Indian J Anim Health (2022), 61(2)- Special Issue: 226-238
DOI: https://doi.org/10.36062/ijah.2022.spl.03222

Research Article

Evaluation of prebiotic potential of nutri-cereals using different probiotic strains

M. Anupama[1], H. C. Devaraja[1*] and K. Jayaraj Rao[1]

[1]Dairy Technology Section, ICAR-National Dairy Research Institute (SRS), Adugodi, Bengaluru-560 030, Karnataka, India

Abstract

Nutri-cereals are excellent sources of soluble fibres, which are expected to influence the growth of probiotics. In the current study, selected nutri-cereals viz. finger millet and sorghum were evaluated for its prebiotic potential on probiotic *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *Lactobacillus acidophilus* strains. The sample without added nutri-cereals served as control. Toned milk incorporated separately with malted finger millet and sorghum at the rate of 3% (w/v) was inoculated with selected probiotic strains at the rate of 3% (w/v) and incubated at 37°C till pH reached to about 4.6±0.2 and, analyzed for acidification rate and probiotic count. The results revealed that the milk incorporated with 24 h germinated finger millet and sorghum flour recorded highest probiotic count compared to non-germinated and 48 h germinated cereal flours. Among selected nutri-cereals, sorghum malt had better prebiotic potential compared to finger millet malt.

Key words: Finger millet, Nutri-cereals, Prebiotics, Probiotics, Sorghum

Highlights

- Malted nutri-cereals exhibited excellent prebiotic potential.
- Among sorghum and finger millet, sorghum exhibited better prebiotic potential.
- Sorghum germinated for 24 h recorded highest probiotic count (12.13 log CFU/mL).
- The culture with mixed strains viz. *L. plantarum* and *L. casei* gave highest probiotic count.

INTRODUCTION

Milk and its fermented products have gained widespread recognition for their nutritional and functional qualities and are now a part of people's daily diets across all age groups. One of the most popular categories of functional foods is fermented dairy products containing probiotic bacteria, and the market for these products is growing every year (Kerry *et al*., 2018) almost by 8.3%. Live microorganisms have been shown to have positive effects on human health in four main areas: gastrointestinal diseases, allergies, infections, and metabolism. Numerous probiotic *Lactobacillus* spp. strains have been linked to immunomodulation and increased pathogen resistance. According to Ma *et al*. (2015), *Lactobacillus casei* is effective in preventing cardiovascular diseases, inhibiting intestinal pathogens, and controlling the immune system. Kort *et al*. (2015) linked *Lactobacillus rhamnosus* with a low incidence of rotavirus-associated diarrhoea and low serum cholesterol levels. Bioactive components are found in abundance in fermented food products, which also have medicinal, antibacterial, antioxidant, probiotic, organoleptic, and cholesterol-lowering properties (Adebiyi *et al*., 2018; Adebo *et al*., 2020). The probiotic fermented milk products can be enriched with nutri-cereals containing prebiotic compounds, which help in growth and maintenance of probiotics. Dietary fibre of nutri-cereals also to some extent fortify the fermented milk products because milk lacks

*Corresponding Author, E mail: dev.rajdsc@gmail.com

in fibre content. India is among the leading producers of nutri-cereals and millets market is set to grow from its current market value of more than $9 billion to over $12 billion by 2025 (Ministry of Agriculture and Farmers Welfare, 2022).

Millets are rich in dietary fibre (both soluble and insoluble) content ranging from 9 to 15% which are effective in management of metabolic disorders like diabetes mellitus, hyperlipidaemia, low glycemic index etc. (Ambati and Sucharitha, 2019). Among the millets, finger millets are of significance because of their high nutritional and dietary fibre contents (15-20%). Sorghum (*Sorghum bicolor*) is another drought-resistant cereal crop which is the fifth most significant cereal produced worldwide and it is the third most widely farmed cereal grain in the African continent (Mabhaudhi *et al*., 2016; Sobowale *et al.*, 2019; Adebo, 2020). The production of sorghum in India was about 8.71 million tonnes in 2021. Sorghum continues to be one of the most significant cereal crops for several human uses (Adebo, 2018; FSSAI, 2020).

A natural and innovative technique to enhance the nutritional and functional qualities of fermented milk can be through combining milk with nutri-cereals, viz. finger millet and sorghum and fermenting the resultant composite mix using suitable probiotic strains. Reduction of anti-nutritional factors due to biochemical processes such as starch hydrolysis and sugar transformation can occur in nutri-cereals during fermentation, resulting in softening of the product. Lactic acid fermentation improves the availability of minerals, increases protein digestibility, and decreases the amount of phytic acid and tannins (Bhandari *et al.*, 2018). According to Palaniappan *et al*. (2017) and Kristek *et al.* (2018), the finger millet's xylobiose and xylooligosaccharides aid in the growth of *Lactobacillus* species. Similar observations were made with sorghum by Majumdar *et al.* (2006). The robust growth of LAB in finger millet and sorghum substrate indicates that this cereal, either by itself or in conjunction with milk, can be an effective probiotic delivery system. The milk-millet fermented product may have health-promoting characteristics by combining the probiotic and prebiotic concepts, with finger millet and sorghum being suitable prebiotic sources. Study of the growth and activity of various probiotic strains in milk in the presence of malted sorghum and finger millet, as well as its impact on the overall fermentation of milk-cereal mix becomes crucial in the development of composite foods. Additionally, there are very few published research papers on the development of probiotic composite dairy products made using milk-millet matrix. The current study aims to evaluate the prebiotic potential of malted finger millet and sorghum on probiotic *L. plantarum*, *L. casei*, and *L. acidophilus* strains.

MATERIALS AND METHODS

Toned milk (3.0% milk fat, 8.5% MSNF) was procured from ICAR-NDRI, SRS campus, Bengaluru, India. Finger millet (dark coloured) and sorghum (white coloured) grains of AGMARK (Grade I) were procured from the local supermarket in Adugodi, Bengaluru, India. The probiotic strains of *Lactiplantibacillus plantarum*, *Lacticasei bacillus casei*, and *Lactobacillus acidophilus* were procured from the National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal, India. The freeze-dried cultures were activated in the sterilized deMann Rogosa Sharpe (MRS) broth. Freeze-dried vials of the probiotic strains were broken, and all the contents were put separately in the MRS broth in tubes and kept at 37ºC for 24 h for activation. Further, sub-culturing of the probiotics was done in the sterilized MRS broth once in a week.

