The Pharma Innovation Journal 2020; 9(1): 427-432

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(1): 427-432

www.thepharmajournal.com
Received: 19-11-2019
Accepted: 21-12-2019

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Study of the physicochemical and proximate composition of cereal based probiotic beverage

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Abstract
The present investigation was carried to Study the physicochemical and proximate composition of cereal based Probiotic beverages made with millet (*Panicum*) and barley (*Hordeum vulgare*) as cereal by fortification with garden cress (*Lepidium sativum*) seed 2 percent and pumpkin(*Cucurbita*) seed powder 2 percent into it fermenting with *Lactobacilli* and *Bifidobacteria* cultures got the results as pH 4.01, total soluble solids 14.6 ^{0}Brix, viscosity 35 ceti-poise and titratable acidity 0.4 percent physical results and Chemical analysis in terms percent as of moisture 81.28, crude fat 1.823, crude protein 3.43, ash 1.27 and total carbohydrate 11.25.

Keywords: Physiochemical and proximate composition, cereal based probiotic beverage

Introduction
Probiotics have been defined as living bacteria and supportive substances that have beneficial effects on the host by improving the bacterial balance in the intestine (Fuller, 1991) [10]. This definition was later expanded to include living bacteria or mixed bacteria that have beneficial effects on the gastrointestinal and respiratory system of the host by improvement of the balance of intestinal flora (Salminen *et al.*, 1998) [24]. Recently, probiotics have been more widely defined as bacteria that work to maintain the host's health (Hozapfel *et al.*, 2002; Saito, 2004; Grajek *et al.*, 2005) [15, 23, 13]. The development of nondairy probiotic products is a challenge to the food industry in its effort to utilize the abundant natural resources by producing high quality functional products. In this respect, probiotic- containing baby foods or confectionery formulations have been developed by adding the strains as additives. (Saarela *et al.*, 2000) [26].

There are some ideal properties of the probiotic strains, which would benefit the human health and could be used in probiotic industry: resistance to acid and bile; attachment to the human epithelial cells; colonization in the human intestine; production of antimicrobial substances, called bacteriocins (Jack *et al.*, 1995) [16].

The most common probiotics are *Lactobacillus* and *Bifidobacterium*. In general most probiotics are gram-positive, usually catalase-negative, rods with rounded ends, and occur in pairs, short, or long chains (Wright *et al.*, 2000) [30]. They are non-flagellated, non-motile and non-spore-forming, and are intolerant to salt. Optimum growth temperature for most probiotics is 37°C but some strains such as *L. acidophillus* prefer 30°C and the optimum pH for initial growth is 6.5-7.0 (Wright *et al.*, 2000) [30]. *L. acidophilus* is microaerophilic with anaerobic referencing and capability of aerobic growth. *Bifidobacterium* is anaerobic but some species are aero-tolerant. Most probiotics bacteria are fastidious in their nutritional requirements (Desmazeaud, 1983, Marshall and Law, 1984) [9, 22]. With regard to fermentation probiotics are either obligate homofermentative (ex. *L. acidophilus*, *L. helvelicas*), obligate heterofermentative (ex. *L. brevis*, *L. reuteri*), or facultative heterofermentative (ex. *L. casei*, *L. plantarum*) (Barrangou *et al.*, 2011) [5]. *Lactobacillus* and *Bifidobacteria* are examples of genera of which some of the species are promising probiotics (Saito, 2004) [23]. These microorganisms are gram-positive lactic acid producing bacteria that constitute a major part of the normal intestinal microflora in animals and humans (De Simone *et al.*, 1993) [8].

Cereals are grown over 73% of the total world harvested area and contribute over 60% of the world food production.Cereals are a major source of fibers in the diet, and the main active component of cereal fibers is β-glucan. Numerous scientific studies demonstrated the hypocholesterolemic effect of this compound, bringing a 20–30% reduction of LDL-cholesterol.

