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Malted Multi-millet based Probiotic Dairy Beverage: Nutritional, Sensory, Physicochemical and Microbiological Characterization

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Abstract

The present study was conducted from November, 2023 to April, 2024, malted multimillet flours (MMF) viz., Finger millet, Foxtail millet and Little millet were used for preparation of probiotic lassi. The millet grains were soaked in water, the steeped grains were incubated at 25ºC 24 h^{-1} for germination and dried in a vacuum tray dryer maintained at 40°C 6 h^{-1}. The dried millet grains were ground into fine powder using commercial flour mill. The equal amount of malted mixed millet flour was used for product preparation.The MMF was incorporated in standardized milk at the rate of 5, 10, 15, 20 and 25% and fermented using probiotic culture containing *Lactobacillus acidophilus, Bifidobacterium and Streptococcus thermophilus*. Control lassi was prepared without addition of multimillet flour. Samples were analysed for proximate composition, sensory, titratable acidity, pH and microbiological parameters. The sensory attributes was measured by using 9 point hedonic scale by panel of semi trained judges. The results revealed that increase in incorporation of multi millet flour had a significant affect (p<0.05) on quality attributes. MMF incorporated products showed higher titratable acidity. The acidity and probiotic count were increased with increased rate of millet incorporation. The probiotic beverage showed above 10^8 CFU ml^{-1} count and improved nutritional and physicochemical profiles, compared to control. Even though the sensory attributes of 15% incorporated MMF probiotic lassi was significantly found superior to control and other concentrations.

Keywords: Finger millet, foxtail millet, little millet, probiotic, lassi

1. Introduction

The development of functional probiotic beverages has shown a marked increase over the last few years, and the consumer's interest and market demands for these products are increasing all over the world due to the health benefits of certain ingredients. Millets are used as food and are widely used in rural areas. India is the largest producer and consumer of millet in the world (Arepally et al., 2023). Finger millet (Ragi) is important millet grown extensively in various regions of India and Africa, constitutes as a staple food for a large segment of the population in these countries (Devi et al., 2014; Kumar and Sivaramane, 2020). It is generally used in the form of the whole meal for preparation of traditional foods, such as roti (unleavened breads or pancake), mudde (dumpling) and ambali (thin porridge) (Hema et al., 2022). Finger millet contains about 5–8% protein, 65–75% carbohydrates, 15–20% dietary fiber and 2.5–3.5% minerals. It has the highest calcium content among all cereals (344 mg 100 g^{-1}). Finger millet as potential prebiotics contains good amount of healthy components (Mitharwa et al., 2021; Sujith et al., 2023).

Nutritional composition of foxtail millet per 100 g includes, carbohydrate (60.9 g), protein (12.3 g), fat (4.3 g), crude fibre (8.0 g), mineral (3.3 g), energy value (351 kcal), iron (2.8 mg), calcium (34 mg) and phosphorus (290 mg). It is rich in magnesium, manganese and phosphorus (Yang et al., 2022). Foxtail millet is good food for the heart as it contain high amount of magnesium (Wandhekar et al., 2021).

Little millet is highly nutrition and may be called little but it is not less in its nutritional content. It has good source of vitamin B, minerals like calcium, iron, zinc, potassium among others. It also provides essential fats to the body, the kind that helps in weight loss. Its high fiber content is yet another positive making it an ideal part of pongal or even kheer instead of rice (Bhat et al., 2018). Additionally, it is being studied for its health benefitting properties such as excellent dietary fibers of these millets can inhibit cardiovascular health risks. Magnesium in millets may prevent heart attacks and migraines, Phytic acid known to lower cholesterol. Potassium helps to lower blood pressure. Millets fiber has potential to decrease cholesterol by removing LDL and boosting the HDL. (Bachate et al., 2022, Patel et al., 2015; Ambati and Sucharitha, 2019).

India is the highest milk producer and ranked first in the world in terms of global milk production, contributing to 24% of total production, India's milk production rose by 4% to 230.58 mt in 2022–23 (Dharmesh et al., 2024). Milk is an excellent medium to carry or generate live and active cultured dairy products. Probiotic fermented milk is one of the major segments amongst fermented milks that have tremendous potential for growth and development. Probiotic has been therapeutically to modulate immunity, improve digestive process, prevent cancer, improve lactose intolerance etc (Fasreen et al., 2017; Kumar et al., 2016). *Lassi* is one of the important ideal fermented milk products for serving with hot dishes as it helps the body to digest the food, itacts as an energizing liquid meal or it provides relief after eating a delicious but hot spicy meal. Thus, *lassi* is a digestive aid for the afternoon meal; it settles the upset stomach and it is the perfect cooling agent and a natural stress buster (Saha et al., 2021). The present study was proposed to optimize nutritious and functional millets based fermented dairy beverage prepared with probiotic culture and evaluate sensory, physico-chemical and microbiological parameters of fortified fermented dairy beverage.

