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# Effect of Supplementation of Prebiotics on Biochemical, Sensory and Microbial Characteristics of Foxtail Millet Based Probiotic Beverage

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ABSTRACT

There is an increasing demand for functional foods from health-conscious consumers. Among the several functional foods types, the probiotic, prebiotic and symbiotic play a significant role in human health and nutrition. Most of the probiotics are lactic acid bacteria and traditional dairy products. Very few attempts are made for development of probiotic beverage / foods using other substrates like millets which are non dairy based favoring the consumption by lactose intolerant consumers. Foxtail millet (*Setaria italica*) is one of the most important food crops of the semiarid tropics, considering its richness of dietary fibres and other carbohydrates. An attempt was made to study the effect of supplementation of prebiotics like honey (2 %), liquid whey (5 & 10 %) and their combinations on bio-chemical, sensory qualities and viability of lactic acid bacteria of foxtail millet based probiotic beverage. The results revealed that foxtail millet malt slurry supplemented with 2 per cent honey + 10 per cent whey fermented with lactic acid bacteria (*Lactobacillus acidophilus* MTCC 10307) resulted in better product in terms of pH (4.15), TSS (14.50 $^{0}$ brix), titrable acidity (0.59 %), sensory attributes, overall acceptability (17.00/20.00) and viable lactic acid bacterial population ($2.30\times10^{11}$cfu/ml) when compared to other treatment combinations. There was a significant difference in nutritional quality and LAB counts due to addition of prebiotic supplements as compared to without supplements.

*Keywords:* Foxtail millet slurry, Supplementation of prebiotics, Whey, Honey, Probiotic beverage

PROBIOTIC beverages / products are usually marketed in the form of fermented milks and yoghurts which are accepted as dairy probiotic beverages. However, with an increase in the consumer vegetarianism, there is a demand for the vegetarian probiotic products. Further more, lactose intolerance and the cholesterol contents are two major drawbacks related to the fermented dairy products. There are a wide variety of traditional non-dairy fermented beverages produced around the world. The non-dairy probiotic beverages may be made from a variety of raw materials, such as cereals, millets, legumes, fruits and vegetables. Probiotics are live microorganisms that when administered in adequate amounts and regularly confer benefit to the host's health, improving the intestinal micro biota balance and defences against pathogenic microorganisms. Prebiotics are defined as non-digestible food ingredients, which are also given regularly and in appropriate amounts and beneficially affect the host because they selectively stimulate the multiplication and activity of probiotic bacteria. Symbiotic means a mixture between probiotics and prebiotics, which is their synergistic combined action on nutrition and human health. Vasudha and Mishra (2013) reviewed the non-dairy probiotic beverages and reported that there was a demand for the vegetarian probiotic products owing to health considerations and from the perspective of cholesterol in dairy products. Very few attempts are made for the development of probiotic beverage / foods using other substrates like millets, cereals, fruits and vegetables which are non dairy based and favouring the consumption by lactose intolerant consumers. Fasreen *et al*. (2017) developed finger millet based probiotic beverage using *Lactobacillus casei* 431.

Foxtail millet (*Setaria italica*) is a good source of proximate minerals and crude fiber and these nutritional properties have made it an important ingredient for preparing noodles, soup, brewing alcoholic beverages, cereal porridges and pancakes in China (Yang *et al.,*

2013). In addition to its nutritional properties, foxtail millet has also shown to possess several health benefits like prevention of cancer, hypoglycemic and hypo lipidemic effects (Zhang *et al.,* 2015). An alternative to increase the nutritional value of millet-based products is by combining millet, prebiotics like honey and whey supplementation. The combinations of millet, milk and honey will have a synergistic effect to provide enhanced nutrition and will ultimately lead to value added functional product. Prebiotics are added in functional probiotic food formulation to increase the viability of probiotics and shelf life of the product. Earlier studies on the effect of honey on the growth of bifidobacteria and found enhancement in the growth, activity and viability of commercial strains of bifidobacteria used in the manufacture of fermented dairy products which were supplemented with the honey. Several research workers have reported on possibility of utilizing the milk whey in the fermented beverage preparation (Shukla *et al*., 2013). Hence, the present study was takeup to determine the effect of supplementation of prebiotics on biochemical, sensory and viability of lactic acid bacteria in the foxtail millet based probiotic beverage.

