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Development of finger millet based probiotic beverage using *Lactobacillus casei*431®

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Abstract

Finger millet (*Eleusine coracana*) based probiotic beverage was formulated which comprises of health benefits from both finger millet and probiotic. Cooked finger millet was inoculated with *Lactobacillus casei*431® and incubated at 37 °C for 2 h, 4 h, and 6 h. The beverage was formulated with the addition of sucrose, fresh cow milk, and cocoa powder, and stored under refrigerated (5±1°C) conditions. Sensory evaluation was conducted to select the best fermented time and the highest acceptability was achieved by the sample fermented for 4 h. Changes in physico-chemical characteristics (pH, titratable acidity, brix, and reducing/non-reducing sugars) and viable cell counts during refrigeration were monitored.

pH was decreased from 7.10 (SD 0.01) to 5.05 (SD 0.00) and titratable acidity was increased significantly ($p<0.05$) due to lactic acid production by probiotic. Reducing and non-reducing sugars were decreased significantly ($p<0.05$) because of usage by probiotics. This study concludes that finger millet based probiotic beverage can be developed with *L.casei*431® and it could be kept in refrigerated (5±1°C) storage up to 5 weeks.

Keywords: Finger millet; Functional foods; Probiotics; Health benefits

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Introduction

Probiotics are defined as live microbial that beneficially affect the hosts by improving its intestinal microbial balance when administrated in adequate amounts (Fuller, 1989). Many researches prove that addition of probiotics to foods leads to several health benefits including the reduction of the level of serum cholesterol, the improvement of gastro intestinal function, the enhancement of the immune system, the inhibition of diarrhoea in young children and the lowering of the risk of colon cancer (Berner & Donnel, 1998). Most of the probiotics are lactic acid bacteria and traditionally dairy products have been the vehicles for the delivery of probiotics. But recently, consumer demand has converted to non-diary based probiotic products such as fruits and vegetables and cereal based products because of cholesterol level, lactose intolerance which is related to diary product consumption and the increased popularity of vegetarianism. The recent investigations carried out by Gamage, Mihirani, Perera and Weerahewa (2016) and Alwis, Perera, and Weerahewa (2016) indicated that incorporation of *Lactobacillus casei* 431 in to carrot juice and beet root juice have proven to be use as an effective synbiotic beverage.

Finger millet (*Eluesine coracana*), is nutritionally an excellent source of minerals, especially Ca, K, P and Mg (Sripriya, Chandrasekharan, Murthy & Chandra,1996) that contributes to a large part of the recommended dietary allowance value. By considering richness of dietary fibres and other carbohydrates in finger millets, probabilities can be made for finger millet based probiotic product developments. However, considerable researches have not been devoted to check viability of probiotics in cereal based products including finger millet. Recently, there has been a growing interest in probiotic cereals, fruits and vegetable products.

Accordingly, the present study aimed to determine the survivability of probiotic bacteria *L.casei*431® in finger millet based beverage throughout refrigerated storage.

Methodology

Preparation of finger millet beverage

Finger millet grains were thoroughly washed and the impurities which appeared on the top of the water was discarded with the water. The grains were roasted for 3 minutes. The roasted finger millet was ground and sieved. Then 25 g of flour was cooked with 500 mL of water in a sterilized container. When the solution was boiled at 78^{o}C, it was retained for a further 10 minutes. When the solution was cooled to

40^{o}C, 0.031 gL^{-1} of *Lactobacillus casei*431® commercial frozen probiotic culture (CH-HANSON - Denmark) was added and incubated at 37^{o}C for 2, 4 and 6 hours respectively. Subsequently, 150 ml of milk was pasteurized (78 ^{o}C1 min) and 46 g/L of sugar, 7.9 g/L of cocoa powder were added to the milk and mixed well. This mixture was added to the fermented finger millet solution which was kept in the incubator previously and mixed well, filled to sterilized glass bottles and sealed.

Sensory evaluation was conducted to select the best fermentation time. Moreover, color, odor, taste, acidity, viscosity and overall acceptability were evaluated using 30 untrained panelists. Finally, the highest scored beverage was selected for the proximate analysis and refrigerated (5 ^{o}C ± 1). Physico-chemical (pH, titratable acidity, brix, total reducing sugars, total sugar, sucrose level, crude fiber content) and microbiological parameters(total viable counts of *L.casei*431® and yeast and mold count) were evaluated once a week.

