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PROBIOTICATION OF FINGER MILLET (*ELEUSINE CORACANA* L.) PORRIDGE FOR ENHANCING ITS FUNCTION AND SAFETY

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Abstract

Finger millet porridge, a traditional lactic acid fermented drink prepared from the minor millet *Eleusine coracana* (L.) has many nutritional and health benefits. In this study, the finger millet porridge was prepared, and probioticated with free and encapsulated cells of the selected probiotic cultures *viz*., *Lactobacillus acidophilus* NCDC 14 and *Lactobacillus casei* NCDC 298 to increase its functionality. The probiotic load of the porridge was 10^{10} cfu/g and based on a 9-point hedonic scale sensory evaluation, a maximum score was obtained for 8 hrs of fermentation time (8.9) irrespective of the treatments. In this study, it was observed that phytic acid decreased whereas the bioavailability of minerals *viz.,* calcium, iron and zinc and reducing sugars were increased. The probiotic cultures were viable (10^9 cfu/g) in the fermented finger millet porridge during 7 days of refrigerated storage with more viability in alginate-encapsulated cells.

Introduction

Finger millet (*Eleusine coracana* (L.)) is one of the common millets in several regions of India. It has traditionally been an important millet staple food in parts of eastern and central Africa and India. Finger millet is a fantastic substitute for persons with celiac disease. Previous research has demonstrated the hypoglycemic, nephroprotective, anti-cataractogenic, antiulcerative, antioxidant, antibacterial, and wound-healing activities of finger millet (Chandra *et al*. 2016). Finger millet are rich in macronutrients, micronutrients, phytochemicals, and possess certain antinutrients to maintain good health and manage diet-related conditions like hypercholesterolemia and protein energy malnutrition.(Olagunju *et al*. 2022).

Finger millet porridge is a naturally fermented food and its microbial succession by lactic acid bacteria develops complex flavors in the final product, giving it a distinctive fermented flavor. Hence it is considered as nutritious and health-promoting drink, but there is little scientific documentation on its nutritive or microbial composition or its probiotic effect (Ilango and Antony 2014). Production of traditional fermented millet-based beverages on a commercial scale is limited and is carried out only at the household level. Now there is considerable interest in extending the range of foods containing probiotic organisms from dairy foods to non-dairy foods especially cereal/millet-based products.

Materials and Methods

The traditional method was followed to prepare the Finger millet porridge. Millet flour was mixed with water (1:10 ratio) to create a slurry, and it was then allowed to ferment for 4, 8, 12 and 16 hrs respectively. The fermented Finger millet mixture was cooked in excess water into a thick porridge and swirled with salt and buttermilk to make it a thin porridge. The prepared porridge was subjected to sensory evaluation and found that 8 hrs of fermentation had better sensory scores.

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In this study, *Lactobacillus acidophilus* NCDC 14 and *Lactobacillus casei* NCDC 298 purchased from the National Dairy Research Institute, Karnal, Haryana, India were taken. The cultures were inoculated in deMan Rogosa Sharpe (MRS) broth and incubated at 37 ± 2°C for 12-16 hrs. The supernatant was disposed of after being centrifuged for 10 min at 3000 rpm. The cell pellets were washed thrice with sterile distilled water by centrifuging for five minutes at 3000 rpm and the cell pellets were suspended in 1ml of sterile water for use as inoculum.

To increase the viability of probiotic strains in porridge, the cultures were encapsulated in sodium alginate polymer. Sodium alginate @ 1 % concentration was used for encapsulation of probiotic cells by using 0.1M calcium chloride as substrate/hardening solution for encapsulation. Probiotic strains were inoculated into 100 mL of broth and incubated at 37 °C for 24-36 hrs and the cells were harvested by centrifugation at 3000 rpm for 10 min and were washed twice with sterile distilled water. Bacterial cultures were suspended in 1 mL of sterile distilled water and mixed with 50 mL of 1 % (w/v) sodium alginate solution which had been sterilized at 121°C for 15 min. The two probiotic cultures were extruded through a syringe needle as droplets into a hardening solution *viz.*, 0.1M calcium chloride and the beads were retained in the calcium chloride solution for 30min then rinsed with sterile distilled water and stored at 4°C (Fig 1). The cross-section of encapsulated cells in alginate beads was stained and observed under a Light Microscope under 40x magnification as reported by Giordano *et al.* (2023) (Fig 1). Freshly prepared alginate microbeads with encapsulated probiotics (1.0 g) were broken down in 9 mL of phosphate buffer solution at pH 7.0 using a sterile pestle & mortar. Dilutions up to 10^8 were made in sterile water blanks and were plated on MRS agar. Probiotic LAB colonies were enumerated following incubation at 37°C for 24 to 36 hrs.