2. Materials and Methods

Cream and skim milk powder was purchased from Amul group, Gujarat, India for standardization of milk.Clean potable drinking water was used for preparation of *lassi* throughout the study period.Good quality commercialgrade cane sugar was used as a sweetener in *lassi*.Good quality Finger millet, Foxtail millet and Little millets were purchased from local market. The probiotic culture containing the *Lactobacillus acidophilus, Bifidobacterium and Streptococcus thermophilus* was procured from Chr. Hansen, Denmark.

2.1. Methodology

2.1.1. Preparation of multi millet flour (MMF)

The foxtail millet, little millet and finger millets grainswere chosen for preparation of probiotic lassi. The Malting of millets was carried out according to Pardhi et al. (2014) with minor modifications. Fresh millet grains were purchased from local market; the dust and dirt particles were removed and cleaned well. The grains were kept for steeping in water (1:3) ratio for 12 h at room temperature. The water was changed periodically for every 4h during steeping.It was then hanged overnight in muslin cloth to remove excess water at room temperature. Steeped grains were incubated at 25±2°C for 24 h for germination. The germinated grains were dried in a vacuum tray dryer maintained at 40°C6 h^{-1}. The dried millet grains were ground into fine powder using commercial flour mill. Subsequently, the finely milled flours were sieved through 100 µm mesh and stored in air-tight containers. The equal amount of malted mixed millet flour was used for product preparation.

2.1.2. Preparation of lassi blended with malted multi millet flour

Fresh buffalo milk was received from the Dairy farm of College of Dairy Technology, Kamareddy. The milk was filtered through muslin cloth and then standardized to 4.5% fat and 8.5% using Pearson's square method. The standardized milk was pre-heated at (60–65°C) and then immediately homogenized (H–102, Goma Pvt. Ltd, Mumbai, India) at 2500 psi –I stage, 500 psi–II stage. The MMF was added slowly to themilk and stirred thoroughly to avoid clot formation of flour. The mixed milk was pasteurized at 90°C for 10 minutes and cooled to 42°C. After cooling 1.5% of probiotic starter culture containing *Lactobacillus acidophilus, Bifidobacterium and Streptococcus thermophilus* was added under aseptic conditions. The inoculated milk was packed (Polystyrene cups) and incubated 42°C for 4–5 hours until firm curd was obtained. Stored at refrigeration temperature (5±1°C) for preparation of lassi.

Five different concentrations of malted MMF was used for preparation of lassi T_1, T_2, T_3, $T_4$and T_5 @ 5, 10, 15, 20 and 25% respectively. The coagulum of curd was broken down by blender with addition of sugar syrup (12% of curd) and water (20%) to obtain homogeneous lassi. The final products (Lassi) was cooled to 5°C and stored in refrigerator (5–7°C) for 2 hours before sensory evaluation. Similarly, T_0 control lassi was prepared using similar procedure without blending of mixed millet flour.

2.2. Sensory evaluation

Sensory evaluation for quality attributes of lassi were evaluated by trained panel of faculty members using a 9-point Hedonic scale viz. colour and appearance, flavour, consistency and overall acceptability.

2.3. Chemical analysis of milk

Proximate composition mainly fat, protein (Kjeldhal method), Carbohydrate (Lane Eynon method), Ash and Total solids content were measured as per procedure given in IS: SP–18, Part XI, (1981). The physico-chemical characteristic such as pH and acidity was determined as per procedure given in IS: SP-18, Part XI, (1981).

2.4. Microbial evaluation of lassi

Lactobacillus acidophillus and Streptococcal count of inoculated lassi samples was determined as per the method described in Indian Standards (IS: 1479, Part III, 1962).

2.5. Statistical design

Statistical analysis of samples was performed using SPSS.23.0 (IBM SPSS, Chicago, USA) software, and the data were assessed using one-way or two-way analysis of variance

(ANOVA) and the treatment means were compared using Post hoc Duncan's multiple range test at 0.05 significance level. The results are presented as means ± standard deviation. All the experiments were performed in triplicate.

