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Formulation and Evaluation of Bajra Millet (PBH-1625) Beverage and Probiotic Beverage

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ABSTRACT

Background: Speciality and functionality of beverages are rising trend and one of major functional requirement is milk alternative. Millets are one of the good source for plant based beverage as they are rich source of micronutrients, especially B vitamins, minerals as well as macronutrients.

Methods: Bajra grain was pressure cooked and ground to a slurry. The slurry was diluted (millet to water ratios - 1:4, 1:5, 1:6, 1:7, 1:8) and best dilution ratio was selected based on sensory evaluation and was used for development of probiotic beverage using *Lactobacillus* culture and fermented (8 hours, 16 hours and 24 hours). The nutrient composition of bajra grains, unfermented bajra beverage and probiotic beverage were analysed.

Result: The results indicate that bajra beverage with millet to water ratio of 1:7 and probiotic beverage fermented for 8 hours was accepted based on sensory evaluation. The pH, total flavonoids and total antioxidant activity of the probiotic beverage decreases and total soluble solids, viscosity, sedimentation index was increased after 8 hours of fermentation in comparison to unfermented bajra beverage. The whiteness index of bajra probiotic beverage increases after fermentation.

Key words: Bajra millet, Beverage, Fermentation, Nutrient composition, Sensory evaluation.

INTRODUCTION

Plant-based beverages or non-dairy beverage alternatives, are a rapidly increasing niche for substitution of mammalian milk. Consumers with lactose intolerance, allergies to cow milk, calorie concerns, hypercholesterolemia and a preference for a vegan diet prefer plant-based milk. Functional foods and nutraceutical components are derived from plant sources and contain health-promoting components such as antioxidants, minerals, vitamins and dietary fibres (Sunny *et al*., 2019). Plant-based milk are liquids formed by the dissolving and disintegration of plant material in water, followed by homogenization for particle size reduction within the range of 5-20 μm, so that they closely mimic bovine milk in appearance and consistency (Sethi *et al*., 2016).

Plant-based milks are typically made from legumes, grains, seeds and nuts because they have favourable properties that make them easy to create a dairy-free, health-promoting, cost-effective, nutritional and pleasant plant-based milk substitute (Kundu *et al*., 2018).

Millets are not only nutritionally comparable to main cereals but they are also excellent providers of carbohydrates, minerals, antioxidants and phytochemicals with nutraceutical benefits (Rao *et al*., 2017). Some millet components, such as phenol, phytates and tannins, have anti-aging properties (Bravo *et al*., 1998). Millet's dietary fibre and micronutrients aid in the prevention of diseases like type 2 diabetes, breast cancer and heart disease. They also help with fat metabolism and tissue repair, as well as issues correlated to blood cholesterol (Gupta *et al*., 2012). Millets being rich source of nutrients, are one of the best source of plant based beverage.

Probiotic strains for cereal substrate fermentation are a good approach for the production of functional foods.

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Cereals and millets have proven to be good lactic acid fermentation substrates and using probiotic bacteria as starter cultures could result in probiotic products. These probiotic supplements are suitable for vegans who want to avoid lactose intolerance problem. It contributes to the functional food category (Gomes and Malcata, 1999).

Lactic acid bacteria make up the majority of probiotics and dairy products have traditionally been used to deliver them. However, because of rising cholesterol levels, lactose sensitivity linked to dairy consumption and the growing popularity of vegetarianism, consumer demand has shifted to non-diary based probiotic products such as fruits and vegetables and cereal based products (Fasreen, 2017).

Probiotics are regarded as important components of functional foods (Bigliardi and Galati, 2013). Cereal grains as an alternative to probiotic milk-based formulas is a promising choice since they can address some of the issues associated with fermented dairy products, such as lactose intolerance and cholesterol effects (Prado *et al*., 2008).

MATERIALS AND METHODS

Raw materials

Bajra (PBH-1625) millet was procured from Regional Agricultural Research Station, PJTSAU, Palem, Nagarkurnool District, Telangana state. Commercial probiotic starter culture consisting of *Lactobacillus, Bifidobacterium, Streptococus* strains was procured for development of probiotic beverages. The study was conducted during the year 2020 to 2021 at Department of Foods and Nutrition, Post Graduate and Research Center, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad.