Fig. 1. Encapsulation of probiotics in alginate beads and its microscopic image under 40x magnification.

Based on sensory evaluation, Finger millet porridge was prepared at a fermentation time of 8 hrs and stored in a bottle at 4°C. Just before bottling, the thoroughly washed probiotic cell pellets suspended in 1 millilitre of sterile water were added to the Finger millet porridge, and one gram of probiotic encapsulated beads was added as per the treatments given in Table 1 and Fig. 2. The changes in chemical composition, microbial load, viability of probiotic cultures and organoleptic characteristics were assessed on 0 day and 7th day during refrigerated storage. The chemical parameters such as protein by Micro kjeldhal method, total sugars, fat, crude fiber, titratable acidity, pH, phytic acid (Ranganna 1979, AOAC 1995, Sadasivam and Manickam 1996, AACC 2000) and minerals such as calcium, iron, zinc by Atomic Absorption Spectrophotometry method. The microbial quality analysis *viz.*, viability of probiotics and product safety by assessing coliform count in Finger millet porridge was performed.

Table 1. Treatment details of probioticated Finger millet porridge.

Treatments	Details
T1	Plain Finger millet porridge (FMP)
T2	Finger millet porridge probioticated with free cells of *Lactobacillus acidophilus* NCDC 14 (FMP-LA)
T3	Finger millet porridge probioticated with free cells of *Lactobacillus casei* NCDC 298 (FMP-LC)
T4	Finger millet porridge probioticated with encapsulated cells of *Lactobacillus acidophilus* NCDC 14 (FMP-LA-E)
T5	Finger millet porridge probioticated with encapsulated cells of *Lactobacillus casei* NCDC 298 (FMP-LC-E)

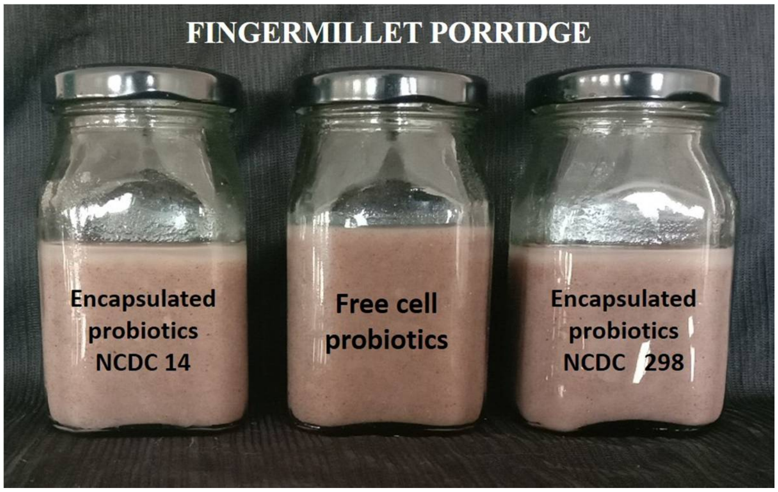

Fig. 2. Bottled probioticated Finger millet porridge.

Results and Discussion

In this study, the acceptable fermentation time for the preparation of Finger millet porridge was standardized at 4 different time intervals 4, 8, 12 and 16 hrs. Based on a 9-point hedonic scale sensory evaluation, the maximum score for the overall acceptability of the product obtained for 8h fermentation time was 8.9 (data not shown). After standardization of fermentation time, the cell pellets (1 ml) of probiotic strains were added just before bottling @ 81 x 10^9 cfu/ml for *L. acidophilus* and 32 x 10^9 cfu/ml for *L. casei* respectively. Whereas, 1g of probiotic alginate beads were added @ 11 x 10^8 cfu/ml for *L. acidophilus* and 9 x 10^8 cfu/ml for *L. casei*, respectively It was then stored at 4°C for 7 days. Hence, the shelf life of the developed product during refrigerated conditions was evaluated for 7 days by estimating chemical, microbial and sensory parameters.