In vitro evaluation of the probiotic attributes of *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidophilus* strains

Acid resistance: Resistance to acidic conditions

was tested as per the method suggested by Clark *et al.* (1993) with some modifications. The culture was grown in MRS broth overnight at 37ºC, added at the rate of 1% to MRS broth, pH adjusted to 2.0 and 3.0 with 1 M HCl and same quantity was simultaneously added to MRS broth with pH 7.0 to serve as control. Survival of organisms was evaluated by spread plating on MRS agar plates after 0, 1, 2 and 3 h of incubation, respectively.

Bile tolerance: Tolerance for bile acids was tested according to the method suggested by Gilliland *et al.* (1984). The culture was grown in MRS broth overnight at 37ºC, and 1% of this broth was added to MRS broth supplemented separately with 0.2% and 0.3% (w/v) ox bile (Himedia Laboratories Pvt. Ltd, Mumbai, India). The sample without supplement was taken as control. Survival of organisms was evaluated by spread plating on MRS agar plates after 0, 1, 2 and 3 h of incubation.

Antibacterial activity: The antagonistic effects of culture supernatants of *Lactiplantibacillus plantarum* (NCDC 344) and *Lacticaseibacillus casei* (NCDC 297) on indicator organisms like *E. coli*, *Bacillus polymyxa*, *Pseudomonas fragi*, *Micrococcus luteus*, *Geobacillus stearothemophillus*, *S. aureus and B. cereus* were tested by the agar-well-diffusion assay. The culture supernatants of *Lactiplantibacillus plantarum* (NCDC 344) and *Lacticaseibacillus casei* (NCDC 297) were prepared by growing the cultures in MRS broth for 16–18 h at 37°C and removing the cells by centrifugation at 8,000xg for 5 min. For testing the antibacterial activity, one part of the supernatant was used at the final pH of the spent broth, while another part was neutralized with 1.0 N NaOH to pH 6.5 before setting the activity. All the solutions were sterilized by filtering through a 0.22 mm pore size cellulose acetate filter membrane before adding to the wells. Pre-poured MRS agar plates were overlaid with 10 mL of soft MRS agar inoculated with 0.3 mL of an overnight culture of the indicator organism grown in M17 broth at 37°C. After allowing the media to solidify at room temperature for 15 min, wells of 7 mm diameter were made with a sterile cork borer. Each well was sealed with one drop of soft agar followed by 100 mL of the cell-free supernatant. The plates were kept undisturbed for few hours so that supernatant can diffuse in the agar. Plates were kept at 37°C for 10–12 h and the diameter of the zone of inhibition was measured.

Antioxidative activity: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated as described by Chooruk *et al.* (2017) with some modifications. DPPH solutions of 0.1 mM and 0.2 mM in methanol were prepared and tested for DPPH radical scavenging efficiency of the selected probiotic strains. To evaluate the antioxidant activity of the LAB strains, 2mL of DPPH solution (0.1 and 0.2 mM) in methanol and 2 mL of bacterial samples were mixed and incubated at 37°C for 30 min in the dark. Ascorbic acid (1 mg/mL) was used as the positive control. The DPPH radical scavenging activity was calculated by measuring the absorbance of the supernatant at 517 nm using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - {}^{A\,\text{sample}}/_{A\,\text{control}}\right) \times 100\%$$

Where, A sample and A control are the absorbance values of the bacterial sample and distilled water at 30 min, respectively.

Preparation of malted finger millet and sorghum malt: Malting of finger millet and sorghum was carried out according to the procedure suggested by Chilakawar *et al.* (2015) with necessary modifications. Finger millet and sorghum grains were weighed and washed well. The grains were kept for steeping in water for 12 h at room temperature. The grains: water ratio was kept at 1:3. During steeping, the water was changed after every 4 h. It was then hung overnight at room temperature to remove excess water. Steeped grains were spread on perforated trays lined with muslin cloth and germinated at 25±2ºC for 24 h in an incubator. The seeds were then spread

uniformly on a stainless-steel tray and dried in a vacuum tray dryer maintained at 60ºC for 6 h. The germinated and dried finger millet and sorghum seeds were ground and made into flour in a commercial flour mill (Milcent flour mill, compression-cum-shearing type (roller) mills, Bengaluru) adjusted to a fine setting. Subsequently, the flour was sieved and stored in air-tight containers at 7±2°C. Raw finger millet and sorghum grains were collected from the local market, cleaned, and turned into flour in a commercial flour mill to make the ungerminated flour.

Preparation of probiotic curd using finger millet malt and sorghum malt: Toned milk (3.0% fat, 8.5% SNF) was preheated to 40ºC and added with malted and ungerminated finger millet flour at three different concentrations, viz., 0%, 3%, and 5% on milk basis and malted powder not soluble in skim milk was used as control. It was mixed to ensure uniform mass. The mixture was heated to 80ºC without holding, cooled to 40ºC and inoculated with probiotic cultures at 1, 2 and 3% (w/v) of the mass. The procured mix was incubated at 37±2°C till titratable acidity reached to about 0.7% LA. The product was cooled to 5±2°C, and the curd was broken to obtain a uniform viscous product.

Titratable acidity: The titratable acidity of the product was determined by the method described in the Indian Standards (IS: 1479, Part I, 1960).

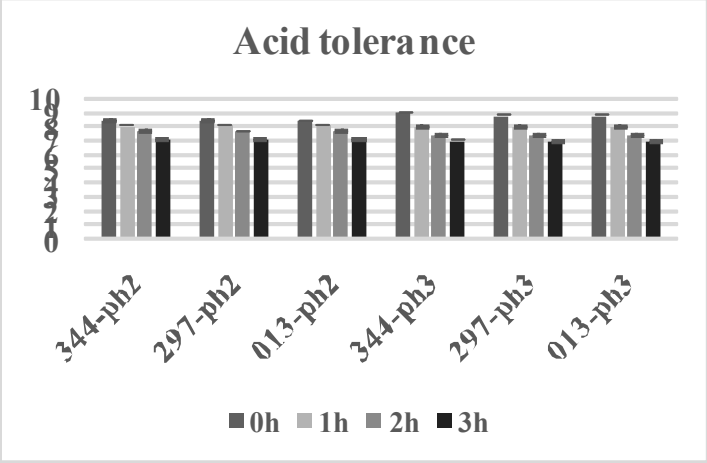
Microbiological analysis: Probiotic count was analyzed according to methods described in Indian Standards (Harrigan *et al.*, 1989).

Statistical analysis: The data was analyzed using SPSS16.0 software and the results are represented as mean ± standard deviation (SD) of three replicate assays. Significant differences were determined by two-way analysis of variance (ANOVA) and compared by Tukey test.