Preparation of bajra millet beverage

Millet grains were cleaned, rinsed with tap water and soaked for 12 hours. After soaking the surplus water was drained and pressure cooked for 18-20 minutes in 1:10 millet to water ratio. The cooked grains were ground to a slurry and diluted to different millet to water ratios (1:4, 1:5, 1:6, 1:7, 1:8) and filtered through a double layer muslin cloth. The filtrate was double boiled up to 80-85°C for 15 minutes and added with 12% of sugar. Finally the product millet beverage was cooled and stored in sterilised glass bottles at 4°C.

Sensory evaluation of unfermented bajra and probiotic beverages

The prepared bajra millet beverages were subjected to sensory evaluation by a semi-trained panel of 20 members using a 9 point hedonic scale (colour, appearance, flavour, taste, consistency and overall acceptability). Acceptability index was calculated from the obtained scores. Based on the sensory scores the millet to water ratio of 1:7 was highly accepted and hence used for development of probiotic beverages.

The developed bajra millet beverage was inoculated with probiotic starter culture at 37°C and incubated for 8, 16 and 24 hours. Based on sensory evaluation scores 8 hours of fermentation was highly accepted. The prepared probiotic millet beverages were filled in sterilised glass bottles and stored at 4°C.

Chemical analysis

Bajra grains were analysed for moisture (IS: 1155-1968 Reaffirmed 2010), Ash (IS: 1155-1968 Reaffirmed 2010), Protein (AOAC 992.23., 32.2.02.), Total Fat (AOAC 922.06-2006), Crude Fibre (AOAC 962.09-2007), Total Carbohydrates (IS 1656:2007 Reaffirmed 2012), Energy content (AOAC 1980), Minerals (AOAC 2015) and bajra beverage was analysed for moisture (AOAC 1990), ash (IS: SP 18 (Part XI) 1981), total fiber (IS 10226-1 1982), fat (IS 1224-2 (1977), total protein content (AOAC 2005) and pH, total soluble solids, viscosity, sedimentation index, whiteness index by (Shunmugapriya *et al.,* 2020a), colour (AOAC 1998), total flavonoids (Lin and Tang, 2007) total phenols (Singleton and Rossie, 1965), total antioxidants (Grau *et al*., 2000).

Statistical studies

Three parallel replicates of mean and standard deviation were calculated. To do pair wise comparisons between quantitative variables, the least significant difference (LSD) approach was used. A 95% confidence interval was used to examine the difference between each level under each variable. ANOVA was calculated for all the attributes of sensory evaluation to test the significance (Johnson, 1972).

RESULTS AND DISCUSSION

As per the results obtained, bajra millet (PBH-1625) grains had moisture, protein, crude fat, crude fiber, total ash, carbohydrates and energy values of 11.46±0.15 g%, 13.07±0.09 g%, 5.74±0.11 g%, 4.43±0.08 g%, 1.40±0.04 g%, 63.89±0.42 g%, 359.51±0.57 Kcal/100g respectively. The results obtained are similar to observations of Reddy *et al*. (2019), Sade, (2009), Singh *et al.* (2018), where as high fiber content was reported by Ali *et al*., (2003). Iron, zinc and calcium content of bajra millet was observed as 4.29±0.05 mg/100 g, 5.84±0.05 mg/100g and 27.45±0.09 mg/100g respectively. Calcium content of 10.0 to 46.0 mg/100g was reported by Himanshu *et al*. (2018), 4 to 8 mg/100g of iron and zinc content were reported by Krishnan and Meera, (2018) and Shankaramurthy and Somannavar, (2019). The large variability of nutrient content among grains could be due to differences in growing environment and genetic differences that influence the nutrient accumulation in food grains.

Based on sensory evaluation, it was observed that among the 5 dilutions of bajra beverage tested, BCM4 was highly acceptable. Highest acceptability index score was observed for BCM4 and lowest for BCM5 due to high dilution, in comparison to control beverage BCM0. BCM4 formula was tested for nutrient composition analysis (Table 1) based on sensory acceptability.

