ±0.02 and was inclined to 1.02±0.01. Similar observations for pH decline and acidity incline were reported by other researchers Devi *et al.,*[19], Shukla *et al.,*[20] and Rafiq *et al.,*[21].

### Change in Sugar Concentration With Time

*Lactobacillus* requires sugar for growth and as the fermentation continues, sugar content of sample was declining gradually. Initially sugar (%) in juice was 9.97±0.09 which was decreased with time. Fig 2 clearly depicted that with microbial fermentation, sugar level decreased with time.

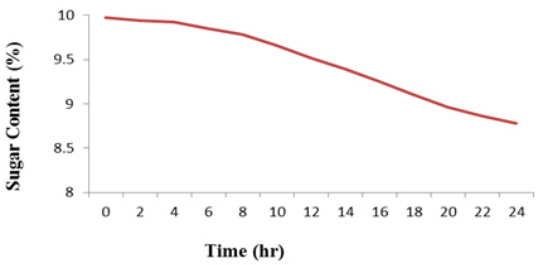


**Fig. 2: Changes in siugar content (%) during fermentation proximate analysis of fresh beetroot juice**

### Proximate Analysis of Fresh Beetroot Juice

Fresh beetroot juice and probiotic drink were evaluated for proximate composition (Table 2) and antioxidant activity. Slight increment in protein value was observed from 3.74±0.008 to 3.77±0.009, this might be due to presence of probiotic microorganisms and their metabolites. Acidity of sample was increased from 0.49±0.02 to 0.78±0.01 (Table 2). There was increase in antioxidant activity, total phenols and flavonoid content was observed. The increase in total antioxidants occurred due to fermentation leading to increase in phenolic and flavonoids compounds due to microbial hydrolysis reaction. Fermentation

also results in structural disintegration of cell walls leading to either liberation or synthesis of different antioxidant compounds. As antioxidants possess free radical scavenging capacity and health promoting benefits, so the probiotic drink is highly valuable. Similar results have been reported by Kazimierczak *et al.,* 2016[22].

**Table 2: Nutritional composition of fresh and probiotic drink**

| Sample | Acidity (%) | pH | Protein (%) | Sugar (%) | Antioxidant acitivity (%) | Total Phenol (%) | Flavonoid content (%) |
|---|---|---|---|---|---|---|---|
| Fresh juice | 0.49±0.02[b] | 6.5±0.03[a] | 3.74±0.08[b] | 9.97±0.09[a] | 75.8±0.05[b] | 22.12±0.02[b] | 3.61±0.01[b] |
| Probiotic drink | 0.78±0.01[a] | 4.75±0.03[b] | 3.77±0.09[a] | 8.66 ±0.09[b] | 78.1±0.05[a] | 24.32±0.04[a] | 3.85±0.02[a] |

*Mean ± SD (n=3)

[a]* Different upper case superscripts in the same column indicate the significant difference ($p<0.05$)

**Sensory Analysis**

The probiotic drink was less accepted (7±0.27) as compared to the fresh one (7.5±0.42), but the benefit of probiotic cultures in beetroot juice adds value in terms of health aspects. The highest mean appearance, aroma, consistency, taste, mouth feel was observed in fresh beetroot juice than the probiotic one (Table 3). Results depicted that microbial fermentation may be used as suitable technology in formulation of health promoting foods.

**Table 3: Sensory analysis of fresh and probiotic juice**

| Sample | Appearance | Aroma | Consistency | Taste | Mouth feel | Overall acceptability |
|---|---|---|---|---|---|---|
| Fresh juice | 7±0.54[a] | 8±0.28[a] | 8±0.36[a] | 7±0.42[b] | 8±0.39[a] | 7.5±0.42[a] |
| Probiotic drink | 7±0.51[a] | 7±0.32[b] | 7±0.42[b] | 7.5±0.28[a] | 7±0.19[b] | 7±0.27[b] |

*Mean ± SD (n=3)

[a]* Different upper case superscripts in the same column indicate the significant difference ($p<0.05$)

**Conclusion and Future Scope**

Mixed culture of *Lactobacillus plantarum, Lactobacillus rhamnnosus* and *Lactobacillus delbrueckii sb*. carried out fermentation and survived in beetroot juice at optimum conditions. Strains possessed good viability in beetroot juice without any specific nutrient supplementation. There was non-significant increase in proximate composition of fresh beetroot juice with formulation of probiotic drink. Probiotic drink was found good in antioxidants, total phenols and flavonoids content. The study depicted that beetroot probiotic drink may act as good alternative to serve lactose intolerant and the persons unable to consume probiotic dairy products due to allergic reactions[23]. Though lot of further research may be conducted regarding sensory improvement, storage stability and commercialization of probiotic drink.

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