RESULTS

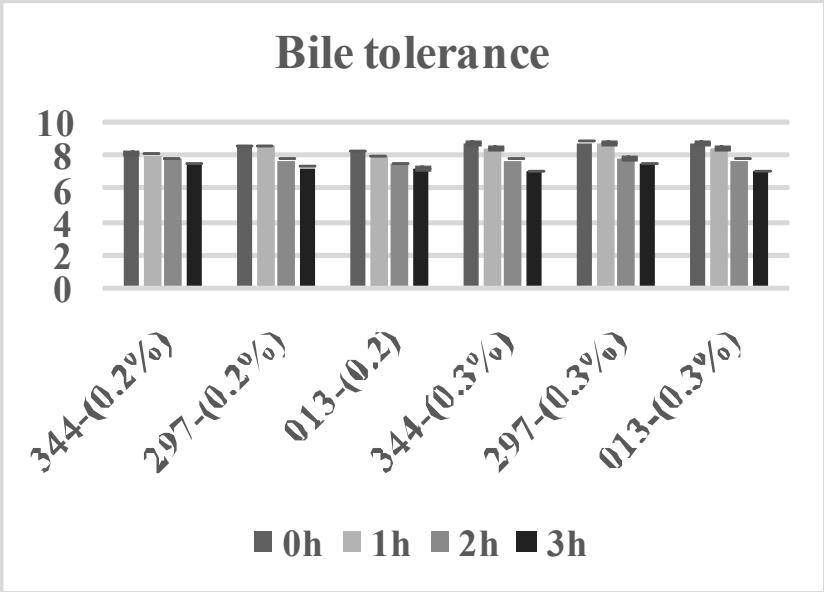
Evaluation of the probiotic attributes of *Lactiplantibacillus plantarum* (NCDC344), *Lacticaseibacillus casei* (NCDC297) and *L. acidophilus* (NCDC013)

Acid tolerance: The acid tolerance of *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidophilus* was evaluated, and it was found that all three



[n=3, results are expressed as Mean ± SD, differ significantly (P<0.05) among samples]

Fig. 1. Acid resistance tests for the *Lactiplantibacillus plantarum* (344), *Lacticaseibacillus casei* (297) and *Lacticaseibacillus acidophilus* (013)



[n=3, results are expressed as Mean ± SD, differ significantly (P<0.05) among samples]

Fig. 2. Bile tolerance tests for *Lactiplantibacillus plantarum* (344), *Lacticaseibacillus casei* (297) and *Lacticaseibacillus acidophilus* (013)

selected strains exhibited good survivability at pH 2 and pH 3 (Fig. 1). As the food takes about 2 to 3 h to pass through stomach during digestion, the acid tolerance was studied for 3h duration. The probiotic count decreased significantly ($p<0.05$) as the duration of incubation increased, but the overall probiotic count, even after 3 h of incubation, was more than 7 log cfu/mL.

Bile tolerance: For survivability of probiotics in the upper digestive tract, they must have the resistance to tolerate the bile juices secreted by liver. Bile salts are the important constituents of bile juices that are secreted by liver to breakdown the fats into fatty acids. The selected strains of *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidophilus* were exposed separately to two different bile salt concentrations of 0.2% and 0.3% for 0, 1, 2 and 3 h. All the selected probiotic strains recorded more than 7 log cfu/mL count in the 0.3% bile salt concentration and incubated for 3h, indicating good bile tolerability (Fig. 2).

Antibacterial activity: The growth inhibition zone diameters to indicate the degree of resistance as per the standards of CLSI (2012) are presented in Table 1. Antibacterial activity of *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei and L. acidophilus* was evaluated using indicator organisms like *Pseudomonas fragi*, *Bacillus polymyxa*, *Geobacillus stearothemophillus*, *S. aurous*, *B. cereus* and *E. coli* (Table 2). The extracellular overnight spent supernatant culture of *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidophilus* exhibited varying zones of inhibition from 16.36 mm to 78.19 mm depending on the tested pathogen. *Pseudomonas fragi* was most sensitive in *Lacticaseibacillus casei*, with 78.19 mm area of inhibition, while *Pseudomonas fragi* showed least sensitivity with a zone of 16.56 mm for *Lactiplantibacillus plantarum* and 16.54 mm in *L. acidophilus.* Lowest sensitivity of *E. coli* with a zone of inhibition of 16.36 mm was noticed in *Lacticaseibacillus casei*.

Table 1. Growth inhibition zone diameter indicating degree of resistance as per CLSI (2012) standards

Disc diffusion method	Zone diameter (mm)
Susceptible	>20
Intermediate	15-19
Resistant	d" 14

Table 2. Growth inhibition zone diameters (mm) indicating antibacterial activity of *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidophillus* against various pathogens

Pathogenic organism	*Lactiplantibacillus plantarum*	*Lacticaseibacillus casei*	*L. acidophilus*
Pseudomonas fragi	16.56±0. 06[a]	78.19±0.63[a]	16.54±0.30[a]
Bacillus polymixa	37.43±0.05[b]	22.57±0.01[b]	37.42±0.15[b]
Geobacillus stearothermophilus	29.71±0.08[c]	46.24±0.01[c]	29.70±0.06[c]
Bacillus cereus	22.59±0.02[d]	64.69±0.02[d]	22.49±0.05[d]
S. aureus	29.74±0.02[e]	46.24±0.03[e]	26.72±0.03[e]
E. coli	55.23±0.05[f]	16.36±0.32[f]	55.21±0.02[f]

n=3, results are expressed as Mean ± SD, with different small letters superscript (a, b, c) within column and capital letters (A, B, C) within row differ significantly (P<0.05) among the samples

Table 3. Antioxidative activity (%) of *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidophilus*

Probiotic organism	0.2 mM concentration of diphenyl picryl hydrazyl	0.1 mM concentration of diphenyl picryl hydrazyl
Control	0.49±0.01[aA]	0.28±0.01[aB]
Lactiplantibacillus plantarum	71.06±0.01[bA]	27.16±0.02[bB]
Lacticaseibacillus casei	53.06±0.02[cA]	22.60±0.01[cB]
L. acidophilus	70.03±0.01[dA]	27.05±0.02[dB]

n=3, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within the column, and capital letters (A, B, C) within the row differ significantly (P<0.05) among the samples (DPPH-Diphenyl picryl hydrazyl)

Antioxidative activity: The DPPH radical scavenging method is widely used to evaluate antioxidant activities because of its simplicity, rapidity, sensitivity and reproducibility compared with other methods (Milardovic *et al.*, 2006). As shown in Table 3, the DPPH scavenging rate of intact cells (activated strains) was higher than that of cell-free extracts (control). The *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidophilus* exhibited the DPPH radical scavenging by 71.06%, 53.06% and 70.03%, respectively in the 0.2 mM DPPH solution, whereas the DPPH scavenging activity of *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidophilus* in 0.1 mM DPPH solution were 27.16%, 22.60% and 27.05%, respectively.