3. Results and Discussion

3.1. Sensory evaluation

The sensory attributes of the control lassi and blended malted MMF lassi samples were measured using 9 point hedonic scale by a panel of semi trained judges in terms of color and appearance, flavor, body and texture, acidity and overall acceptability using 9 point hedonic scale by a panel of trained judges. The results pertaining to sensory are presented in Figure 1. Rate of increasing level of MMF addition had a significant (p<0.05) effect on all the sensory parameters of the products. Control lassi (T_0) scored higher (8.50) on hedonic scale, highest flavor score (8.46) was obtained for lassi fortified with treatment 15% of MMF (T_3) which differ significantly (p<0.05) from other concentrations that score being 8.25, 8.34, 8.31, 8.20 for T_1, T_2, T_4 and T_5 respectively which were at par with each other indicating that the flavour of MMF added products were accepted and liked same as control by the expert panel of judges. No significant difference (p<0.05) was found between Treatment T_3 and T_0 with respect to flavor. These results are in agreement with Vila-Real et al. (2022), reported the synbiotic fermented finger millet-based yoghurt like beverage improved sensory properties compared to the unfermented control.

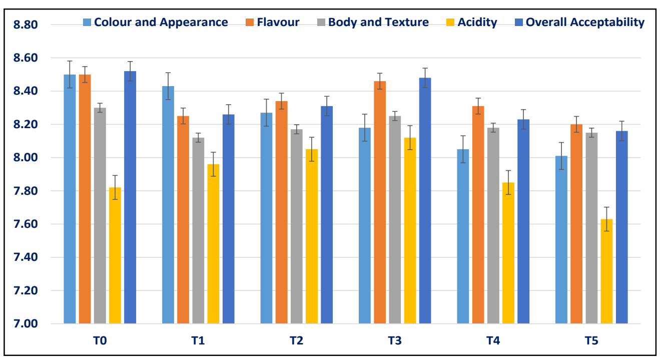

Figure 1: Effect of rate of addition of malted MMF on sensory attributes of probiotic lassi

The colour and appearance score for (T_0) showed significantly higher (8.5) over the treatments. Among all the products body and texture of control sample scored the maximum score (mean value 8.30). This may be because the control lassi and MMF lassi products differed particularly in terms of smoothness of the body. MMF fortified lassi increased from 5 to 15% (8.12 to 8.25) further increased rate of addition about 20 and 25% decreased the body and textural characteristics due to more viscous and consistency of the product. Similar results were reported by Jayashri et al., 2022, who prepared misti dahi by addition of finger millet and foxtail millet flour at 2, 4 and 6%. They reported that addition of 4% mixed millet flour showed highest overall acceptability when compared with other treatments and control. The acidity score for T_0, T_1, T_2, T_3, T_4 and T_5 lassi was 7.82, 7.96, 8.05, 8.12, 7.85 and 7.63, respectively. The acidity increased with increase in the level of millet flour. The acidity score for T_3 sample was highest while lowest score was observed in the sample T_5. The overall acceptability of 15% incorporation of MMF lassi (8.48) showed no significant difference when compared with control sample (8.52). These results are in agreement with Anil and Anamika (2015), reported that the overall acceptability of millet lassi increased with increase in level of sorghum flour.

3.2. Proximate composition

Based on the sensory evaluation upto 15% addition of MMF was showed acceptable sensory score. Therefore, the control lassi and MMF lassi samples (5, 10 and 15% addition) were assessed for its composition and results are represented in Table 1. The fortification of lassi with MMF increased the nutritive value of the product. The total solid content of lassi ranged from 24.54±0.95 to 29.94±0.36%, the values were significantly different among the samples. Fat content varies significantly (p<0.05) for the samples T_0 to T_3 except for T_1 and T_2. The lower fat content was found for control lassi (3.78±0.18) while the highest for 15% MMF lassi (3.96±0.21). This might be due to high fat content in little millet (4.7 g) and foxtail millet (4.3 g) (Gowda et al., 2021). The increasing trend of protein content of the lassi samples were in the range of 3.67, 4.53, 5.39 and 6.61%, while the carbohydrate content was 16.25, 18.64, 19.91 and 21.37% for T_0, T_1, T_2 and T_3 samples respectively. All the lassi samples had significantly different protein, carbohydrate and ash content. Millets are rich source of protein, carbohydrate and ash content (Rana and Bhandari, 2023), with sample T_3 having the highest values. On increasing the proportion of MMF, there was significant difference in total solids, fat, protein, carbohydrate and ash content of lassi from T_0 to T_3.

