

Original Research Article

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Standardization and Quality Evaluation of Non-Dairy Probiotic Beverage Prepared from Finger Millet and Foxtail Millet

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ABSTRACT

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Probiotics are microbial food supplements containing live organisms which exact beneficial effects on consumer's health. The great interest has been focused on the development of non-dairy probiotic foods. It was made to develop such kind of probiotic food which can be used as functional food. Finger and foxtail millet malt based probiotic beverage was formulated which comprises of health benefits from both millet malt and probiotics. Pasteurized malt having 10° Brix was inoculated with *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* and incubated at 37°C for 6 hrs. The beverage is formulated with addition of sucrose and stored under refrigerated (4°C) conditions. Changes in physicochemical characteristics (pH, acidity, brix) and viable cell count during refrigeration were monitored. Titrable acidity was increased significantly due to lactic acid production by probiotics. Reducing and non reducing sugars were decreased significantly because of usage by probiotics. The study concluded that finger and foxtail malt based probiotic beverage can be developed with *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* and it could be kept in refrigerated (4°C) condition up to 25 days.

Introduction

The word probiotic means “for life”, and it was given by Kollath in 1950, It was first invented by Lilly and Stillwell to live microorganisms (bacteria and spores) as a supplement for living beings that should help in restricting the use of antibiotics in animal

husbandry. The very first definition of probiotic is “These are the live microorganism feed supplement which has benefits to the host body by balancing the microbiota of intestine of the living beings” (Fuller, 1992). Probiotics offer increased resistance to infection by harmful microorganisms in the intestine, reduces the

duration of diarrhea. Probiotics promote the digestion of the lactose in the intestine, increases the bioavailability of the nutrients i.e. it increases the nutritional value but better digestibility increases the vitamin absorption and also absorption of minerals they regulate the gut function (Irritable bowel syndrome, constipation) reduces the blood cholesterol concentration, prevents the risk of cancer, maintain the mucosal integrity of intestine reduce the carcinogen production in the body (Orrhage and Nord, 2000).

Cereals and millets had been world's most important sources of the food. About one-third of the diet worldwide is fermented food (Campbell, 1994). Millets have been cultivated for a long time in different regions of North Asia and Africa. Millets contain about 10-12% of proteins. The protein content in millets is of good quality than that of wheat and corn i.e. millet protein is rich in amino acids. The gluten content of the millets is lower; therefore, it is suitable for many products. *Lactobacillus* species are known as a complex microorganism, these microorganisms require fermentable carbohydrates, B vitamin, amino acids, nucleic acid, minerals for their growth. From this, cereals may represent the best way to obtain the substrate for the growth of microorganisms which are beneficial for health.

For the development of functional food, probiotic strains for the fermentation of cereal substrate area good approach. Cereals and millets proved to be suitable substrates for lactic acid fermentation, and the use of probiotic microorganisms as starter cultures could result in probiotic products. These probiotic products are appropriate for vegan peoples, to avoid the lactose intolerance problem. It contributes to the functional food group (Gomes and Malcato, 1999).

Materials and Methods

Raw material

The raw material required for the preparation of Millet based Probiotic Beverage such as finger millet, foxtail millet, sugar etc. are procured from local market. The culture of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* was obtained from Food Microbiology and Safety Department

Preparation of finger millet and foxtail millet malt

Finger millet and foxtail was procured from local market. Foreign matter was removed (dirt, dust, etc). Then both millets are soaked in water (proportion 1:3, SO₂ added 0.2%). Grains were kept in a muslin cloth for 4 days for sprouting and then water sprinkled after every 10 hrs to prevent drying, after that the germinated grains were sun dried. Dried grains were grounded by using grinder.

Preparation of starter culture

The starter culture was prepared with the help of method described by Mousavi *et al.*, with small change. *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* was cultivated separately in the MRS broth for 24 hrs at 37°C. To obtain the biomass, 10ml of separately cultivated MRS broths were mixed in equal proportion (1:1) and centrifuged at 4000rpm for 10 min. The obtained biomass was washed with sterile saline solution twice to remove the residual MRS media. Thus, inoculum was prepared. It was then introduced into pasteurized millet-based beverage (100ml) for making it 10% concentration of probiotics. The inoculated beverage was then incubated at 37°C for 6hrs and was treated as starter culture for preparation of final beverage.

Preparation of millet based probiotic beverage

Firstly, the sugar was dissolved in the 100ml water then this water was slightly heated. In this water 5% malt (Finger millet malt: Foxtail millet malt in 3:2 proportion) was added. After that pasteurization of this malt blend was takes place. Then the stabilizer xanthan gum was added into the blend. After pasteurization the blend was kept for the cooling upto room temperature. When the blend was cooled inoculation of starter culture (10%) in this blend was done. It was allowed to ferment in incubator at 37°C for 6hrs. After incubation, the beverage was kept at refrigeration temperature (4°C) for future use.

Physico-chemical analysis of millet based probiotic beverage

Total Soluble Solids (TSS), titrable acidity and pH

TSS was measured immediately after extraction using hand refractometer. Titrable acidity, expressed as percent lactic acid, was determined by titration against 0.1% NaOH using phenolphthalein as an end point indicator. The pH value was obtained by using a digital pH meter (ELICO LI612) after standardizing it with buffers of pH 4.0 and 9.0 (Ranganna, 1991).

Glucose and fructose

The glucose and fructose content were determined in beverage by phenol sulfuric acid method (Nielson S. 2010).