Effect of germination of sorghum and finger millet on probiotic count of the curd

Effect of germination of sorghum and finger millet on probiotic count in the curd is presented

Table 4. Effect of germination of select nutri-cereals on probiotic count (log cfu/mL) of the curd

Type of fermented product	Incubation period (h)			
	0	4	8	12
(NCDC 344) Probiotic count (log cfu/mL)				
Skim milk	4.32±0.03^{aA}	6.11±0.02^{aB}	7.23±0.01^{aC}	8.13±0.01^{aD}
Ungerminated sorghum	5.54±0.01^{bA}	7.22±0.04^{bB}	8.38±0.02^{bC}	8.23±0.02^{bD}
Ungerminated finger millet	5.44±0.03^{cA}	7.12±0.04^{cB}	8.33±0.04^{cC}	8.15±0.04^{cD}
24 h germinated sorghum	5.64±0.01^{dA}	7.26±0.01^{dB}	9.38±0.06^{dC}	10.13±0.02^{dD}
24 h germinated finger millet	5.54±0.02^{bA}	7.16±0.03^{eB}	9.18±0.01^{eC}	9.83±0.02^{eD}
48 h germinated sorghum	4.27±0.04^{eA}	6.11±0.01^{fB}	7.23±0.04^{fC}	8.13±0.01^{fD}
48 h germinated finger millet	4.22±0.01^{fA}	6.01±0.00^{gB}	7.05±0.03^{gC}	8.05±0.02^{gD}
(NCDC 297) Probiotic count (log cfu/mL)				
Skim milk	4.22±0.04^{aA}	6.06±0.03^{aB}	8.24±0.01^{aC}	8.36±0.00^{aD}
Ungerminated sorghum	5.16±0.01^{bA}	7.16±0.04^{bB}	8.28±0.06^{bC}	9.13±0.01^{bD}
Ungerminated finger millet	5.12±0.03^{cA}	7.06±0.02^{cB}	8.15±0.01^{cC}	9.03±0.03^{cD}
24 h germinated sorghum	5.46±0.02^{dA}	7.26±0.01^{dB}	8.98±0.01^{dC}	10.73±0.01^{dD}
24 h germinated finger millet	5.36±0.01^{eA}	7.16±0.03^{bB}	8.65±0.01^{eC}	9.85±0.04^{eD}
48 h germinated sorghum	4.26±0.03^{fA}	6.1±0.04^{eB}	7.25±0.01^{fC}	8.14±0.02^{fD}
48 h germinated finger millet	4.06±0.04^{gA}	6.06±0.02^{fB}	7.16±0.01^{gC}	8.03±0.01^{gD}
(NCDC 013) Probiotic count (log cfu/mL)				
Skim milk	3.32±0.02^{aA}	5.06±0.03^{aB}	6.02±0.01^{aC}	6.46±0.02^{aD}
Ungerminated sorghum	4.16±0.01^{bA}	6.26±0.01^{bB}	7.18±0.06^{bC}	7.33±0.01^{bD}
Ungerminated finger millet	4.12±0.02^{bA}	6.06±0.04^{cB}	7.12±0.01^{cC}	7.23±0.04^{cD}
24 h germinated sorghum	4.46±0.04^{cA}	6.79±0.12^{dB}	7.80±0.03^{dC}	8.22±0.01^{dD}
24 h germinated finger millet	4.36±0.01^{dA}	6.57±0.05^{eB}	7.65±0.03^{eC}	8.13±0.04^{eD}
48 h germinated sorghum	4.26±0.03^{eA}	6.16±0.04^{fB}	6.25±0.01^{fC}	7.12±0.02^{fD}
48 h germinated finger millet	4.06±0.01^{fA}	6.06±0.02^{gB}	6.16±0.05^{gC}	7.08±0.03^{gD}
(NCDC 344+NCDC297) Probiotic count (log cfu/mL)				
Skim milk	4.32±0.03^{aA}	6.06±0.02^{aB}	8.24±0.02^{aC}	8.66±0.04^{aD}
Ungerminated sorghum	6.16±0.01^{bA}	7.76±0.01^{bB}	8.28±0.06^{aC}	9.13±0.03^{bD}
Ungerminated finger millet	6.12±0.03^{bA}	7.46±0.03^{cB}	8.15±0.03^{bC}	9.03±0.02^{cD}
24 h germinated sorghum	7.46±0.11^{cA}	8.68±0.04^{dB}	11.05±0.12^{cC}	12.13±0.13^{dD}
24 h germinated finger millet	7.36±0.13^{dA}	8.46±0.02^{eB}	10.84±0.05^{dC}	11.53±0.04^{eD}
48 h germinated sorghum	5.26±0.01^{eA}	6.16±0.04^{fB}	7.25±0.04^{eC}	8.24±0.04^{fD}
48 h germinated finger millet	5.06±0.02^{fA}	6.06±0.02^{aB}	7.16±0.03^{fC}	8.03±0.01^{gD}

n=3, results are expressed as Mean ± SD, with different small letters superscript (a, b, c) within column and capital letters (A, B, C) within row differ significantly (P<0.05) among the samples
(NCDC344-*Lactiplantibacillus plantarum*, NCDC297-*Lacticaseibacillus casei*, NCDC013-*Lactobacillus acidophilus*)

in Table 4. Incorporation of malted millet increased the probiotic count significantly (P<0.05). Germination of millets for 24 h significantly (P<0.05) increased the prebiotic potential of the selected millets (viz. finger millet and sorghum) whereas, germination of millets

for 48 h significantly (P<0.05) reduced the probiotic count in the curd. The sorghum germinated up to 24 h recorded the highest probiotic count (*L. casei*) of 10.73±0.01 log cfu/mL, whereas the finger millet germinated for 48h recorded significantly (P<0.05) lower counts (9.85±0.02 log cfu/mL) than the former. Amongst the selected strains, *Lacticaseibacillus casei* recorded significantly (P<0.05) higher counts compared to *Lactiplantibacillus plantarum* and *L. acidophilus*. The culture containing mixed strains of *Lactiplantibacillus plantarum* and *Lacticaseibacillus casei* (1:1) exhibited significantly (P<0.05) higher count of 12.13±0.01 log cfu/mL after 12 h incubation at 37°C when compared with the other period of incubation.