## Material and Methods

### Collection and Preparation of Foxtail Millet Substrate for Probiotication

Foxtail millet grains were collected from the AICRP on Millets Scheme, University of Agricultural Sciences, GKVK, Bengaluru for the experimentation. The collected foxtail millet grains were cleaned and washed thoroughly and were imbibed in distilled water for 24 hrs under room temperature. The imbibed grains were kept for germination in a tray using sterile muslin cloth for 48 hrs under room temperature with frequent spraying of water. After 48 hrs, the sprouts /germinated grains were kept for drying under tray drier at 50 $^0$C for 24 hours. Dried sprouts were de husked using millet de husking machine. De husked grains were separated from husk and processed by milling under domestic millet flour mill to get flour, sieved and was used as a substrate for the preparation of probiotic beverage. The process flow chart for the preparation of foxtail millet malt slurry (Musturi Begum, 2003) is presented in Fig 1.

Washing of raw grains of foxtail millet
↓
Soaking in water (24 hrs)
↓
Germination of grains (48 hrs)
↓
Tray drying at 50 - 55º C (48 hrs)
↓
De husking of grains
↓
Milling de husking grains in grinder
↓
Sieving (18 mm mesh)
↓
Mixing of flour with water in the ratio of 1: 15 w/v
↓
Addition of sugar to attain 15º brix
↓
Autoclaving
↓
Sterilized foxtail millet malt slurry as substrate

Fig. 1: Flow chart for the preparation of foxtail millet malt slurry

### Preparation of Probiotic LAB Starter Culture

A loopful inoculum of purified probiotic lactic acid bacteria *Lactobacillus acidophilus* was inoculated to conical flask containing 100 ml of sterile MRS broth and incubated at 37 $^0$C for 48 hour.

### Experimental Setup

An experiment to assess the influence of supplementation of prebiotics like honey and whey on nutritional quality and viable counts of LAB of the foxtail millet based probiotic beverage was conducted (Plate 1) with 10 per cent substrate concentration, 15 per cent sugar concentration, inoculum level of 7.5 per cent and fermentation period of 24 hrs with following treatments in triplicates- Treatments : 7, Replications : 3

T1 : Foxtail millet slurry control

T2 : Foxtail millet slurry + LAB

T3 : Foxtail millet malt slurry + 2% honey + LAB

T4 : Foxtail millet malt slurry + 5% liquid whey + LAB

T5 : Foxtail millet malt slurry + 10% liquid whey + LAB

T6 : Foxtail millet malt slurry + 2% honey + 5% liquid whey + LAB

T7 : Foxtail millet malt slurry + 2% honey + 10% liquid whey + LAB

Note: Foxtail millet Substrate – 10%, Sugar – 15%, Inoculum concentration – 7.5% and Fermentation period- 24 hrs

Plate 1 : Experimental set up for fermentation of foxtail millet malt slurry supplemented with prebiotics

*Honey*: Commercial Dabur honey was supplemented to the foxtail millet slurry at 2 per cent in the treatments where ever it is required.

*Liquid whey*: The prepared whey was supplemented to the foxtail millet slurry at 5 and 10 per cent in the treatments wherever it is required.

**Bio-Chemical Analysis**

The pH of the sample was measured using digital pH meter (Digital pH meter type MK-VI). Total titrable acidity was expressed as per cent lactic acid (Srivastava and Kumar, 1993). The total soluble solids of the sample were determined with the help of 'ERMA' hand refractometer having a range of 0 to 35º brix at room temperature. The assessment of microbial population of the fermented samples was done by employing standard plate count method (Hoben and Somasegaran, 1982) and the results were expressed in terms of colony forming units / ml of the sample (cfu / ml).

**Sensory Evaluation**

The blended foxtail millet fermented beverages were subjected to sensory evaluation with respect to color/ appearance, taste, sweetness, mouth feel and overall acceptability by a semi-trained panel of 10 judges using 20 point hedonic scale (Walts *et al.,* 1989).