The overall effect is reduction of cardiovascular disease risk. (Gallaher 2000; Wrick, 1994) [11]. Beta-glucan is also considered a prebiotic as it can support the growth of some beneficial bacteria in the colon (Stark and Madar, 1994) [27]. The cereals with highest beta-glucan content are oats and barley Cereal grains are an important source of protein, carbohydrates, vitamins, minerals and fiber for people all over the world, and can be used as sources of non-digestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of *Lactobacilli* and *Bifidobacteria* present in the colon, thereby acting as prebiotics. (Manthey 1999, Wood and Beer, 1998) [21, 31].

Cereals contain water-soluble fiber (such as β-glucan and arabinoxylan), oligosaccharides (such as galacto and fructo oligosaccharides) and resistant starch, and thus have been suggested to fulfill the prebiotic concept. (Anderson *et al.*, 2001) [3] The beneficial effects of food with added live microbes (probiotics) on human health are being increasingly promoted by health professionals. Probiotic products available in the markets today, are usually in the form of fermented milks and yoghurts; however, with an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian probiotic products. And, owing to health considerations, from the perspective of cholesterol in dairy products for the developed countries, and economic reasons for the developing countries, alternative raw materials for probiotics need to be searched.

Materials and Methods

The present investigation was conducted in the Department of Food and Industrial Microbiology, with collaboration of Department of Food Chemistry and Nutrition, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani during the year 2013-2015. Materials used and methods adopted for the present investigation are presented in this chapter under suitable headings.

Materials

Cereals and other ingredients

Cereals like sorghum, barley, pumpkin seeds and garden cress seeds were used and they were collected from local market Parbhani.

Sugar: Granulated cane sugar was obtained from the Parbhani local market.

PET bottles: PET bottles for storage of beverage sample were purchased from local market of Parbhani.

Chemicals

All the chemicals used in this investigation were of analytical grade. They were obtained from Department of Food and Industrial Microbiology and Department of Food Chemistry and Nutrition, College of Food Technology, V.N.M.K.V., Parbhani.

Processing and analytical equipments

The processing and analytical equipments included, hot air oven, muffle furnace, BOD incubator, soxhlet apparatus, microkjeldhal assembly, glass wares, Brookfield viscometer DV-E for viscosity, an electronic balance with the accuracy of 0.0001g for weight measurements were obtained from College of Food Technology, V.N.M.K.V., Parbhani.

Methods

Preparation of starter culture

The starter culture was prepared with the help of the method described by Ghadge *et al.*, (2008) [12] with some modifications.

Preparation of MRS medium

All the ingredients were suspended in distilled water and heated to dissolve the medium completely. The medium was sterilized in autoclave at 15 lbs pressure for 15 minutes (De Mann *et al.*, 1960) [7].

Isolation of Lactic acid bacteria from the commercial Yoghurt sample

The samples of yoghurt was used for isolation of culture on MRS agar. The serially diluted sample was inoculated on MRS agar and incubated at 37 ^{0}c for 24 hours. Then selected colonies was again inoculated in to MRS broth for 24-48 hours. After vigorous growth of culture then again inoculated and incubated on MRS agar to get pure culture. This culture grown on nutrient agar by standard procedure.

Purity of the cultures

The staining of the obtained pure cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was carried out by using Gram positive staining technique for their identification (Harley and Prescott, 2002) [14].

Sub-culturing of pure culture

The pure cultures i.e. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were sub-cultured on slants prepared from MRS media in laminar air flow. This was incubated at 37 ºC for 24 hours in incubator. It is having microbial count nearly about 32 X 10^7 cfu/ml.

Chemical characteristics of cereal flour

Proximate analysis of cereal flour

All samples were analyzed for moisture, crude protein, crud fat, crude fiber, total ash and total carbohydrate contents according to their respective standard methods as described in (A.O.A.C., 2000) [2].

Preparation of Probiotic cereal beverage

The production of probiotic cereal beverage was carried out in the Department of Food and Industrial Microbiology, College of Food Technology, V.N.M.K.V., Parbhani by using *Lactobacillus acidophilus* and *Bifidobacterium bifidum* starter culture. The recepie used for preparation of ceral based probiotic beverage are mentioned below in Table 4 and the three standard compositions of composite flour are represented in Table 3 and pure LAB cultures used in recepie in different concentration not shown.