Effect of germination on titratable acidity and pH of the curd

Effects of germination of sorghum and finger millet on titratable acidity and pH of the curd are presented in Table 5 and 6, respectively. The selected treatments, viz. type of flour, duration of germination, the bacterial strain and duration of incubation had significant (P<0.05) influence on the acidity and pH of the curd. The product mix containing 24 h germinated flours recorded significantly (P<0.05) higher acidity and lower pH compared to other product mix combinations. The finger millet germinated for 24 h, inoculated with *Lacticaseibacillus casei* and incubated for 12 h had the lowest pH of 4.30 and highest acidity of 0.73% LA. Similarly, when the culture with mixed strains of *Lactiplantibacillus plantarum* and *Lacticaseibacillus casei* were used for the same substrates with skim milk as control, significantly (P<0.05) higher acidity for these cultures was obtained as compared to other incubation periods.

The pH of the curd (Table 6) varied between 4.22 to 5.24 after 12 h incubation with selected bacterial strain and millets combination. The finger millet germinated for 24 h recorded significantly (P<0.05) lower pH of 4.30 when incubated for 12 h with *L. casei* as compared to other durations of incubation. The culture containing mixed strains of *L. plantarum* and *L. casei* (1:1) exhibited significantly (P<0.05) lower pH of 4.22 in milk containing 24 h germinated finger millet malt upon incubation for 12 h in comparison with the incubation periods of 0, 4 and 8 h.

DISCUSSION

Evaluation of the probiotic attributes of *Lactiplantibacillus plantarum* (NCDC344), *Lacticaseibacillus casei* (NCDC297) and *L. acidophilus* (NCDC013)

Acid and bile tolerance: The acid tolerance of the *Lactobacilli* species can be correlated with the presence of gradient between extracellular and cytoplasmic pH. According to Kumar *et al.* (2020), the count of certain probiotic bacteria reduces and eventually gets killed when the threshold level of pH is reached. The authors noticed that there was a significant reduction in the cell count at pH 1 for *Lactiplantibacillus plantarum and Lacticaseibacillus casei* strains, whereas a non-significant reduction was observed in pH range 3-7 upon incubation for 90 min. Similar results were also observed by Vinderola *et al*. (2011) for resistance of *Lacticaseibacillus casei* to acidic conditions during storage. The acidic conditions similar to stomach conditions were simulated in the *in-vitro* acid resistance test, and no decrease in count was observed at pH 3 in 90 min in the presence of pepsin because of the resistant nature of the probiotic strain. But, without pepsin at pH 2.5 for 90 min, there were 5 log reductions in the counts.

Antibacterial activity: The antibacterial activity of probiotics against the pathogens could be due to acidic condition produced by *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei* and *L. acidhophillus*. LAB including *Lactiplantibacillus plantarum* and *L. acidophilus* produce lactic acid and acetic acid, which can serve as potent antibacterial agents against pathogens like

Table 5. Acidity of products as affected by addition of finger millet and sorghum

Type of fermented product	Incubation period (h)			
	0	4	8	12
(NCDC 344) Acidity (% LA)				
Skim milk	0.12±0.01^{aA}	0.35±0.02^{aB}	0.46±0.02^{aC}	0.52±0.07^{aD}
Ungerminated sorghum	0.13±0.04^{bA}	0.37±0.02^{bB}	0.47±0.04^{bC}	0.58±0.09^{bD}
Ungerminated finger millet	0.13±0.05^{bA}	0.39±0.03^{bB}	0.48±0.13^{cC}	0.60±0.05^{cD}
24 h germinated sorghum	0.14±0.03^{cA}	0.43±0.04^{cB}	0.49±0.04^{dC}	0.64±0.17^{dD}
24 h germinated finger millet	0.16±0.03^{dA}	0.45±0.12^{dB}	0.53±0.02^{eC}	0.68±0.06^{eD}
48 h germinated sorghum	0.14±0.04^{cA}	0.35±0.04^{aB}	0.48±0.01^{cC}	0.58±0.07^{bD}
48 h germinated finger millet	0.16±0.02^{dA}	0.43±0.05^{cB}	0.54±0.03^{fC}	0.64±0.14^{dD}
(NCDC 297) Acidity (% LA)				
Skim milk	0.13±0.08^{aA}	0.41±0.04^{aB}	0.53±0.07^{aC}	0.60±0.06^{aD}
Ungerminated sorghum	0.13±0.07^{aA}	0.41±0.05^{aB}	0.56±0.08^{bC}	0.60±0.15^{aD}
Ungerminated finger millet	0.14±0.08^{bA}	0.43±0.07^{bB}	0.58±0.05^{cC}	0.65±0.07^{bD}
24 h germinated sorghum	0.15±0.09^{cA}	0.48±0.09^{cB}	0.60±0.08^{dC}	0.70±0.04^{cD}
24 h germinated finger millet	0.16±0.17^{dA}	0.50±0.04^{dB}	0.62±0.16^{eC}	0.73±0.07^{dD}
48 h germinated sorghum	0.15±0.09^{cA}	0.46±0.03^{eB}	0.54±0.09^{bC}	0.61±0.04^{aD}
48h germinated finger millet	0.15±0.08^{cA}	0.48±0.06^{fB}	0.59±0.06^{eC}	0.66±0.08^{eD}
(NCDC 013) Acidity (% LA)				
Skim milk	0.12±0.03^{aA}	0.32±0.04^{aB}	0.47±0.06^{aC}	0.56±0.08^{aD}
Ungerminated sorghum	0.13±0.12^{bA}	0.34±0.06^{bB}	0.42±0.04^{bC}	0.53±0.07^{bD}
Ungerminated finger millet	0.14±0.05^{cA}	0.35±0.04^{cB}	0.45±0.06^{bC}	0.58±0.14^{cD}
24 h germinated sorghum	0.14±0.07^{cA}	0.35±0.16^{cB}	0.44±0.08^{cC}	0.62±0.16^{dD}
24 h germinated finger millet	0.16±0.02^{dA}	0.38±0.08^{dB}	0.47±0.07^{aC}	0.63±0.08^{eD}
48 h germinated sorghum	0.15±0.04^{eA}	0.26±0.16^{eB}	0.41±0.09^{dC}	0.51±0.04^{fD}
48 h germinated finger millet	0.16±0.03^{dA}	0.29±0.05^{fB}	0.45±0.04^{eC}	0.57±0.03^{cD}
(NCDC 344+NCDC297) Acidity (% LA)				
Skim milk	0.14±0.07^{aA}	0.43±0.08^{aB}	0.56±0.08^{aC}	0.65±0.08^{aD}
Ungerminated sorghum	0.14±0.15^{aA}	0.43±0.06^{aB}	0.56±0.18^{aC}	0.65±0.07^{aD}
Ungerminated finger millet	0.15±0.06^{bA}	0.45±0.07^{bB}	0.58±0.05^{bC}	0.68±0.09^{bD}
24 h germinated sorghum	0.15±0.06^{bA}	0.48±0.15^{cB}	0.68±0.07^{cC}	0.75±0.15^{cD}
24 h germinated finger millet	0.16±0.08^{cA}	0.51±0.09^{dB}	0.70±0.08^{dC}	0.78±0.07^{dD}
48 h germinated sorghum	0.15±0.09^{bA}	0.45±0.03^{bB}	0.58±0.09^{bC}	0.63±0.06^{eD}
48 h germinated finger millet	0.15±0.17^{bA}	0.48±0.06^{cB}	0.66±0.05^{cC}	0.68±0.03^{bD}