Total sugars and reducing sugar

Total carbohydrates were estimated by standard procedure using phenol sulphuric acid. (Nielson S. 2010). The reducing sugar content of the probiotic beverage was

calculated Nelson-Somogyi method (Syed *et al.*, 2007).

Viscosity

Viscosity of the Millet based Probiotic Beverage was estimated by using Brookfield viscometer DV-E at constant speed of 100rpm and changing temperature. Viscosity expressed in terms of centipoises(cP). (Weaver *et al.*, 2005).

Microbial analysis of millet based probiotic beverage

The viable count of mixed culture was determined by the slanted plate count method using MRS agar. The obtained results were expressed in CFU/ml. The yeast and mold count of prepared beverage was determined by PDA agar and the coliform and E-coli count was determined by using MacConkey's agar. Plates were incubated at 37°C for 48-72 hrs (Chris *et al.*, 2006)

Results and Discussion

Physico-chemical characteristics of millet based probiotic beverage

The Millet based Probiotic Beverage was used to analyze its chemical characteristics because the nutrients present in beverage would be a source of food for probiotic microorganisms to maintain the viability in product besides quality of product. The chemical properties of the prepared probiotic beverage presented in Table 1.

It is observed from the Table 1, that the TSS of the Millet based Probiotic Beverage was found to 10⁰Bx as it was changed during the fermentation time of beverage. During fermentation was due to lactic acid production the sugar value was decreased. The acidity of the beverage increases due to utilization of

sugar by the microorganisms which get converted into lactic acid. The titrable acidity is a measure of shelf life of the product and guard against the attack of microbes. It also helps to ensure some chemical changes in the during preparation (Swientek, 1998) and storage (Langthasa, 1999) of the product. The pH is inversely proportional to acidity. The pH value of the prepared product was 4.47 and the titrable acidity of the prepared probiotic was 0.42.

Textural characteristics of millet based probiotic beverage

The viscosity is an important characteristic to determine in food industry because it is related to the appearance and density of product. In the present research, the effect of different temperatures on the viscosity of prepared Millet based Probiotic Beverage was

estimated. The information related to viscosity (consistency) change is given in following Table 2.

As shown in the viscosity Table 2, the consistency i.e. viscosity of the prepared Millet based Probiotic Beverage changed significantly with the increase in temperature. At temperature 20⁰C, the viscosity of beverage was significantly higher (13.60 cP) than that observed at 40⁰C temperature (8.767 cP). The viscosity observed at 25, 30 and 35⁰C were found be 12.40, 10.133 and 9.267 cP showing a declining trend as the temperature increased. The reason for viscosity reduction was that the heat causes the molecules to speed up as they bump and move around each other. Hence more temperature means more movement of molecules and thus reducing their resistance to flow (Manerramov *et al.*, 2007).

Table.1 Physico-chemical properties of Millet based Probiotic Beverage

Sr. No.	Parameter	Control	Probiotic Beverage
1	Total Soluble Solids (°Brix)	12	10
2	Titratable Acidity (% citric acid)	0.16	0.42
3	pH	6.56	4.47
4	Total Sugars (%)	12.51	10.14
5	Reducing Sugars (%)	10.31	9.42
6	Non reducing Sugars (%)	0.87	0.72

Table.2 Effect of temperature on textural characteristics of probiotic beverage

Sr. No.	Temperature (°C)	Viscosity (cP)
1	20	13.667
2	25	12.400
3	30	10.133
4	35	9.267
5	40	8.767

Table.3 Microbial analysis of millet based probiotic beverage

Sr.No.	Parameter	Observations
1	Total Plate Count (CFU/mL)	8.6 x10 ⁹
2	Yeast and Mold Count (CFU/mL)	ND
3	Coliform Count (MPN/mL)	ND

Microbial analysis of millet based probiotic beverage

The growth of harmful and unwanted microorganisms will spoil the prepared product and may lead to different types of food borne diseases affecting the human body. Therefore, microbial analysis of the prepared beverage is mandatory in probiotic based products to prevent the product from spoilage and also maintain the safety. The data related to microbiological analysis of probiotic beverage is given in Table 3.

In the present work, the count of beneficial bacteria was detected as 8.6 x10⁹ CFU/ml and yeast and mold count was not detected in a beverage. This count was in suitable range as observed by (Shah N.P 2001) in probiotic food products. On the other hand, the yeast and mold count and coliform count was also determined and they were not detected in the sample, which showed that the product was free of any pathogenic microorganisms and safe for consumption.

In conclusion, the present investigation focuses on the development of probiotic beverage which imposes potential health benefits of finger millet and foxtail millet. For achieving this, the probiotic strains of LAB (*L. acidophilus* and *L. bulgaricus*) were subcultured after staining and microscopic study. Then the method of malting of finger millet and foxtail millet was standardized and it was found that malting method improves and increases the nutritional value of the beverage. The pasteurized millet malt blend was inoculated with probiotic culture (10%)

of *L. acidophilus* and *Bulgaricus* (1:1) and fermented for 6 hrs. Then, the prepared beverage was analyzed for chemical, textural (consistency) and microbiological characteristics. It was then stored at suitable refrigeration temperature (4°C). Results showed that the chemical parameters were in sufficient amount for providing nutrition and other components to consumers. Microbiological analysis found that the beverage contained desired amount of probiotic cultures which is helpful for maintaining the health of gastro intestinal tract. Further the prepared beverage did not contain any traces of yeast and molds and also coliform bacteria, thus indicating that beverage is containing only health beneficial bacteria.

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