**Statistical Analysis**

The data emerged from the investigation was subjected to analysis of variance using WASP 2.0 tool by Completely Randomized Statistical Design. The data obtained from the experiments were subjected to statistical analysis to evaluate treatment effects.

## Results and Discussion

The experimental results pertaining to changes in pH, TSS, titrable acidity and total bacterial counts of probioticated foxtail millet beverage (Plate 2) as influenced by supplementation with honey and liquid whey are presented in Table 1.


Plate 2 : LAB fermented foxtail millet probiotic beverage supplemented with honey and liquid whey combinations

*pH:* The initial pH of foxtail millet malt slurry was 5.82. LAB fermentation of foxtail millet malt slurry supplemented with honey and whey for 24 hours decreased the pH to 4.15 and 4.29, respectively when compared to initial pH. However, highest reduction in pH (4.15) was observed in foxtail millet supplemented with 2 per cent honey + 10 per cent liquid whey (T7). Changes in pH among the different treatments after LAB fermentation may be due to the ability of bacteria to convert sugars into organic acids (lactic acid) and reduction in pH is a clear indication of utilization of sugars by the probiotic bacteria. Reduction in pH was found more in prebiotic supplemented fermented foxtail millet probiotic beverage compared to treatment without supplementation. This may be due to the consumption of blended substrates (honey and liquid whey) and production of acids during LAB fermentation. These results were on par with the results reported by Sharma *et al*., 2017 in the production of whey-oat based product using lactic acid bacteria. Similar results were found in the research conducted in the fermentation of oat-based mash using *Lactobacillus plantarum* B28. Similarly, Sangita Ganguly (2013) obtained pH between 4.0-4.20 in whey-cereal based probiotic beverage.

TABLE 1

Changes in biochemical and microbiological parameters of LAB fermented foxtail millet slurry as influenced by supplemented with Honey and Whey

| Treatments | pH | TSS (°brix) | T.A (% lactic acid) | LAB population (cfu/ml) |
|---|---|---|---|---|
| T1 : Foxtail millet slurry control | 5.82 $^{a}$ | 19.00 $^{a}$ | 0.09 $^{d}$ | 0 |
| T2 : Foxtail millet slurry + LAB | 4.29 $^{b}$ | 18.00 $^{b}$ | 0.42 $^{c}$ | $1.70\times10^{9}$ |
| T3 : Foxtail millet malt slurry + honey (2%) + LAB | 4.21 $^{cd}$ | 18.00 $^{b}$ | 0.47 $^{b}$ | $6.11\times10^{10}$ |
| T4 : F. millet malt slurry + 5% liquid whey + LAB | 4.25 $^{bc}$ | 17.00 $^{c}$ | 0.45 $^{bc}$ | $1.14\times10^{11}$ |
| T5 : F. millet malt slurry + 10% liquid whey + LAB | 4.24 $^{bc}$ | 17.00 $^{c}$ | 0.46 $^{bc}$ | $3.76\times10^{11}$ |
| T6 : F. millet malt slurry + honey (2%) + 5% liquid whey + LAB | 4.19 $^{cd}$ | 17.50 $^{bc}$ | 0.55 $^{a}$ | $1.40\times10^{11}$ |
| T7 : F. millet malt slurry + honey (2%) + 10% liquid whey + LAB | 4.15 $^{d}$ | 17.50 $^{bc}$ | 0.59 $^{a}$ | $2.30\times10^{11}$ |