n=3, results are expressed as Mean ± SD, with different small letters superscript (a, b, c) within the column, and capital letters (A, B, C) within the row differ significantly (P<0.05) among the samples
(NCDC344-*Lactiplantibacillus plantarum*, NCDC297-*Lacticaseibacillus casei*, NCDC013-*Lactobacillus acidophilus*)

coliforms, Salmonella and *Clostridia* spp. (Kaushik *et al*., 2009). *Lactiplantibacillus plantarum* and *L. acidophilus* were exported to exhibit strong antagonistic effect against pathogens due to their capability to produce organic acids (Wilson *et al*., 2005).

Antioxidative activity: The results showed that

Table 6. pH of products as affected by addition of finger millet and sorghum

Type of fermented product	Incubation period (h)			
	0	4	8	12
	(NCDC 344) pH			
Skim milk	6.56±0.02^{aA}	5.72±0.01^{aB}	5.06±0.05^{aC}	4.65±0.02^{aD}
Ungerminated sorghum	6.53±0.12^{bA}	5.52±0.04^{bB}	5.07±0.03^{aC}	4.62±0.11^{bD}
Ungerminated finger millet	6.53±0.01^{bA}	5.46±0.03^{cB}	4.17±0.04^{bC}	4.58±0.04^{cD}
24 h germinated sorghum	6.43±0.13^{cA}	5.35±0.16^{dB}	4.85±0.04^{cC}	4.42±0.02^{dD}
24 h germinated finger millet	6.43±0.05^{cA}	4.82±0.04^{eB}	4.63±0.11^{dC}	4.38±0.01^{eD}
48 h germinated sorghum	6.53±0.08^{bA}	5.62±0.06^{fB}	4.83±0.06^{eC}	4.56±0.07^{fD}
48 h germinated finger millet	6.56±0.01^{aA}	5.42±0.02^{gB}	4.86±0.05^{fC}	4.41 ±0.09^{gD}
	(NCDC 297) pH			
Skim milk	6.50±0.04^{aA}	5.61±0.06^{aB}	4.96±0.05^{aC}	4.44±0.05^{aD}
Ungerminated sorghum	6.50±0.06^{aA}	5.56±0.14^{bB}	4.85±0.07^{bC}	4.45±0.03^{aD}
Ungerminated finger millet	6.50±0.07^{aA}	5.46±0.01^{cB}	4.77±0.03^{cC}	4.38±0.02^{bD}
24 h germinated sorghum	6.50±0.09^{aA}	5.32±0.05^{dB}	4.73±0.08^{dC}	4.34±0.03^{cD}
24 h germinated finger millet	6.50±0.02^{aA}	5.20±0.01^{eB}	4.66±0.03^{eC}	4.30±0.01^{dD}
48 h germinated sorghum	6.50±0.04^{aA}	5.22±0.03^{fB}	4.93±0.08^{fC}	4.45±0.04^{aD}
48 h germinated finger millet	6.50±0.03^{aA}	5.50±0.08^{gB}	4.86±0.02^{bC}	4.48±0.02^{eD}
	(NCDC 013) pH			
Skim milk	6.60±0.05^{aA}	6.21±0.02^{aB}	5.66±0.08^{aC}	5.24±0.01^{aD}
Ungerminated sorghum	6.53±0.04^{bA}	6.13±0.05^{bB}	5.53±0.04^{bC}	5.13±0.09^{bD}
Ungerminated finger millet	6.56±0.05^{cA}	6.06±0.07^{cB}	5.43±0.07^{cC}	4.85±0.02^{cD}
24 h germinated sorghum	6.50±0.02^{dA}	6.22±0.09^{dB}	5.58±0.13^{dC}	5.04±0.04^{dD}
24 h germinated finger millet	6.52±0.06^{bA}	5.80±0.04^{eB}	5.16±0.04^{eC}	4.61±0.02^{eD}
48 h germinated sorghum	6.60±0.07^{aA}	6.22±0.07^{aB}	5.43±0.06^{cC}	5.24±0.05^{aD}
48 h germinated finger millet	6.60±0.08^{aA}	5.62±0.05^{fB}	5.26±0.04^{fC}	5.15±0.02^{fD}
	(NCDC 344+NCDC297) pH			
Skim milk	6.50±0.06^{aA}	5.51±0.04^{aB}	4.66±0.06^{aC}	4.38±0.03^{aD}
Ungerminated sorghum	6.50±0.04^{aA}	5.43±0.08^{bB}	4.63±0.08^{bC}	4.34±0.01^{bD}
Ungerminated finger millet	6.50±0.07^{aA}	5.36±0.06^{cB}	4.58±0.04^{cC}	4.25±0.12^{cD}
24 h germinated sorghum	6.50±0.03^{aA}	5.22±0.09^{dB}	4.56±0.08^{dC}	4.28±0.01^{dD}
24 h germinated finger millet	6.50±0.08^{aA}	5.20±0.05^{eB}	4.46±0.04^{eC}	4.22±0.08^{eD}
48 h germinated sorghum	6.50±0.04^{aA}	5.32±0.07^{fB}	4.55±0.08^{fC}	4.30±0.06^{fD}
48 h germinated finger millet	6.50±0.08^{aA}	5.25±0.08^{gB}	4.46±0.07^{eC}	4.26±0.02^{gD}