Proximate composition analysis

Protein content (Kjeldhal method), crude fat content (Soxhlet method), crude fiber content, carbohydrate content and ash content were analyzed using A.O.A.C. (2000) methods.

Physico-chemical analysis of the beverage

The pH (pH meter (OHAUS- USA), acidity based on lactic acids (acid- base titration method (Sharma, 2006), total soluble solids expressed as sucrose amount refractometer (ATAGO- Japan) (Sharma, 2006) and presence of added sucrose were detected using A.O.A.C. (2000) methods.

Microbiological analysis of the beverage

Viable *Lactobacillus casei*431® bacteria was enumerated by spread plate count technique on MRS (De Man, Rogosa, Sharpe agar) (HiMedia - India). Plates were incubated as inverted position at 37 ^{o}C in an anaerobic condition using an anearogen sachet for 24 h. Spread plate count technique on Potato dextrose agar (HiMedia - India) was used to measure yeast and mold count (Collins, Lyne&Grange, 2004).Catalase test (Nelson &George, 1995), Gram staining test (Collins et al., 2004) and endospore staining test (Gerhardt, Murray, Wood & Krieg, 1994) were used as confirmation tests for *L.casei*.

Shelf life Determination

Sensory evaluations on color, odor, acidity flavor and overall acceptability were conducted once in two weeks. pH, titratable acidity, viable *L. casei* and yeast and mold counts were examined to evaluate the shelf life of probiotic finger millet beverage.

Statistical analysis

Non-parametric tests were performed to determine the statistical difference of the sensory data, and where appropriate, T-tests were performed for comparison of two means. Significant differences between the results were assessed by analysis of variance (ANOVA) with the help of SPSS software. The results of proximate analysis were the average of triplicate experiments and were expressed as mean ± SD. Differences at 0<0.05 was considered statistically significant for all analyses.

Results

Sensory evaluation for best fermentation time selection

Table 1 & Table 2: Kruskal Wallis test for five point hedonic scale tests for the treatments

Ranks

	treatment	N	Mean Rank
scale	2	180	292.90
	4	180	241.99
	6	180	276.61
	Total	540	

Test Statistics[a,b]

	scale
Chi-Square	11.982
df	2
Significant (P) value	.003

a. Kruskal Wallis Test

b. Grouping Variable: treatment (hours)

According to the results of Kruskal-Wallis test, overall the lowest mean score was recorded by the 4 h fermented sample while the highest mean score was recorded by 2 h fermented sample. According to the ranks given in the scale, the lowest mean value of ranks should be obtained by the best sample and it was shown by 4 h fermented sample which was selected as the best time of fermentation.

Proximate composition analysis

Table 3: Proximate composition of the product

Component	Percentage
Moisture	89.53±0.11
Total Solids	10.47±0.11
Carbohydrate	8.27±0.14
Protein	1.80±0.07
Crude fat	0.08±0.03
Crude fiber	0.91±0.13
Ash	0.55±0.12

Table 3 shows the proximate composition of the final product. Because of being a beverage, it has a higher moisture content. It was recorded as 89.53 g per 100 g of the beverage. The total solids of the beverage were recorded as 10.47 g per 100 g of the beverage. This amount was low because of the high moisture content of the beverage. Moreover total carbohydrate, protein, crude fat, crude fiber and ash content of the product were 8.27 g, 1.80 g, 0.08 g, 0.91 g and 0.55 g per 100 g of the beverage respectively.