Table 1: Effect of incorporation of different levels of malted mixed millet flour on proximate composition of probiotic lassi

Samples	Total solids (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)
Control lassi	24.54±0.95^a	3.78±0.18^a	3.67±0.57^a	16.25±0.66^a	0.85±0.26^a
5% MMF lassi	26.82±0.72^b	3.81±0.10^b	4.53±0.36^b	18.64±0.78^b	0.93±0.14^b
10% MMF lassi	27.71±0.65^c	3.89±0.15^b	5.39±0.68^c	19.91±0.35^c	1.07±0.19^c
15% MMF lassi	29.94±0.36^d	3.96±0.21^c	6.61±0.71^d	21.37±0.83^d	1.13±0.35^d

The values bearing different alphabets significantly differ (p<0.05) among the rows

3.3. Titratable Acidity and pH

The effect of mixed millet flour on titratable acidity of the probiotic lassi is shown in Figure 2. The mean acidity value of products prepared using millets, were in the range of 0.68 to 0.73% LA respectively. On the other hand, rate of incorporation of mixed millets had a significant (p<0.05) effect on acidity of the products. Compared to control lassi (T_0), the MMF incorporated lassi showed a higher titratable acidity and the value increased with increase in the rate of addition. The increase of acidity might be due to more carbohydrate content in millets (60–72 g), as a result hydrolysis of some complex organic molecules during germination. Moreover, higher total solids per cent in germinated lassi sample contributes to more acidity when compared to control (Gyawali, 2021). Highest acidity 0.73% LA was observed in product prepared using 15% MMF. Results are in close concord with Rani et al. (2018) who reported germinated whey pearl millet based fermented beverage (Lassi) increased acidity when compared with control and soaked pearl millet lassi. Titratable acidity results had shown reverse trend to pH. The pH of prepared lassi samples was in the range of 4.76 to 4.58 (Figure 2). Millet incorporated lassi samples had significantly (p<0.05) lower value of pH as compared to control.

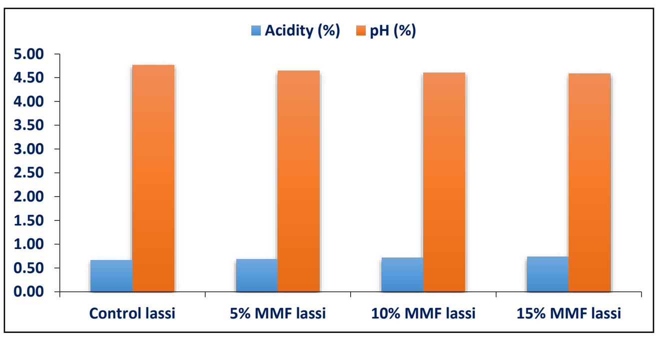

Figure 2: Effect of rate of addition of MMF on titratable acidity and pH of lassi samples

3.4. Probiotic and streptococcal counts

Effect of different levels of MMF on viability of *Streptococcus thermophilus* and probiotic *Lactobacillus acidophilus*is shown in Table 2. The rate of addition of MMF had a significant (p<0.05) effect on viability of both strains. All the probiotic lassi samples blended with MMF showed *Streptococcal count and L. acidophilus* above 10^8 log cfu g^{-1}.The increase in counts of both strains was linear with increase in concentrations of MMF from 0 to 15%. This may be due to good source of carbohydrate and total solids in the MMF lassi samples as compared to control. During germination, the amylases partially hydrolyze the starch to simple sugars, lower molecular weight carbohydrates such as disaccharides and oligosaccharides (Shobana et al., 2013). The lactic acid bacterial strains can utilize these simple sugars for their growth.

Table 2: *Streptococcal* and probiotic count of lassi prepared using different rates of incorporation of MMF

Samples	Streptococcal count (log cfu g^{-1})	L.acidophilus (log cfu g^{-1})
Control lassi	9.35±0.05[a]	9.57±0.11[a]
5% MMF lassi	10.21±0.02[b]	10.30±0.08[b]
10% MMF lassi	10.48±0.08[c]	10.81±0.13[c]
15% MMF lassi	10.93±0.07[d]	10.90±0.16[d]

The values bearing different alphabets significantly differ (p<0.05) among the rows

4. Conclusion

The use of malted multimillet flour improved the product nutrition and quality attributes in comparison to control. Rate of incorporation of MMF had shown a significant (p<0.05) effect on all quality attributes. The total solids, carbohydrate, protein, minerals, probiotic counts, titratable acidity increased with increase in concentrations of MMF. Therefore, it may be concluded that more nutritive and acceptable probiotic lassi could be prepared by addition of 15% MMF to eradicate the malnutrition problems.

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