*TSS*: The initial TSS of the blended foxtail millet malt slurry was 19º brix. LAB fermentation of foxtail millet malt slurry varied from 17.0 to 18.0º brix between the treatments after 24 hours of fermentation. Highest reduction in TSS (17.0º brix) was observed in foxtail millet malt slurry supplemented with 5 - 10 per cent of liquid whey respectively (T4 and T5). Least reduction in TSS (18.0º brix) was achieved in foxtail millet malt slurry without blending (T2) and with supplementation of 2 per cent honey (T3), which may be due to the sweetness of the added honey. TSS reduction in foxtail millet malt slurry supplemented with 2 per cent honey + 5 per cent of liquid whey (T6) and supplemented with 2 per cent honey + 10 per cent of liquid whey (T7) were on par with each other. Hence, these results indicate that addition of liquid whey influenced the efficiency of LAB in utilization of sugar. Changes in TSS between LAB fermented honey and whey supplemented treatments might be due to change in fermentation efficiency and sugar conversion by the lactic acid bacteria. Similar results were obtained from the research conducted by Shukla and Kushwaha (2017) in the development of probiotic beverage from Whey and Orange juice using *Lactobacillus acidophilus*.

*Titrable acidity*: The initial titrable acidity of blended foxtail millet malt slurry was 0.09 per cent (% lactic acid). After LAB fermentation for 24 hours, titrable acidity of treatments varied between 0.42 and 0.59 (Fig 2). There was a significant difference in titrable acidity between blended and non-blended LAB fermented beverages, which might be due to influence of blending substrates (honey and liquid whey) in fermentation efficiency which may be attributed to the enhancement in titrable acidity. These results were in support with work of Sasikumar (2015) who reported that increase in acidity to 0.525 per cent in fermented whey blended with Aloe vera juice. Similarly, developed a probiotic cabbage juice using lactic acid bacteria and observed an increase in titrable acidity. Fermentation has been reported to cause decrease in pH with a simultaneous increase in titrable acidity in several fermented products prepared from pearl millet, wheat, barley, rice, corn, cowpea, chickpea, soybean and their blends. These studies have concluded that increase in titrable acidity of the beverage may be due to the production of organic acids (lactic acids) which adds to the sourness and acidity taste in the fermented beverage and its benefits to the human health. Similar is the case in the present study wherein, fermentation of foxtail millet slurry with prebiotic supplements like whey and honey resulted in significant increase in the titrable acidity of the beverage.

*Viable counts of lactic acid bacteria*: The results of the present study revealed that foxtail millet malt slurry supplemented with 10 per cent of liquid whey (T5)

revealed highest LAB population of ($3.76\times10^{11}$cfu/ml) followed by 2 per cent honey + 10 per cent whey ($2.30\times10^{11}$cfu/ml) (T7) (Fig. 2 and Plate 3), which were more than the population recorded by Shukla *et al.,* 2013 in the pineapple whey based probiotic beverages. Foxtail millet malt slurry without supplementation (T2) had lowest population of $1.70\times10^{9}$ cfu/ml which is less than the population recorded in oat based probiotic drink by LAB fermentation where total LAB population observed was $7.5\times10^{10}$cfu/ml.

The population of lactic acid bacteria (*Lactobacillus acidophilus*) increased from $10^{9}$ to $10^{11}$cfu/ml in foxtail millet slurry supplemented with 10 per cent liquid whey; this could be attributed to millet malt slurry and supplemented whey were a source of good media for bacterial growth. Increase in the bacterial population was mainly due to the blending of millet malt with whey protein materials. Liquid whey at 5 per cent had provided a very good nutrient for the multiplication of lactic acid bacteria. The required standard codex

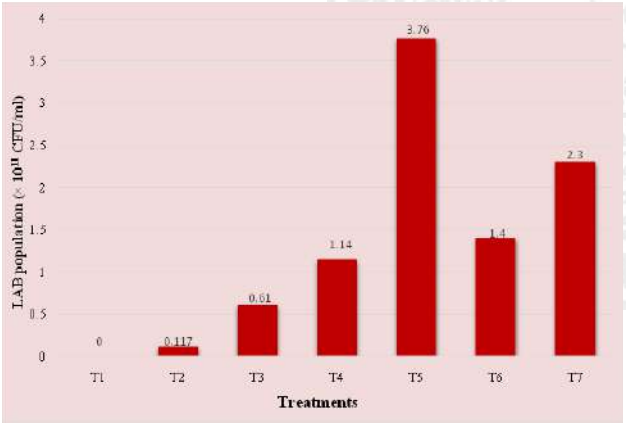


Fig. 2 : Variation in lactic acid bacterial population in foxtail millet based beverage influenced by supplementation of Honey and Whey