Bajra millet (PBH-1625) beverage was found to contain 84.63±0.06 g% moisture, 1.72±0.05 g% protein and 1.46±0.03 g% fat. The bajra millet protein content was higher with the protein content of coconut milk reported by Sunny *et al*. (2019) and almond milk reported by Kundu *et al*. (2018). The fat content in bajra millet (PBH-1625) beverage is similar to the fat content of (0.50±0.34 g%) millet milk developed by Sunny *et al*. (2019). The developed millet beverage was not a very rich source of ash 0.24±0.01 g% and fiber 0.36±0.02 g% content and similar observations were reported by Sheela *et al*. (2018) in little millet milk and kodo millet milk. The carbohydrate content was 11.57±0.02% and energy content was 66.44±0.27 k.cal/100 ml in bajra millet (PBH-1625), which is in agreement with observations of Sethi *et al*. (2016).

The formula BCM4 was used for development of probiotic beverage. Probiotic starter culture was added to

Table 1: Sensory scores of bajra beverages.

Sample	Sensory attributes						Acceptability
code	Colour	Appearance	Flavour	Taste	Consistency	Overall acceptability	index (%)
BCM0	8.85±0.35^{d}	8.70±0.71^{d}	8.75±0.69^{d}	8.70±0.45^{d}	8.70±0.55^{e}	8.75±0.53^{d}	97.13^{d}
BCM1	8.10±0.83^{c}	7.75±0.62^{b}	7.70±0.78^{bc}	7.80±0.81^{bc}	7.65±0.85^{cd}	7.30±0.64^{b}	85.74^{bc}
BCM2	8.05±0.38^{c}	7.65±0.72^{b}	7.40±0.66^{ab}	7.45±0.58^{b}	7.20±0.60^{ab}	7.30±0.45^{b}	83.42^{b}
BCM3	7.65±0.90^{b}	7.60±0.48^{ab}	7.75±0.76^{bc}	7.55±0.73^{b}	7.30±0.64^{bc}	7.45±0.66^{b}	83.89^{b}
BCM4	8.05±0.58^{c}	8.20±0.60^{c}	8.15±0.85^{c}	8.05±0.80^{c}	8.05±0.86^{d}	8.00±0.83^{c}	88.37^{c}
BCM5	7.05±0.73^{a}	7.25±0.69^{a}	7.10±0.70^{a}	6.75±0.69^{a}	6.85±0.65^{a}	6.75±0.69^{a}	77.29^{a}
Grand mean	7.95	7.85	7.80	7.71	7.62	7.59	85.97
SE of mean	0.07	0.07	0.08	0.08	0.08	0.08	0.78
CD	0.34	0.37	0.46	0.41	0.43	0.37	3.50
CV	6.95%	7.63%	9.39%	8.57%	0.43%	7.92%	6.49%

Note: Values in the columns are expressed as mean±standard deviation. Means with similar letters with in the column do not significantly differ at (P<0.05). BCM0 (Control badam milk), Bajra beverages-BCM1 (1:4 millet to water ratio), BCM2 (1:5 millet to water ratio), BCM3 (1:6 millet to water ratio), BCM4 (1:7 millet to water ratio), BCM5 (1:8 millet to water ratio).

BCM4 formula at 37°C and was incubated for 8, 16 and 24 hours. The three probiotic beverages were subjected to sensory evaluation and the results are presented in (Table 2).

Results of sensory evaluation of the probiotic bajra beverages indicate that, as the fermentation time increased, sensory acceptability decreased. The probiotic beverage fermented for 8 hours was rated to be best among the others and was at par with the control probiotic beverage (commercial probiotic beverage), indicating that bajra probiotic beverage is equivalent to a commercial probiotic beverage. It was also observed that as the fermentation time increased, the sourness in the beverage also increased, due to which the sensory acceptability decreased. The most acceptable probiotic beverage (as per sensory evaluation results-BBP1) was subjected to quality analysis and the results of the same are presented in (Table 2).

The results of pH, total soluble solids, viscosity and sedimentation index is given in (Fig 1). The pH of unfermented bajra beverage was 6.57±0.01 and probiotic bajra beverage was 4.52±0.01, which were significantly (p<0.05) different. The results obtained are similar to the reported values of Shunmugapriya *et al*. (2020a) and Hassan *et al*. (2012). The conversion of acetic acid to lactic acid during fermentation leads to decrease in pH and increase in titrable acidity (Fasreen *et al.,* 2017).