Table 2. Determination of chemical parameters of plain and probioticated Finger millet porridge.

Sl. No	Treatments	0 days of storage						7 days of storage					
		Lactic acid (%)	pH	Protein (%)	Fat (%)	Fibre %	Total sugars (g/100g)	% lactic acid	pH	Protein (%)	Fat (%)	Fibre (%)	Total sugars (g/100g)
1.	T_1-FMP	0.98±0.15	4.40±0.10	5.07±0.02	6.7±0.52	1.7±0.05	2.23±0.22	1.09±0.11	4.15±0.09	5.25±0.05	4.8±0.11	2.2±0.29	2.17±0.15
2.	T_2–FMP-LA	0.97±0.25	4.38±0.19	4.90±0.07	5.8±0.23	2.0±0.02	2.11±0.29	1.25±0.05	3.95±0.05	5.25±0.09	3.0±0.39	2.1±0.23	2.04±0.15
3.	T_3–FMP-LC	0.92±0.11	4.39±0.25	4.11±0.12	5.7±0.45	2.1±0.14	2.10±0.45	1.59±0.09	3.80±0.12	4.90±0.07	4.5±0.59	2.0±0.15	2.02±0.15
4.	T_4–FMP-LA-E	0.99±0.10	4.29±0.22	4.46±0.22	5.0±0.33	1.7±0.22	2.25±0.21	1.12±0.19	4.03±0.10	4.28±0.10	4.6±0.11	1.8±0.15	2.03±0.15
5.	T_5–FMP-LC-E	0.91±0.21	4.32±0.11	4.28±0.32	5.4±0.12	2.1±0.12	2.23±0.11	1.07±0.21	4.06±0.17	4.37±0.22	3.1±0.25	2.2±0.15	2.04±0.15

Table 3. Determination of phytic acid, minerals and probiotic count of plain and probioticated Finger millet porridge during storage.

Sl. No	Treatments	0 days of storage (mg/100g)					7 days of storage (mg/100g)				
		Phytate P	Calcium	Iron	Zinc	Probiotic count (log 9 cfu/ml)	Phytate P	Calcium	Iron	Zinc	Probiotic count (log 8 cfu/ml)
1.	T_1-FMP	125±0.21	240±0.09	2.54±0.12	0.70±0.09	0.32	115±0.02	250±0.15	2.59±0.06	0.76±0.12	0.00
2.	T_2–FMP-LA	135±0.15	250±0.02	2.37±0.18	0.65±0.11	1.13	128±0.29	300±0.11	2.45±0.09	0.74±0.15	0.33
3.	T_3–FMP-LC	140±0.12	280±0.05	2.30±0.23	0.42±0.15	1.41	135±0.10	320±0.15	2.44±0.13	0.53±0.09	0.00
4.	T_4–FMP-LA-E	110±0.11	260±0.11	2.48±0.21	0.62±0.22	0.82	105±0.11	310±0.24	2.52±0.19	0.71±0.16	0.67
5.	T_5–FMP-LC-E	132±0.20	290±0.16	2.01±0.19	0.58±0.29	0.73	128±0.21	320±0.18	2.20±0.20	0.69±0.22	0.53

The results of the chemical parameters of the porridge are given in Table 2. The lactic acid % ranged from 0.91 ± 0.21 to 0.99 ± 0.10 and the pH values ranged from 4.29 ± 0.22 to 4.40±0.10. Among the treatments, there was an increase in lactic acid % (1.59 ± 0.09) and a decrease in pH values (3.80 ± 0.12) in free cell-added finger millet porridge which might be due to the addition and action of probiotic cell-pellets. However, there was a very slight change in lactic acid and pH values in probiotic-encapsulated porridge. Geetha and Gurumurthy (2013) reported pH and titratable acidity of fermented finger millet flour alone were 6.0 ± 0.09 and 0.5 ± 0.15%, respectively on 5 hrs and 5.1 ± 0.05 and 1.8 ± 0.11%, respectively after 15 hrs of fermentation. There is an increase in protein content during storage whereas total sugars decreased during 7 days of storage which might be due to the fermentation by lactic acid bacteria (Table 2). The higher values of fat may be due to the addition of buttermilk which is in the range reported by Saleh *et al.* (2013). The crude fibre content in finger millet porridge ranged from 1.7 ± 0.05 to 2.1 ± 0.12%. The fermented Finger millet beverage showed an increase in protein from 7.6 to 10.5% and some free aminoacids and vitamins. Significant amounts of bioavailable minerals *viz.,* Calcium, phosphorus and iron in the fermented Finger millet beverage were also found (Basappa *et al.* 1997). The phytic acid decreased from 110-140 (0 days of storage) to 105- 135 (7 days of storage). The bioavailability of minerals *viz*., calcium, iron and zinc was found to be increased (Table 3) and values are within the range reported by previous researchers (Nakarani *et al.* 2021).