Physico-chemical analysis of the beverage

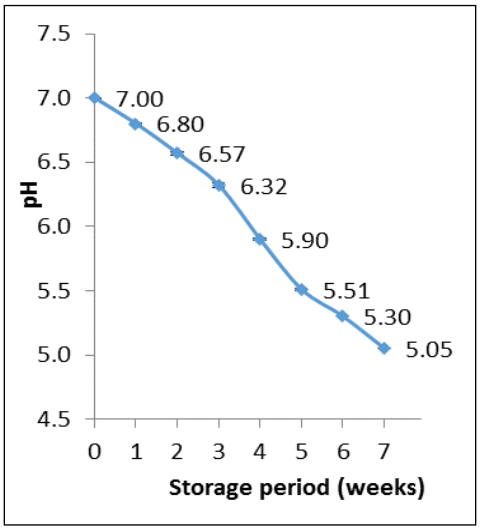

Figure 1: pH value changes of the beverage during storage period

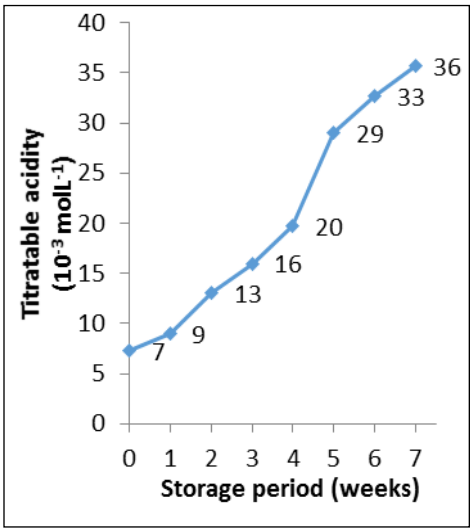

Figure2: Changes of titratable acidity of beverage during storage period

Changes in lactic acid concentration produced by probiotic bacteria leads to the changes in titratable acidity of the beverage. It was significantly ($p \leq 0.05$) increased during storage period of 7 weeks (Fig.2).

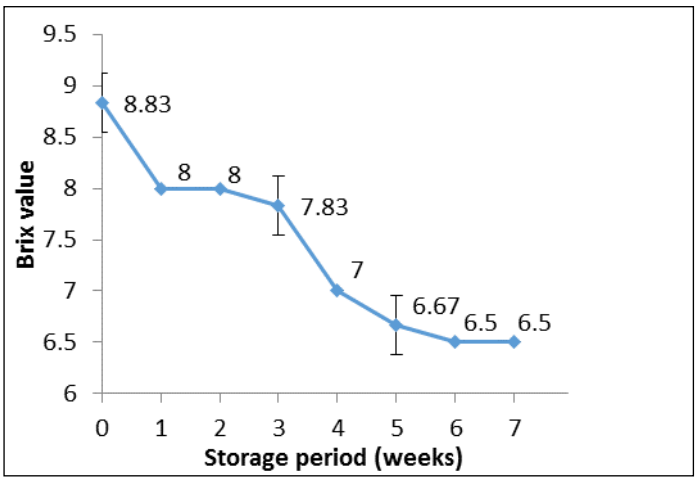

Figure 3: Brix value changes of the beverage during storage

Brix value was significantly reduced with a high reduction rate in 1st and 4th week. After 6th week brix value became a constant (Fig.3).

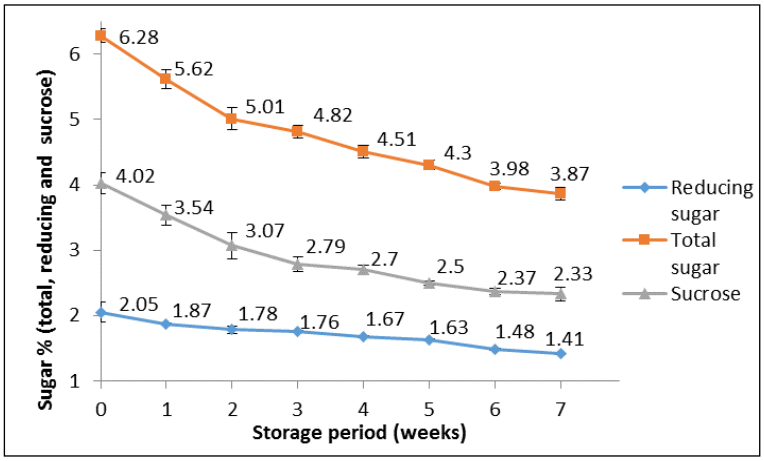

Figure 4: Changes of percentage of total sugars, reducing sugars and sucrose of the beverage during storage period

Percentage of total sugars (reducing and non-reducing sugars), reducing sugars and sucrose as non-reducing sugar showed high reduction rates. Although there was a significant ($p \leq 0.05$) reduction in percentage of all sugars, the reduction rate was lowered after 2nd week (Fig.4).