Note : T1 : Foxtail millet slurry control;
T2 : Foxtail millet slurry + LAB;
T3 : Foxtail millet malt slurry + honey (2%) + LAB;
T4 : Foxtail millet malt slurry + 5% liquid whey + LAB;
T5 : Foxtail millet malt slurry + 10% liquid whey + LAB;
T6 : Foxtail millet malt slurry + honey (2%) + 5% liquid whey + LAB;
T7 : Foxtail millet malt slurry + honey (2%) + 10% liquid whey + LAB;

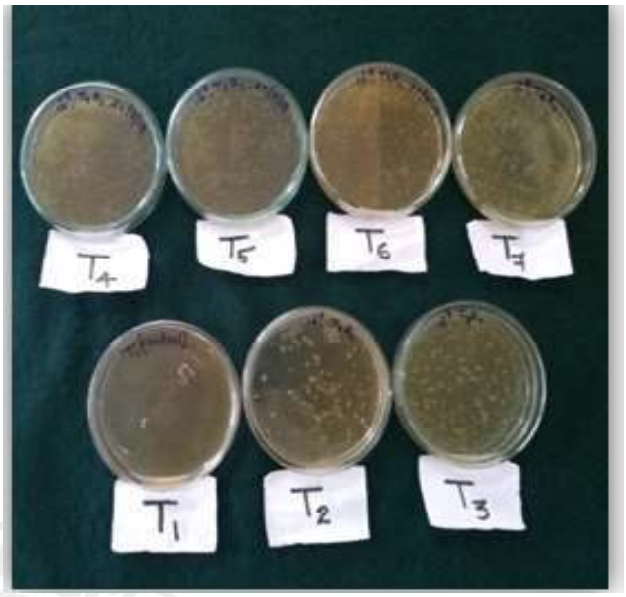


Plate 3: Lactic acid bacterial population in the foxtail millet probiotic beverage as influenced by the supplementation of Honey and Whey

Note: T1 : Foxtail millet slurry control;
T2 : Foxtail millet slurry + LAB;
T3 : Foxtail millet malt slurry + honey (2%) + LAB;
T4 : Foxtail millet malt slurry + 5% liquid whey + LAB;
T5 : Foxtail millet malt slurry + 10% liquid whey + LAB;
T6 : Foxtail millet malt slurry + honey (2%) + 5% liquid whey + LAB;
T7 : Foxtail millet malt slurry + honey (2%) + 10% liquid whey + LAB;

population for probiotic beverage was well maintained by probiotic lactic acid bacteria in all the foxtail millet based beverage treatments. Hence, fermentation of foxtail millet malt slurry by *Lactobacillus acidophilus* was found very much suitable for the production of probioticated millet based beverages. These results support the work of Gupta *et al*. (2010) who reported that viability of lactic acid bacteria in the cereal based fermented drink was 10.4 log cfu/ml.

**Changes in sensory characteristics of LAB fermented foxtail millet malt slurry supplemented with honey and whey are shown in Table 2**

*Organoleptic Evaluation* : Fermented beverage used for human consumption should be evaluated by organoleptic procedure as they have pleasant aesthetic refreshing qualities and attract due to their color and

TABLE 2

Sensory attributes of LAB fermented foxtail millet slurry blended with Honey and Whey (20 points hedonic scale)