Process flow chart

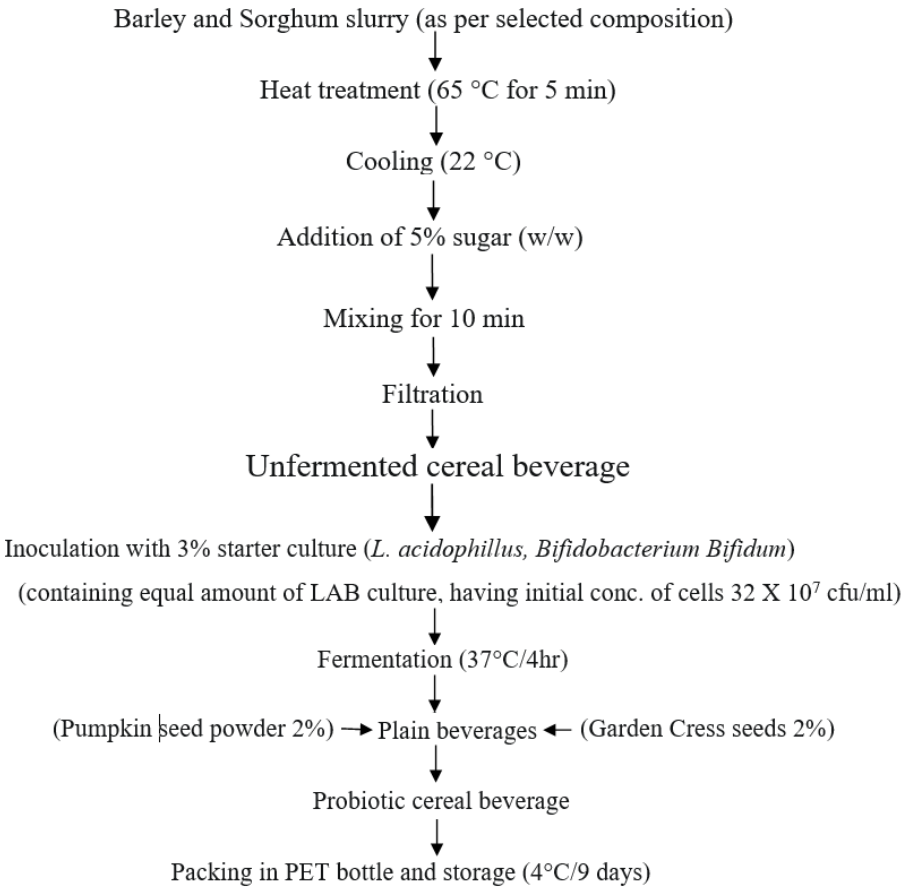

Fig 1: Flow Charts Preparation of Cereal - Based Probiotic Beverages

Physicochemical analysis of product

pH

The pH of beverage was measured through electronic digital pH meter. Buffer solution of pH 4 and 9 were used to calibrate the pH meter. Beverage sample was taken in a beaker; electrode of pH meter was immersed in the sample to determine pH.

Titratable acidity

The acidity of sample was calculated by standard A.O.A.C. method (1990) [1].

Total soluble solid

The content of total soluble solid (T.S.S.) of Probiotic beverage, were determined with the help of hand refract meter corrected at 20^0C. Care was taken that the prism of refract meter was washed with distilled water and wiped dry before every reading.

Viscosity

Viscosity was determined to check the flowing nature and thickness or viscosity (resistance to flow) of beverage which is one of the quality criteria of probiotic cereal beverage determined by using the Brookfield viscometer DV-E at constant speed 100 rpm and at constant temperature with a spindle number S-63 and it was expressed in terms of centipoises (cP).