Gabaza *et al.* (2017) reported that compared to the raw finger millet grains, antinutritional factors *viz*., phenolic compounds and tannins were reduced by up to 41% and 35% respectively in fermented porridge, while phytic acid was reduced by 22 - 54%. Iron and zinc bioaccessibility was 6 and 13%, respectively, in the porridges. Antony *et al.* (1998) reported that fermentation of finger millet flour using endogenous grain microflora showed a significant reduction of phytates by 20% and tannins by 52% and trypsin inhibitor activity by 32% at the end of 24 hrs. There was a simultaneous increase in mineral availability (Calcium-20%, Phosphorous-26%, Iron-27% and Zinc-26%).

The microbiological quality of the fermented plain and probioticated Fingermillet porridge was evaluated and the results are elaborated in Table 3. The viability of probiotic cultures added was found by plating in MRS agar. The cell load was in the range of 0.32 to 1.41 cfu/ml in 10^9 dilutions on 0 days of storage, whereas the microbial load reduced during storage in free cell-added porridge but there the population was maintained in encapsulated probiotic porridge (0.53-0.67 cfu/ml). It confirmed that encapsulation protects the bacterial cells thereby increasing their viability. Giordano *et al.* (2023) reported that microcapsules of alginate and chitosan decreased the acidification of *Lacticaseibacillus casei.* The coliform count in Violet Red Bile Agar was absent throughout the storage period which may be due to hygienic handling. Ilango and Antony (2014) also reported that in porridge preparations, LAB was found to be dominant and yeast-mould counts were comparatively lower.

Nine-point hedonic scale was followed for conducting the sensory evaluation of plain and probiotic Finger millet porridge. The change in the quality attributes directly influenced the organoleptic evaluation scores (Fig. 3). The changes noted in each quality attribute of the beverages influenced the overall acceptability score values. Thereafter, the score gradually decreased during storage. After 7 days of refrigerated storage, encapsulated Finger millet porridge after 8 hrs of fermentation recorded a maximum score of 8.1 followed by plain Finger millet porridge whose value was 7.9. Free cell added Finger millet porridge recorded a comparatively lesser score (6.9) due to increased acidity.

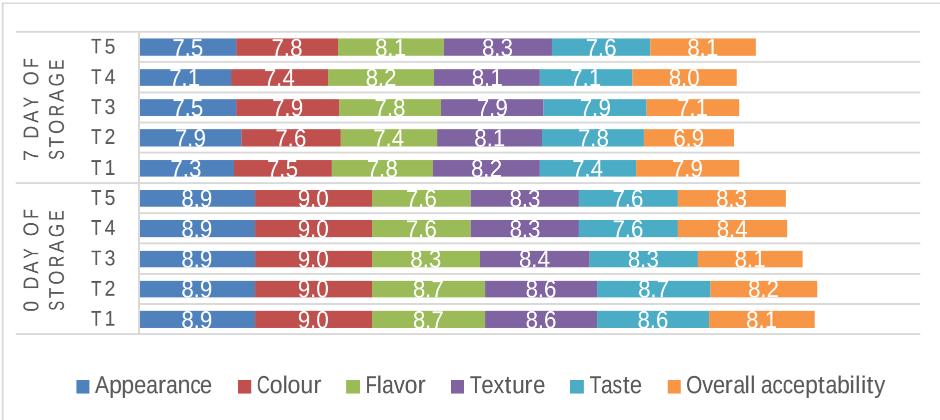

Fig. 3. Sensory attributes of plain and probioticated Finger millet porridge treatments during storage.

This study showed that the nutritional quality, function and safety of finger millet porridge can be improved by adding probiotic lactic acid bacteria (*Lactobacillus acidophilus* NCDC 14 and *Lactobacillus casei* NCDC 298). The product can be commercialized by encapsulating the probiotic cells with no change in sensory attributes.

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