There was no significant ($p > 0.05$) reduction in the crude fiber content of the beverage from initial to 3rd week. At the initial stage, the mean value of crude fiber was recorded as 0.91% (from the wet basis of the beverage). At the 1st,2nd and 3rdweek, amount of crude fibers was recorded as 0.91%, 0.908% and 0.905% respectively.

Microbiological analysis of the beverage

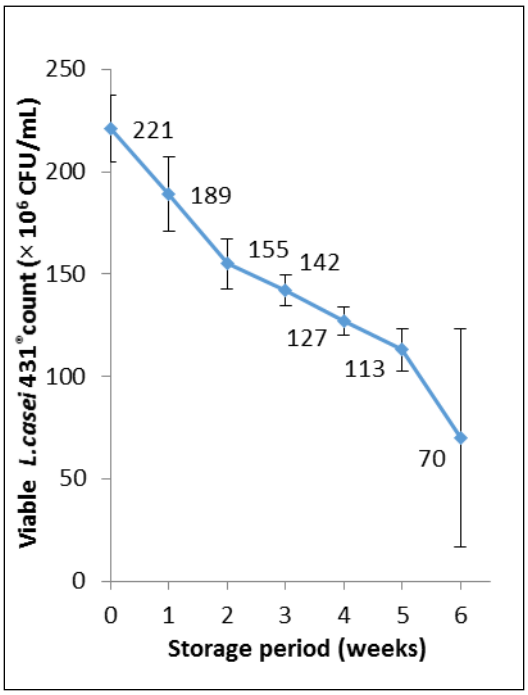

Figure 5: Changes of viability of L.casei431® in the beverage during storage period

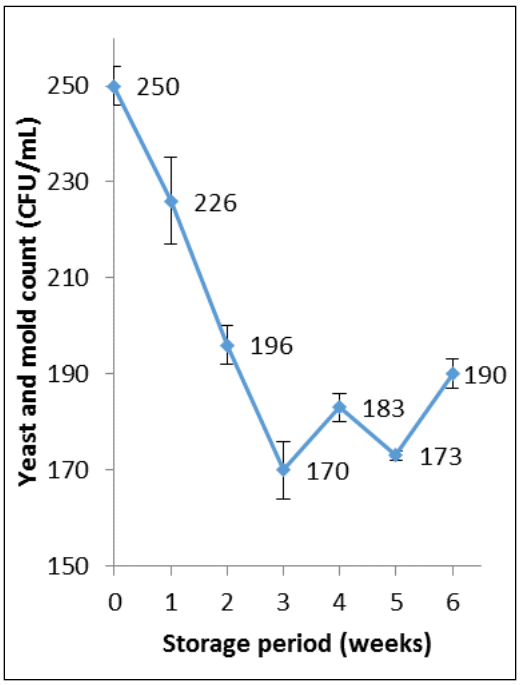

Figure 6: The count of yeast and mold in the beverage during storage period

After fermentation there was 2.21×10^8 CFU mL^{-1} of *L.casei*431® presented in the beverage (Fig.5). The reduction of viable cells of the beverage at refrigeration (5 ± 1 °C) was significant ($p < 0.05$) but the reduction rate was very low in the middle of the storage while the rate was high at the initial and end of the storage (Fig. 5). Finally,

at the 6th week viable count was recorded as 7.0×10^7 CFU mL^{-1}.The viability of *L.casei*431® in this beverage was in an appreciable level under refrigerated condition (5 ± 1°C) for 5 weeks but subsequently, the count was exceedingly reduced. According to this result, the finger millet beverage can be considered as a functional beverage up to 5 weeks under refrigerated condition (5 ± 1°C).

The count of yeast and mold did not show a gradual increment or decrement throughout the storage period. Until the 3rd week, the count was decreased but after that, there was no any order in count change. Initially the count was 250 CFU/mL (Fig.6) comparatively a higher value for a beverage.

Shelf life evaluation

Titratable acidity was increased and pH was reduced at the end of the storage period of 7 weeks. But it was remained in tolerable acidity and pH level to the viable L.casei431®bacteria. There were no changes in sensory attributes until 5thweek. Therefore, this beverage could serve as a ready to drink functional beverage under refrigerated (5 ^oC ± 1) storage up to 5 weeks.