Parameters used for viscosity measurement of probiotic cereal beverage was as follows

Spindle – S 63

Shear rate - 3.2

Speed – 100 rpm

Temperature – 25 ^{0}C

Proximate analysis of the beverage

Beverage was analyzed for proximate composition; moisture, ash, protein, fat, fiber and total carbohydrates according to their respective methods (A.O.A.C., 2000) [2].

Moisture

The moisture content of flour was determined according to method No. 44-15 A of (A.O.A.C, 2000) [2]. 5g of flour sample was taken in tarred crucible and dried in a hot air oven at 100 ± 5 ºC till to a constant weight. The moisture content was calculated by the formula given below.

$$\% \text{ Moisture} = \frac{\text{Initial weight} - \text{final weight}}{\text{Total weight of sample}} \text{ X } 100$$

Ash

The ash content was determined as a total inorganic matter by incineration of the samples at 600ºC according to method No. 08-01 of (A.O.A.C., 2000) [2]. Remaining inorganic materials are reduced to their most stable form, oxides or sulphates and are considered as 'ash'.

Procedure

Oven dried 5 g sample was taken in a pre-weighed crucible and charred on the burner. Then it was ignited in the muffle

furnace at 550-600ºC for 5-6 hours or till constant weight of grayish ash was obtained. The ash of sample was calculated through following formula.

$$\% \text{ Ash} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty Crucible}}{\text{Total weight of sample}} \times 100$$

Fat

The method employed was that of solvent extraction using a Soxhlet extraction as described in method No. 30-10 (A.A.C.C., 2000) [2]. 2 g of flour was taken in a thimble and placed in extraction tube of Soxhlet apparatus. About 250 ml of Hexane was added in 500 ml bottom flask of the apparatus and connected to Soxhlet apparatus. The fat was extracted by running Hexane over the sample at the rate of 3-4 drops per sec for about 5 hr. The solvent was recovered and the flask was kept in hot air oven for 10 min at 40-50ºC. The flask was cooled in desiccator and weighed. Fat percentage was calculated according to the following formula.

$$\% \text{ fat} = \frac{\text{Final weight of flask} - \text{Empty weight of flask}}{\text{Weight of sample}} \times 100$$

Protein

The protein was determined by the Kjeldhal's method as described in method No. 46-10 of (A.A.C.C., 2000) [1]. This is based on the fact that on digestion with concentrated sulphuric acid and catalysts, organic compounds are oxidized and the nitrogen is converted to ammonium sulphate. Upon making the reaction mixture alkaline, ammonia is liberated, removed by the steam distillation, collected and titrated.

Procedure

The nitrogen content of samples was determined by using micro Kjeldhal's method. The sample was first digested in digestion flask with H_2SO_4 in presence of digestion mixture for 3-4 hr till the contents of digestion flask get transparent color. The samples were then diluted with distilled water up to 250 ml in a volumetric flask. The ammonia from the samples was liberated through distillation after adding 40% NaOH solution and collected in flask containing 4% boric acid solution using methyl red as an indicator. The nitrogen content in the samples was determined by titrating against standard 0.1 N H2SO4 solution and the crude protein percentage was calculated by using following formula

$$\%N = \frac{(\text{Sample-Blank}) \times \text{N of } H_2SO_4 \times 0.014 \times \text{D.F.}}{\text{Wt. of sample (g)}} \times 100$$

Crude fiber

Crude fiber content was determined by following the method No. 32-10 as described in (A.O.A.C., 2000) [2]. 2 g fat and moisture free sample was taken and placed in 1000 ml beaker. 200 ml solution of 1.25% H_2SO_4 was added in the beaker. The sample was then digested by boiling for 30 min. Then it was filtered by using suction apparatus. The residue was washed with hot water until become acid free. The residue was then again transferred to 1000 ml beaker and boiled with 200 ml solution of 1.25% H_2SO_4 for 30 min. It was again filtered and the residue was transferred to pre-weighed crucible and dried in an oven at 100 ºC of 24 hr till constant weight was obtained. Then the dried residue was charred on a burner and ignited into muffle furnace at 550-600 ºC for 5-6 hr, cooled in desiccator and weighed. The loss in weight during incineration represents the weight of crude fiber in sample. The crude fiber percentage was calculated by using the following formula.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Total carbohydrate

Carbohydrates were calculated by difference method as follows.

$$\text{Carbohydrates} = 100 - \% \text{ (Moisture + Fat + Protein + Ash +Crude Fiber)}$$

Result and Discussion

The quality parameters selected for investigation include pH, titratable acidity, total solids and viscosity which are related to stability of bioactive components in plant derived products and the obtained results are presented in table 1.