n=3, results are expressed as Mean ± SD, with different small letters superscript (a, b, c) within column and capital letters (A, B, C) within row differ significantly (P<0.05) among the samples
(NCDC344-*Lactiplantibacillus plantarum*, NCDC297-*Lacticaseibacillus casei*, NCDC013-*Lactobacillus acidophilus*)

intact cells of three strains exhibited higher anti-oxidative activity in scavenging DPPH radical. Many Lactobacillus strains were previously reported to survive under oxidative stress (exposed to hydrogen peroxide and hydroxyl radicals) to different extents. It was shown that *Lacticaseibacillus casei*, *Lacticaseibacillus casei* KCTC 3260 and *L. rhamnosus* GG

survived after 8 h of 1.0 mmol L^{-1} hydrogen peroxide incubation (Lee *et al.*, 2005), while *L. fermentum* E-3 and E-8 survived for 180 and 150 min, respectively (Kullisaar *et al.*, 2002). *Lactiplantibacillus plantarum* C88, C10 and K25 exhibited strong resistance to hydrogen peroxide, while *Lactiplantibacillus plantarum* S5-2, S5-6 and S7-2 were sensitive to it (Li *et al.*, 2012).

Effect of germination of sorghum and finger millet on probiotic count of the curd

As per the results, 48 h germinated sorghum and finger millet had significantly ($P<0.05$) lower probiotic count compared to ungerminated and 24 h germinated flours. Amongst the selected millets, sorghum was found to be the best substrate for the probiotic than the finger millet. All selected strains showed the highest growth in 24 h germinated product mix compared to other mix. This may be due to the presence of more amylases in 24 h germinated flour which hydrolyze starch to produce lower molecular weight carbohydrates like oligo- and disaccharides. Shaikh *et al.* (2017) also noted that addition of finger millet significantly ($P<0.05$) increased the *Streptococcus thermophilus* MTCC 5460 and probiotic *Lactobacillus helveticus* MTCC5463 count in the product. The oligo- and disaccharides produced by the amylases are majorly attributed to the prebiotic potential of the malted millets.

Effect of germination on titratable acidity and pH of the curd

The curd made with germinated finger millet produced greater acidity. This may be due to the presence of amylases in malted flour, which partially hydrolyze starch to produce lower molecular weight carbohydrates like oligo- and disaccharides (Ganguly and Sabikhi, 2012). These lower molecular weight carbohydrates could be responsible for increased microbial activity (Shaikh *et al.*, 2017) and increased acidity in the product. Similar findings were reported by Pardhi *et al.* (2014), who prepared finger millet lassi with finger millet flour added at concentrations of 2%, 3%, and 4%. When mixed cultures of *Lactiplantibacillus plantarum* and *Lacticaseibacillus casei* were used for the same substrates with skim milk as control, the significantly ($P<0.05$) lower pH were found in the 24 h malted finger millet. Mridula and Gupta (2013) also reported a decrease in pH with an increase in the content of sprouted wheat flour and oat flour on fermentation with *L. acidophilus*. Among the various studied strains, most rapid change in pH was observed in the samples cultured with *L. acidophilus* followed by *L. rhamnosus* and *Lacticaseibacillus casei.*

The current study revealed that the nutri-cereals possess excellent prebiotic potential. Germination of the nutri-cereals significantly ($P<0.05$) increases its prebiotic potential when compared with un-germinated nutri-cereals. Amongst the selected nutri-cereals, sorghum exhibited best prebiotic potential. Amongst the select probiotic strains, mixed strains of *Lactiplantibacillus plantarum* and *Lacticaseibacillus casei* gave the maximum probiotic count. Incubation of toned milk fortified with 3% sorghum malt and inoculated with 4% of mixed culture containing *Lactiplantibacillus plantarum* and *Lacticasei bacilluscasei* (1:1) recorded a probiotic count of 12.13 log cfu/mL after12 h of fermentation at 37°C.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: MA: Performed the experiments and drafted the manuscript; DHC: Conceived the idea and guided the technical program; JRK: Supervised the work and edited the manuscript.

ACKNOWLEDGEMENT

All the authors extend their sincere thanks to the authority of the National Dairy Research Institute for funding this research work. Research fellowship offered by the Deemed University, ICAR-NDRI, Karnal to first author is gratefully acknowledged.

REFERENCES

Adebiyi JA, Obadina AO, Adebo, OA and Kayitesi E, 2018. Fermented and malted millet products in Africa: expedition from traditional/ethnic foods to industrial value-added products. Crit Rev Food Sci Nutr, 58(3): 463-474, doi: 10.1080/10408398.2016.1188056

Adebo OA and Gabriela Medina-Meza, 2020. Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. Molecules, 25(4): 927, doi:_10.3390/molecules25040927

Adebo OA, 2020. African sorghum-based fermented foods: past, current and future prospects. Nutrients, 12(4): 1111, doi: 10.3390/nu12041111

Adebo OA, Njobeh PB, Mulaba-Bafubiandi AF, Adebiyi JA, Desobgo ZSC *et al*., 2018. Optimization of fermentation conditions for ting production using response surface methodology. J Food Process Preserv, 42(1): e13381, doi: 10.1111/jfpp.13381

Ambati K and Sucharitha KV, 2019. Millets- review on nutritional profiles and health benefits. Int J Recent Sci Res, 10(7): 33943-33948, doi: 10.24327/IJRSR.2019.1007.3786

Bhandari L, Chawla P, Dhull SB, Sadh PK and Kaushik R, 2018. Technological and functional aspects of yoghurt cheese. In Advances in Animal Biotechnology and its Applications (Gahlawat SK, Duhan JS, Salar RK, Siwach P, Kumar S *et al*., Eds.). Springer (Singapore) pp 259-267, doi: 10.1007/978-981-10-4702-2_16

Chilakawar PM and Pawar VS, 2015. Preparation of health food from finger millet malt. Bioinfolet, 12(2 A): 403- 404

Chooruk A, Piwat S and Teanpaisan R, 2017. Antioxidant activity of various oral Lactobacillus strains. J Appl Microbiol, 123(1): 271-279, doi: 10.1111/jam.13482

Clark PA, Cotton LN and Martin JH, 1993. Selection of Bifidobacteria for use as dietary adjuncts in cultured dairy foods. II. Tolerance to simulated pH of human stomachs. Cult Dairy Prod J, 28(4): 11-14