Discussion

With regard to the objectives of this study, *Lactobacillus casei*431®, was found to be growing well on cooked finger millet. Fermentation period of 4h at 37 ^oC, *L. casei*431® showed a rapid significant growth and it reached nearly 2.0×10^8 CFU/mL. The viability of the probiotic was significantly reduced during the storage period of 6 weeks but until that viability was appreciable. Viable count at 6th week still stood at nearly 7.0×10^7 CFU/mL (Table 4).

Table 4: Changes of viability of L.casei431® of the beverage during refrigerated storage

Week	Viable *L.casei431®* ($\times10^6$ CFU/mL)
0	221
1	189
2	1555
3	142
4	127
5	113
6	70

For the maximum health benefits, the minimum number of probiotic organism in a food product should be 10^6 CFU/mL and to be considered as a functional product the count should be 10^8 CFU/mL (Shah, 2001). According to Table 4, at the 0to 5th week, this beverage enumerated the functional level.

There are several reasons for the reduction of viability of probiotics e.g. lack of essential nutrients (minerals, peptides) for *L.casei*431®, antibacterial substances present in the finger millet, level of oxygen in products, oxygen permeation of the package, fermentation time, and storage temperature (Shah, 2001). Viability also depends on lactic acid concentration. Titratable acidity increases, and the accumulation of lactic acid, leads to pH reduction. After tolerable acidity and pH level, lactic acid acts as an inhibitory substance for probiotics. In this study, during the storage period of 5 weeks, pH was significantly reduced. Although it was a significant reduction, reduction was 7 to 5.05. At the initial to 7th week, pH was in tolerable level for probiotics (7-4.5)(Shah, 2007). Consequently titratable acidity showed a significant increment from 0 to 4th week as 0.9% to 2.0% but that was in tolerable level for the probiotics (0.3-1.9%) (Shah, 2007). Therefore, it can be understood that there was a better survivability of *L.casei*431® in this finger millet based beverage.

Addition of probiotics changes the organoleptic properties of the beverage by fermentation. Fermentation is a metabolic process of deriving energy from organic compounds without involvement of any exogenous oxidizing agent (Bourdichon, 2012). *L.casei* 431® is a homo fermentative bacteria and by fermentation of carbohydrates and prebiotics it only produces lactic acid. The time or extent of fermentation and the temperature affects sensory attributes. In this study, solution was incubated at 37 ^oC which was the optimal temperature for *L.casei*431®(Shah, 2001). Consequently 4 h fermentation was enough to maintain an overall sensory quality and functional level of the beverage which was proven by the results obtained from sensory evaluations.

USDA has proclaimed that 100 CFU/ mL as the maximum yeast and mold level for ready to serve beverages. But in this beverage, more than that amount was recorded. Literature reveals that there are native yeasts and mold present in the raw finger millets (Mousa, et al., 2015). Although finger millet was boiled, spores of yeasts and molds remained in the beverage. Further yeast and mold can survive in a wide range of temperature and pH values. This yeast and mold might be helpful for the fermentation process. But undesirable yeast and mold can be contaminated

during the preparation of beverage, especially at the filling step. Even though the product was pasteurized, hot filling was not practiced because probiotic culture should not be added at high temperatures. If bottles were filled under sterile environment, this problem could have been overcome.

Reduction of total sugar content is the main reason for the reduction of the brix value because of fermentation by *L.casei*431®. This was further proved by the results of sugar levels including total sugars, reducing sugars and sucrose as non-reducing sugars, during the storage period. All three sugar levels were significantly reduced when beverage was at the 7thweek.This supports and adds to the findings of Nighswonger, Brashears and Gilliland (1996) that fermentation of the beverage has been limited by keeping the beverage in the refrigerator (5 ± 1 °C).

Conclusion

Based on the obtained results of this study, it can be concluded there was a better survivability of probiotic *Lactobacillus casei* 431® in finger millet based beverage. The count of probiotic organism was within the level of standards (10^8 CFU/ mL) until the end of 5 weeks. Finally, this research has revealed that sensory acceptable finger millet based fermented probiotic beverage can be developed using *L.casei431®* by inoculating 0.031 g L^{-1} inoculum of frozen *Lactobacillus casei* 431®and could serve as a ready to drink functional beverage in refrigerated (5 ± 1 °C) storage up to 5 weeks.

Acknowledgement

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