



Short paper

## Development of synbiotic beverage from beetroot juice using beneficial probiotic *Lactobacillus Casei* 431

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**Abstract.** A beetroot beverage with *Lactobacillus casei* 431 as the probiotic microorganism was tested for sensory acceptability at 3 fermentation periods of 2, 4 and 6 hours at 37°C. The beverage fermented for 2 hours yielded the highest overall sensory acceptability. *L. casei* 431 grew well and reached nearly 10<sup>8</sup> CFU/ mL after 2 hours of fermentation at 37°C. Although the lactic culture in the fermented beetroot beverage gradually lost its viability during cold storage, viable cell count of lactic acid bacteria remained at 10<sup>6</sup>–10<sup>8</sup> CFU/mL after 4 weeks of cold storage at 4°C. Titratable acidity increased significantly from 5.5±0.05 to 3.45×10<sup>8</sup> (P≤ 0.05) during storage. This study concludes that this beetroot-based synbiotic fermented beverage could be developed as a ready-to-drink product and kept for 6 weeks under refrigerated storage, meeting the standard 10<sup>8</sup>–10<sup>10</sup> CFU/mL of a functional drink.

**Keywords.** Functional food; *Lactobacillus casei*; prebiotics; probiotics; synbiotic beverage.

### 1 Introduction

Interest in functional foods has recently increased among consumers due to an increasing consciousness towards health and nutrition, as well as the need to prevent rather than cure diseases and also the increasing scientific evidence of their effectiveness. The concept of functional foods originated in Japan and are defined as being similar in appearance to conventional foods and used as

part of a normal diet, but demonstrating nutritional functions beyond those considered basic, physiological benefits or reducing the chronic risk of disease: these are known as 'Food for Specified Health Use' (Hasler, 1998). Hence, food products containing probiotics and prebiotics are considered as functional food. Much research has focused on evaluating the addition of probiotics and prebiotics to obtain synbiotic food with improved final quality.

Prebiotic oligosaccharides are Non-Digestible Oligosaccharides (NDO) and low calorific compounds stimulating the growth and development of gastro-intestinal microflora described as probiotic bacteria. Dietary carbohydrates that show prebiotic ability include fructans-fructooligosaccharides (FOS) and inulin, galactooligosaccharides (GOS), polydextrose, resistant starch, soyoligosaccharides, xylooligosaccharides, isomaltooligosaccharides, and lactulose (Patel and Goyal, 2012).

Probiotics are defined as selected, viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human organism through their effects in the intestinal tract. Some selected strains of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus* and *Saccharomyces* have been promoted in food products because of their reputed health benefits (Sharma *et al.*, 2012). Various researchers have discovered the use of different fruits, vegetables and cereals for producing synbiotic beverages in different countries of the world (Manzon *et al.*, 2012; Gokavi *et al.*, 2005).

The beetroot (*Beta vulgaris*), apart from consumption in its fresh form, is also a valuable vegetable used in the food industry to produce dried and frozen food, non-concentrated and concentrated juices as well as natural colorants (betalains) which are used as additives in food manufacturing. In many countries there is a growing interest in foods preserved in natural ways. Lactic fermentation is one of the methods of natural preservation and thus production of foods with the highest nutritive value. Based on the above information the present study was aimed to evaluate the production of a synbiotic beverage using beetroot juice fermented by *Lactobacillus casei*.

## 2 Materials and Methods

This research was carried out in the Food Processing Laboratory of the Department of Food Science and Technology, Wayamba University of Sri Lanka. Beetroot variety 'Crimson Globe' and other ingredients were purchased from a local store at Pannala. After being washed, the beetroots were peeled and cut in to small cubes with an approximate thickness of 0.5 cm. The juice was prepared using a juice extractor (PANASONIC 800W-

Osaka, Japan). Coarse particle content was separated gravimetrically by centrifuging beetroot juice at 1500 x g (model 2-16 K-Braunschweig, Germany) for 20 minutes. Juice was electrically heated at a moderate temperature of 50°C and grounded sucrose (40 g/ L) was added. Beetroot beverage was pasteurized at 90°C for 1 minute. At 43°C, a commercial frozen probiotic culture, *L. casei* 431 (CHR-HANSON - Denmark) (0.1g/L) was added. The beetroot beverage was allowed to ferment in a thermostat (Hengzi HH-B11.600-S-II, Shanghai, China) at 37°C for 0, 2, 4 and 6 hours.

pH of the beverage samples was measured using a pH meter (OHAUS Starter 300 Portable- Parsippany, USA). Titratable acidity was determined by titrating 10 mL of sample against 1.0N NaOH in the presence of Phenolphthalein as an indicator (Sharma, 2006). The total soluble solid content was measured by using a refractometer (ATAGO- Tokyo, Japan). Determination of moisture content, ash, protein, fat, total fiber, total sugars, reducing sugars and % sucrose was performed according to A.O.A.C. (2000).