The results also indicate that there was a significant (p<0.05) increase in total soluble solids content of probiotic bajra beverage (12.00±0.14°brix) compared to unfermented bajra beverage (10.86±0.04°brix). Robles *et al*. (2019) reported similar results of increase in total soluble solids in ready-to-drink (RTD) ‘saba’ banana beverage after fermentation. The presence of α-amylase aids the release of free glucose and maltose units from the starches, resulting in higher soluble sugar levels. Budhwar *et al*. (2020) reported that at the beginning of fermentation process, sugars levels seem to be higher but as fermentation time increases, the sugar levels exhibit downward trend, which is due to prolonged period of fermentation resulting in utilization of sugar by fermenting microflora. The viscosity of unfermented bajra beverage was 36.83±1.1cP and probiotic bajra beverage was 39.17±1.7 cP, indicating no significant (p<0.05) difference. Hassan *et al*. (2012) reported that the viscosity behaviour was shown to be related to pH, with maximum values occurring at the lowest pH.

The results indicate that the sedimentation index of unfermented bajra beverage was 0.17±0.01 g/20 ml and probiotic beverage was 0.19±0.00 g/20 ml indicating no significant (p<0.05) variation between unfermented bajra beverage and probiotic beverage. Sudha *et al*. (2016) reported that sedimentation index of millet milk was in range of 0.4 to 0.85 ml/10 ml. Daneshi *et al*. (2013) reported that sedimentation value is related directly to acidity and with increasing acidity, the amount of sedimentation also increases.

The L* value of unfermented bajra beverages and probiotic beverages ranged from 60.78±0.02 to 65.78±0.02 and 69.43±0.03 to 75.51±0.01; a* value ranged from -1.73±0.00 to -0.54±0.01 and -1.66±0.02 to 0.98±0.00; b* value ranged from 11.61±0.02 to 14.44±0.03 and 9.87±0.01 to 14.24±0.02 and DE* value ranged from 26.09±0.07 to 27.70±0.02 and 30.27±0.01 to 34.41±0.01 given in (Fig 2). A significant difference (p<0.05) was observed between the colour values of unfermented bajra beverages and probiotic beverages. The L* value of bajra beverages decreased as the dilution ratio increased and increased as the fermentation time increased. DE* and b* value of probiotic beverages increased as the fermentation time increased. The colour differences in plant-based milk are caused by changes in their composition and structure which occur as a result of the various ingredients and unit operations involved to make them. The concentration and size of any particle matter which scatters light determines the lightness of a plant-based milk substitute (Mc Clements, 2020).

The whiteness index (%) values of bajra beverages ranged from 58.26±0.01 to 63.84±0.02%, which is lower than undiluted bovine milk that has highest whiteness index of 81.89% (Jeske *et al*., 2017). Highest whiteness index (%)

Table 2: Sensory scores of bajra probiotic beverages.

Sample code	Sensory attributes: Colour	Appearance	Flavour	Taste	Consistency	Overall acceptability	Acceptability index (%)
BBP0	8.20±0.81[c]	8.10±0.88[b]	7.70±0.84[bc]	7.45±1.07[b]	8.00±1.04[c]	8.00±0.83[c]	87.86[c]
BBP1	7.85±0.72[bc]	7.65±0.57[a]	8.00±0.63[c]	7.95±0.73[c]	7.90±0.70[c]	7.95±0.66[c]	87.68[c]
BBP2	7.60±0.91[ab]	7.40±0.66[a]	7.40±0.96[b]	7.25±0.99[b]	7.45±0.73[b]	7.20±0.74[b]	81.94[b]
BBP3	7.35±0.79[a]	7.45±0.84[a]	6.85±0.79[a]	6.70±0.95[a]	6.95±0.86[a]	6.70±0.84[a]	77.68[a]
Grand Mean	7.75	7.65	7.48	7.33	7.57	7.46	83.79
SE of Mean	0.09	0.09	0.10	0.11	0.10	0.10	1.00
CD	0.37	0.36	0.42	0.49	0.40	0.40	3.74
CV%	7.66%	7.52%	0.42%	10.71%	8.41%	8.55%	7.05%

Note: Values are expressed as mean±standard deviation. Means with in the column followed by a common letter are statistically similar at 0.05% level. BBP0 (Commercial control probiotic drink), BBP1(8 hours fermentation), BBP2 (16 hours fermentation), BBP3 (24 hours fermentation).