| Treatments | Appearance (2) | Color (2) | Aroma (2) | Bouquet (2) | Acidity (2) | Sweetness (2) | Body (2) | Astringency (2) | Flavour (2) | Quality (2) | Overall accept (20) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T1 | 1.30 | 1.30 | 1.00 | 1.00 | 0.50 | 1.80 | 1.50 | 1.00 | 1.00 | 1.00 | 11.40 |
| T2 | 1.50 | 1.50 | 1.20 | 1.40 | 1.80 | 1.50 | 1.40 | 1.50 | 1.50 | 1.40 | 14.70 |
| T3 | 1.65 | 1.65 | 1.45 | 1.45 | 1.60 | 1.60 | 1.50 | 1.40 | 1.70 | 1.50 | 15.50 |
| T4 | 1.75 | 1.75 | 1.43 | 1.50 | 1.80 | 1.40 | 1.70 | 1.55 | 1.80 | 1.70 | 16.38 |
| T5 | 1.65 | 1.65 | 1.45 | 1.55 | 1.82 | 1.45 | 1.60 | 1.60 | 1.70 | 1.65 | 16.12 |
| T6 | 1.68 | 1.68 | 1.50 | 1.50 | 1.82 | 1.55 | 1.60 | 1.60 | 1.75 | 1.60 | 16.28 |
| T7 | 1.70 | 1.70 | 1.60 | 1.60 | 1.85 | 1.55 | 1.75 | 1.70 | 1.80 | 1.75 | 17.00 |

Note:

T1 : Foxtail millet slurry control T2 : Foxtail millet slurry + LAB T3 : Foxtail millet malt slurry + honey (2%) + LAB

T4 : Foxtail millet malt slurry + 5% liquid whey + LAB T5 : Foxtail millet malt slurry + 10% liquid whey + LAB

T6 : Foxtail millet malt slurry + honey (2%) + 5% liquid whey + LAB T7 : Foxtail millet malt slurry + honey (2%) + 10% liquid whey + LAB

tactile sensation. Therefore, it is essential to go for organoleptic evaluation. Sensory evaluation was carried out for the developed probiocated blended foxtail millet beverages by following 20 point hedonic scale by a panel of 10 judges and recorded the data on organoleptic evaluation (Table 2). The results revealed that the foxtail millet slurry supplemented with 5 per cent liquid whey only (T4) and supplemented with honey (2%) and whey (10%) (T7) showed highest sensory attributes with respect to appearance (1.75 & 1.70 /2.00), color (1.75 & 1.70 /2.00), aroma (1.43 & 1.60 /2.00), bouquet (1.5 & 1.6 /2.00), acidity (1.80 & 1.85 /2.00), sweetness (1.40 & 1.55 /2.00), body (1.70 & 1.75 /2.00), astringency (1.55 & 1.70 / 2.00), flavor (1.80 & 1.80 /2.00) and quality (1.70 & 1.75 /2.00) respectively compared to other treatment combinations including control treatment.

*Overall Acceptability*: Sensory evaluation scores with respect to overall acceptability for LAB fermented beverages influenced by supplementation of honey and whey were in between 11.40 to 17.00 /20.00 between treatments. However, foxtail millet slurry supplemented with 5 per cent liquid whey only (T4) and supplemented with honey (2%) and whey (10%) (T7) fermented by lactic acid bacteria which showed highest sensory attributes with respect to over all acceptability (16.38 & 17.00 /20.00) respectively and least sensory score (11.40) was recorded in control treatment (T1). Organoleptic evaluation of LAB fermented beverage from foxtail millet supplemented with honey and whey showed higher acceptability for consumption. Higher sensory score for overall acceptability might be due to LAB fermentation and blending with honey may be helping in acidity modulation for higher acceptability for consumption. These results were in support of results reported by Latha *et al.* (2014) reported in kokum juice blended with honey.

Most of the probiotic foods are dairy based products which are usually marketed in the form of fermented milk, yoghurt, cheese *etc*. However, with an increase in the consumer demand by vegetarian, there is also demand for the non-dairy based (millets, fruits and vegetables) probiotic products and also favour for consumption by lactose intolerant consumers. The present study results conclude that the foxtail millet malt slurry supplemented with 5 per cent liquid whey

and 2 per cent honey fermented by probiotic lactic acid bacteria resulted in better product in terms of biochemical (pH, TSS, titrable acidity), sensory attributes, overall acceptability and with highest viable lactic acid bacterial population. The study indicates that there was a significant difference in nutritional quality, acceptability for consumption and LAB counts due to addition of prebiotic supplements as compared to without supplements. Hence, this minor millet *viz.,* foxtail millet could be used as a substrate for the production of non-dairy based probiotic beverage using lactic acid bacteria.

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