Sensory evaluation was conducted using 31 semi-trained panelists who were requested to make a score on colour, consistency, taste, odor, and overall acceptability on a 5 point hedonic scales (1 = dislike extremely, 5 = like extremely).

Viable cell counts (CFU/mL) of the inoculum were determined by the standard plate method with MRS Agar (Merck Millipore- Massachusetts, USA) medium after 48 hours of incubation at 30°C. Coliform counts were estimated using Mac Conkey Agar (Merck Millipore- Massachusetts, USA) plates incubated at 37°C. Yeast and mold were enumerated by a surface spread plate technique using Potato Dextrose Agar (HiMedia- Maharashtra, India) plates in triplicate. Samples were taken at weekly intervals to examine the effect of cold storage on probiotic cell viability in the beet juice.

Significant differences among treatments were analysed using ANOVA with the help of the SAS software version 9.4. Results were expressed as mean  $\pm$  SD. Non-parametric tests (5 point hedonic scale, Kruskal Wallis Test) were performed to determine the statistical difference of the sensory data, where appropriate. Differences at  $P < 0.05$  was considered statistically significant for all analyses.

### 3 Results and Discussion

The present study was carried out to investigate the ability of *L. casei* to survive in beetroot juice throughout the refrigerated storage of 6 weeks. The *Lactobacillus* bacteria used in this study (*L. casei* 431) is a novel bacterial

strain developed by Chr.Hansen, Denmark. The initial pH of the beetroot juice was 5.6, a closer value to the optimum pH for the *L. casei* 431 strain (Paraschiv *et al.*, 2011). However, in this study *L. casei* 431 reduced the pH of beet juice from an initial value of 5.6 to lower than 5.2 while increasing titratable acidity after a 2 hour of fermentation due to their ability to produce lactic acid prominently. In the fermentation process the rate of pH decrease is very important. The resultant low pH minimizes the influence of spoilage bacteria, particularly at the beginning of the fermentation when the substrate is rich in sugars. Table 1 illustrates the changes in pH, titratable acidity and viable count of *L. casei* 431 after 2, 4 and 6 hour fermentation periods.

Table 1: Changes in pH, titratable acidity, and viability of *L. casei* 431 in Beet root juice at different time intervals (mean±SD).

Time (h)	pH	Titratable acidity (%)	CFU/mL
0	5.5±0.05 <sup>a</sup>	0.16±0.06 <sup>a</sup>	2.45×10 <sup>7</sup>
2	5.2±0.04 <sup>a</sup>	0.19±0.11 <sup>a</sup>	2.50×10 <sup>8</sup>
4	5.0±0.02 <sup>a</sup>	0.22±0.04 <sup>a</sup>	3.05×10 <sup>8</sup>
6	4.9±0.01 <sup>a</sup>	0.23±0.23 <sup>b</sup>	3.45×10 <sup>8</sup>

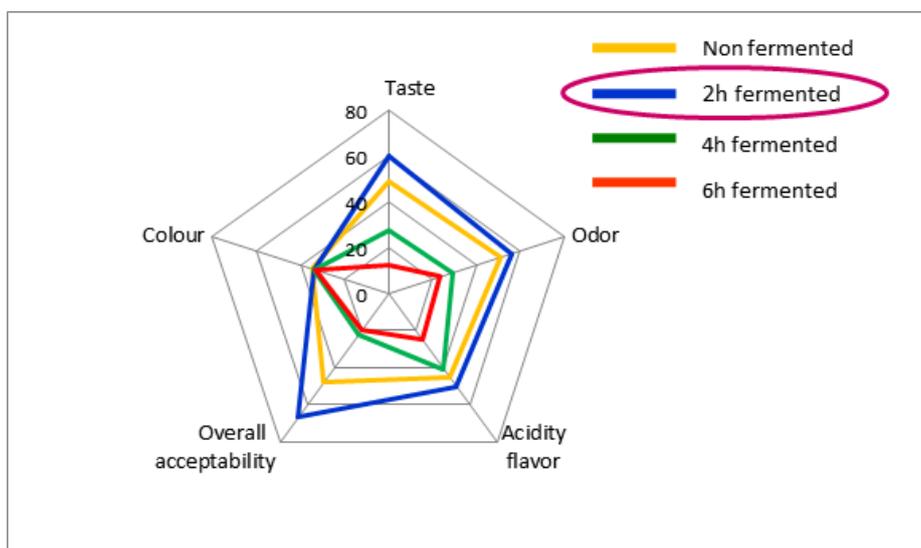
The means with different superscripts in a column differ significantly ( $P \leq 0.05$ ).