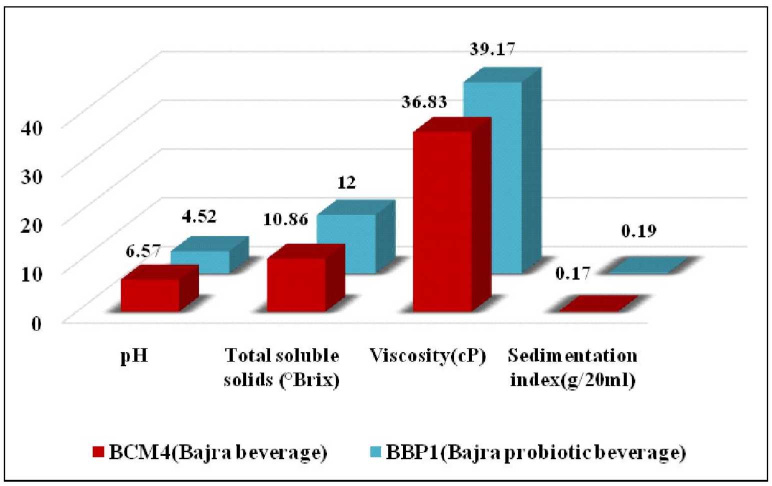

Fig 1: Quality attributes of unfermented bajra and probiotic beverages BCM4 (1:7 millet to water ratio) and probiotic beverage BBP1 (8 hours of fermentation).

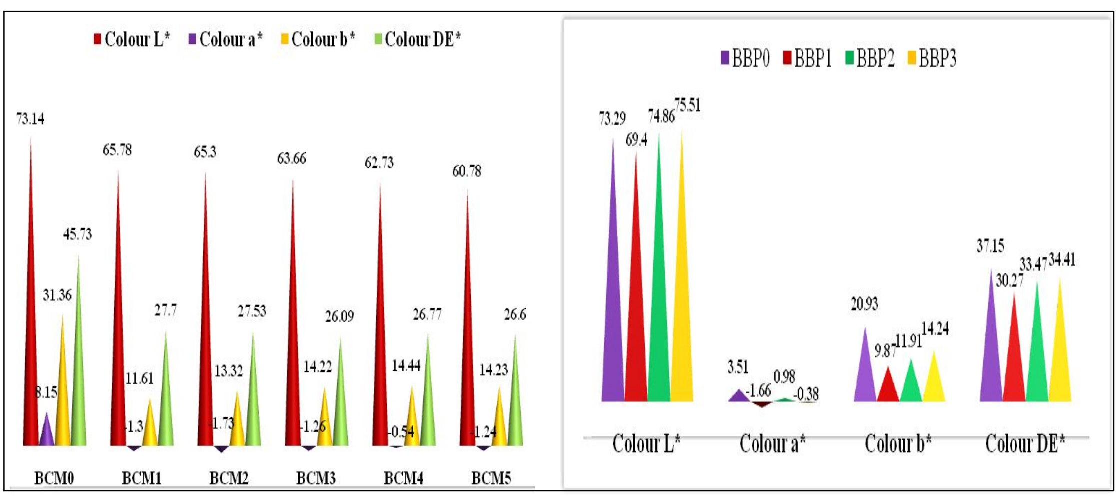

Fig 2: Colour values of bajra beverages and probiotic beverages. a) L* (whiteness/brightness), a* (redness/greenness), b* (yellowness/blueness) and DE* (Total colour difference). Control badam milk (BCM0), Bajra beverages- BCM1 (1:4 millet to water ratio), BCM2 (1:5 millet to water ratio), BCM3 (1:6 millet to water ratio), BCM4 (1:7 millet to water ratio), BCM5 (1:8 millet to water ratio). BBP0 (Commercial control probiotic drink), BBP1 (8 hours fermentation), BBP2 (16 hours fermentation), BBP3 (24 hours fermentation).

Table 3: Bioactive compounds in unfermented bajra beverage and probiotic beverage.

Treatment	Total flavonoids (mg QE/100 g)	Total phenols (mg GAE/100 g)	Antioxidant activity (µg/100 g)
BCM4 (Bajra beverage)	116.7±1.63b	30.98± 0.39a	429.7±1.27b
BBP1 (Bajra probiotic beverage)	86.7±1.63a	34.03±1.28b	321.9±1.27a
Grand mean	102.00	32.50	375.77
SE of mean	7.19	0.80	24.11
CD	8.60	2.89	6.71
CV%	2.40%	2.53%	0.50%

Note: All the values are expressed as mean±standard deviation. Values within the columns with similar letter do not differ significantly at p<0.05. BCM4 (1:7 millet to water ratio), BBP1 (8 hours of fermentation).