FSSAI, 2020. (Food Safety and Standards Authority of India), 2020. Available in: https://archive.fssai.gov.in/dam/jcr:6f183856-e806-4ff9-87ca-a4c257e69e49/Nutraceuticals_Regulations. pdf. [Accessed on 16 April, 2020]

Ganguly S and Sabikhi L, 2012. Fermentation dynamics of probiotic *Lactobacillus acidophilus* NCDC-13 in a composite dairy-cereal substrate. Int J Food Ferment Technol, 1(1): 33-46

Gilliland SE, Staley TE and Bush LJ, 1984. Importance of bile tolerance of *Lactobacillus acidophilus* used as dietary adjunct. J Dairy Sci, 67: 3045-3051, doi: 10.3168/jds.S0022-0302(84)81670-7

Harrigan P, Zieman JC and Macko SA, 1989. The base of nutritional support for the gray snapper (*Lutjanus griseus*): An evaluation based on a combined stomach content and stable isotope analysis. Bull Mar Sci, 44(1): 65-77

IS (Indian Standards): 1479, Part I (1960) Methods of test for dairy industry, chemical analysis of milk. Indian Standards Institution, New Delhi

Kaushik JK, Kumar A, Duary RK, Mohanty AK, Grover S *et al*., 2009. Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum*. PLoS One, 4(12): e8099, doi: 10.1371/journal.pone.0008099

Kerry RG, Patra JK, Gouda S, Park Y, Shin HS *et al*., 2018. Benefaction of probiotics for human health: A review. J Food Drug Anal, 26(3): 927-939, doi: 10.1016/j.jfda.2018.01.002

Kort R, Westerik N, Mariela SL, Douillard FP, Gottstein W *et al*., 2015. A novel consortium of *Lactobacillus rhamnosus* and *Streptococcus thermophilus* for increased access to functional fermented foods. Microb Cell Factories, 14(1): 1-14, doi: 10.1186/s12934-015-0370-x

Kristek A, Schär MY, Soycan G, Alsharif S, Kuhnle GGC *et al*., 2018. The gut microbiota and cardiovascular health benefits: A focus on wholegrain oats. Nutr Bull, 43(4): 358-373, doi: 10.1111/nbu.12354

Kullisaar T, Zilmer M, Mikelsaar M, Vihalemm T, Annuk H *et al*., 2002. Two antioxidative Lactobacilli strains as promising probiotics. Int J Food Microbiol, 72(3): 215-224, doi: 10.1016/s0168-1605(01)00674-2

Kumar A, Kaur A and Tomer V, 2020. Process optimization for the development of a synbiotic beverage based on lactic acid fermentation of nutricereals and milk-based beverage. Lebensm Wiss Technol, 131: 109774, doi:_10.1016/j.lwt.2020.109774

Lee J, Hwang KT, Chung MY, Cho DH and Park CS, 2005. Resistance of *Lactobacillus casei* KCTC 3260 to reactive oxygen species (ROS): role for a

metal ion chelating effect. J Food Sci, 70(8): 388-391, doi: 10.1111/j.1365-2621.2005.tb11524.x

Li S, Zhao Y, Zhang L, Zhang X, Huang L *et al*., 2012. Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. Food Chem, 135(3): 1914-1919, doi: 10.1016/j.foodchem.2012.06.048

Ma C, Ma A, Gong G, Liu Z, Wu Z *et al*., 2015. Cracking *Streptococcus thermophilus* to stimulate the growth of the probiotic *Lactobacillus casei* in co-culture. Int J Food Microbiol, 210: 42-46, doi: 10.1016/j.ijfoodmicro.2015.04.034

Mabhaudhi T, Reilly PO, Walker S and Mwale S, 2016. Opportunities for underutilised crops in southern Africa's post-2015 development agenda. Sustainability, 8(4): 302, doi: 10.3390/su8040302

Majumdar TK, Premavalli KS and Bawa AS, 2006. Effect of puffing on calcium and iron contents of ragi varieties and their utilization. J Food Sci Technol, 43(5): 542-543

Milardovic S, Ivekovic D and Grabaric BS, 2006. A novel amperometric method for antioxidant activity determination using DPPH free radical. Bioelectrochemistry, 68(2): 175-180, doi: 10.1016/j.bioelechem.2005.06.005

Ministry of Agriculture and Farmers Welfare, 2022. Millet Production. Retrieved November 20, 2022 from. https://pib.gov.in/PressReleasePage.aspx?PRID=1796559

Mridula D, Gupta RK and Manikantan MR, 2013. Effect of incorporating sorghum flour to wheat flour on quality of biscuits fortified with defatted soy flour. Am J Food Technol, 2: 428-434, doi: 10.3923/ajft.2007.428.434

Palaniappan A, Balasubramaniam VG and Antony U, 2017. Prebiotic potential of xylooligosaccharides derived from finger millet seed coat. Food Biotechnol, 31: 264-280, doi: 10.1080/08905436.2017.1369433

Pardhi PS, Desale RJ, Mule PR, Ghule BK, Tambe DR *et al*., 2014. Studies on finger millet lassi. Asian J Dairy Foods Res, 33(4): 255-258, doi: 10.5958/0976-0563.2014.00613.7

Shaikh AM, Sreeja V and Desai RR, 2017. Effect of malted and unmalted finger millet flour and its rates of incorporation on quality attributes of finger millet enriched probiotic fermented milk product. Int J Curr Microbiol Appl Sci, 6(9): 2258-66, doi: 10.20546/ijcmas.2017.609.277

Sobowale SS, Adebo OA and Mulaba-Bafubiandi AF, 2019. Production of extrudate pasta from optimal sorghum-peanut flour blend and influence of composite flours on some quality characteristics and sorption isotherms. Trans R Soc S Afr, 74(3): 268-275, doi: 10.1080/0035919X.2019.1639563

Vinderola G, Cespedes M, Mateolli D, Cardenas P, Lescano M *et al.*, 2011. Changes in gastric resistance of *Lactobacillus casei* in flavored commercial fermented milks during refrigerated storage. Int J Dairy Technol, 64(2): 269-275, doi: 10.1111/j.1471-0307.2010.00659.x

Wilson AR, Sigee D and Epton HA, 2005. Anti-bacterial activity of *Lactobacillus plantarum* strain SK1 against *Listeria monocytogenes* is due to lactic acid production. J Appl Microbiol, 99: 1516-1522, doi: 10.1111/j.1365-2672.2005. 02725.x

Received- 22.10.2022, Accepted- 24.11.2022, Published- 01.12.2022 (Print)
Section Editor: Prof. M. K. Sanyal, Member, Editorial Board