International standards stipulate that probiotic products should contain a minimum of 10<sup>8</sup> viable probiotic bacteria per mL of the product at time of consumption for health and functional claims (Samona and Robinson, 1991). However, in this study, fermented beetroot juice has reached around 10<sup>8</sup> viable probiotic bacteria per mL at the end of the 6<sup>th</sup> week. Nighswonger *et al.* (1996) revealed that there was a slight fermentative activity by the probiotic organism even at 4°C. Although the lactic acid cultures in the fermented beet juice gradually reduced their cell viability during refrigerated storage (4°C), the viable cell counts of the lactic acid bacteria in the fermented beet juice still remained at 10<sup>6</sup>–10<sup>8</sup> CFU/mL (average of 10<sup>7</sup> CFU/mL) after 4 weeks of storage at 4°C.

Some studies have revealed that several factors such as inoculum level, incubation temperature, inhibitors, and presence of hydrogen peroxide and oxygen concentration of metabolites, buffering capacity of the media, storage temperature and availability of nutrients may affect the survival of lactic acid bacterial strain (Hayek and Ibrahim, 2013). In this study, even after 6 weeks of storage at 4°C, *L. casei* was capable of surviving in the fermented beetroot juice at low pH. This pH value can still be adequate to positively affect host health. This effect was previously demonstrated for other *Lactobacillus* bacterial strains for which survival under analogous conditions was enhanced

by presence of carbohydrate present in the vegetable products (Yoon *et al.*, 2005).

Sensory evaluation results show that beetroot juice fermented for 2 hours gives the highest ranks for colour, taste, odor, consistency and overall acceptability. Mainly, the colour of this synbiotic beetroot beverage gained higher consumer acceptance. There was an unappealing odour in the beetroot juice after 6 weeks of storage in the refrigerator.



**Fig 1. Mean scores for Kruskal Wallis Test at different times of fermentation**

The amount of reducing sugar, sucrose and total sugars were significantly reduced ( $P \leq 0.05$ ) during storage and the least reduction was observed after 6 weeks of storage. Sugar consumption of *L. casei* is correlated with the lactic acid accumulation in the beverage. Therefore, sucrose addition is desirable for the purpose of maintaining of native prebiotics in the beverage. The Brix value of the beverage has slightly decreased from 5 to 4 with storage period. The reduction of total sugar content due to the fermentation process of *L. casei* is the main reason for reduction of Brix during storage.

Beetroot contains both insoluble and soluble fiber components in a desirable ratio. Mainly insoluble fibers can be considered as the prebiotics. *L. casei* is a probiotic which can metabolize above prebiotics and can live by using those prebiotics as substrates. To be a synbiotic beverage, the amount of prebiotics should be maintained until point of consumption as there are no external prebiotics introduced to the product. Therefore, the addition of

sucrose to the beetroot beverage appeared to help retain the viability of the desired prebiotics in the final product. According to data, there was no significant reduction ( $P < 0.05$ ) in the fiber content during the storage period. It is evident that prebiotics in the beverage have been preserved until the end of shelf life.

#### 4 Conclusion

Based on the results of the present study, it can be concluded that beetroot could serve as a raw material for the production of probiotic beet juice through lactic acid fermentation with *L. casei*. The fermented beet juice has a pH value less than 5.5 (high acid) and contains a significant number of beneficial lactic acid bacteria ( $10^8$  CFU/mL). Symbiotic beetroot beverage also showed sensory characteristics acceptable to health-conscious consumers.

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