Table 4: Mineral composition of unfermented bajra beverage and probiotic beverage.

Treatment	Copper (mg/100 ml)	Magnesium (mg/100 ml)	Manganese (mg/100 ml)	Cobalt (mg/100 ml)	Iron (mg/100 ml)	Zinc (mg/100 ml)	Calcium (mg/100 ml)
BCM4 (Bajra beverage)	$0.21±0.03^a$	$3.03±0.01^b$	$0.06±0.02^a$	$0.05±0.01^a$	$0.27±0.03^a$	$0.31±0.02^a$	$16.28±0.08^a$
BBP1 (Bajra probiotic beverage)	$0.28±0.02^b$	$2.17±0.01^a$	$0.02±0.00^a$	$0.07±0.01^a$	$0.30±0.03^a$	$0.37±0.03^b$	$16.37±0.03^a$
Grand mean	0.25	2.60	0.04	0.06	0.29	0.34	16.32
SE of mean	0.02	0.19	0.01	0.00	0.01	0.01	0.03
CD	0.04	0.03	0.05	0.02	0.01	0.04	0.36
CV%	4.86%	0.41%	33.96%	10.60%	1.40%	3.56%	0.63%

Note: All the values are expressed as mean±standard deviation. Means within the columns followed by similar superscripts do not differ significantly at p<0.05. BCM4 (1:7 millet to water ratio), BBP1 (8 hours of fermentation).

value was observed in BCM1 and lowest in BCM5 indicating that, as the dilution ratio increases, the whiteness index of the beverages decrease which was similar with the findings of Shunmugapriya *et al*. (2020b). The whiteness index values of probiotic bajra beverage values ranged from 67.84±0.02 to 72.16±0.01%. The results indicate that whiteness index % increased as the fermentation hours of probiotic beverages increased.

The significant (p<0.05) decrease in total flavonoid content and antioxidant activity and significant (p<0.05) increase in total phenols content was observed in bajra probiotic beverages (Table 3). The increase in phenol content after fermentation of beverages was similar with the results of obtained by Hassan *et al*. (2012). Dlamini *et al*. (2007) stated the decrease in antioxidant concentration after fermentation is related to processing, that alter the extraction of total phenols and tannins. These modifications were thought to have occurred as a result of interactions between tannins, phenols, proteins and other substances in the grain. Decrease in total flavonoid content, total phenol content and total tannin content was possibly due to phenolic compounds degradation and hydrolysis of bioactive compounds. Extractability was affected due to the breakdown of tannin related molecules into lower molecular weight compounds.

Mineral content was evaluated in the probiotic milk (Table 4). A significant (p<0.05) increase of copper and zinc content, whereas no significant difference in manganese, cobalt, iron and calcium content and significant (p<0.05) decrease was observed in magnesium content in probiotic bajra beverage as compared to unfermented bajra beverage. A study by Sowonola *et al*. (2005) reported 0.08% calcium, 0.31% phosphorous, 0.0021% iron and 0.18% magnesium in pearl millet based Kunnu beverage. Hassan *et al*. (2012) reported the iron, zinc, calcium content as 14.7 mg/L, 2.7 mg/L, 130 mg/L in unfermented rice milk and 15.4 mg/L, 2.7 mg/L, 204 mg/L in fermented rice milk. Though the millets grains are rich in mineral composition, the beverages made with the same millets resulted in very less amount of minerals, that could be due to the dilution of millet extracts with water during processing.

CONCLUSION

Bajra millet can be used for preparation of bajra beverage and probiotic beverage with good nutritional composition and sensory acceptability. The probiotic bajra beverage can be formulated by inoculating probiotic culture followed by 8 hours of fermentation. The beverages had good amount of minerals like calcium, zinc and iron along with bioactive components, which is an indication that plant based milk substitutes like bajra beverages are good sources of nutrients. Millet based beverages can be promoted as a plant based milk alternatives and can also be used for formulating probiotic beverages.

Conflict of interest: None.

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