Table 1: Physicochemical Characteristics of Cereal Based Probiotic Beverage

Parameter	Values (%)
PH	4.01
T.S.S (^{0}Bx)	14.67
Viscosity (cP)	35
Terrible acidity (%)	0.4

* Each value is average of three determinations.

From the results presented in table 8, it has been observed that the pH, titratable acidity, total solids and viscosity of accepted beverage sample were found to be 4.01, 0.44 per cent, 14.67 per cent and 35 centipoise respectively. Decrease in pH might be due to the less acidic nature of pumpkin seeds and garden cress seeds. Martin *et al.*, (2003) [20] reported that adding probiotic starter culture caused decrease in pH value of the beverage relative to control; at the same time titratable acidity was found to be increased. These results are also corroborated with findings of Salwa *et al.*, (2000) [25] for pH as there is an inverse relationship between acidity and pH. The increase of acidity may be attributed to the production of lactic acid as a result of microbial fermentation of lactose (Tamine and Robinson, 2004) [28] Further due to addition of probiotic bacteria caused increase in viscosity of sample, increase in viscosity take place atacidic pH value (Martin *et al.*, 2003) [20].

Chemical composition of probiotic beverage sample

Table 2: Chemical Analysis of Cereal Based Probiotic Beverage

Parameter	Values (%)
Moisture	81.28
Crude fat	1.823
Crude protein	3.43
Ash	1.270
Total carbohydrate	11.25

* Each value is average of three determinations.

Data presented in table 2 revealed that the beverage sample contained 81.28 per cent moisture, 3.43 per cent crude protein. Higher protein content in probiotic beverage might be might be due to the protein content presented in pumpkin seed powder and garden cress seeds. It was observed that ash content of probiotic sample was 1.270 percent. Ash content increased significantly due to fermentation process and fortification with pumpkin and garden cress seed. It was observed that fat content of probiotic beverage was 1.823 per

cent. It was increased due to the fortification of pumpkin seed powder and garden crees seeds. It was observed that the carbohydrate content of prepared probiotic beverage was 11.25 per cent. In the case of cereals, the fermentation with probiotic microorganisms could be beneficial due to the decrease of non-digestible carbohydrates (poly- and oligosaccharides), the improvement of the quality and level of lysine, the availability of the vitamin B group, as well as the degradation of phytates and release of minerals like manganese, iron, zinc, and calcium (Blandino *et al.*, 2003) [4]. Some authors have indeed observed that the traditional fermentation of cereals reduced the content of phytic acid and polyphenols to significantly improve protein quality while increasing the lysine content, the availability of essential amino acids, the *in vitro* protein digestibility, the carbohydrates digestibility, the availability of iron, minerals and B-group vitamins including thiamine, niacin, folic acid (Kayode, 2006; Katongole, 2008; Chelule *et al.*, 2010; Lyumugabe *et al.*, 2012) [18, 17, 6, 19]. Fermentation also leads to a decrease in the content of carbohydrates, trace and non-digestible polysaccharides.

Sensory evaluation of probiotic cereal beverage for judging the different beverage samples

The sensorial quality characteristics of cereal based probiotic beverage play a vital role in attracting consumers to purchase the product. Consumer judges beverage quality on the basis of its sensory parameters such as color, flavor, taste, texture etc. Sensorial evaluation was done using hedonic scale. Probiotic drink was evaluated for acceptability based on characteristics such as color, taste, flavor and texture.

Conclusion

Thus in light of scientific data of the present investigation, it may be concluded that barley, sorghum, garden cress seed & pumpkin seed are highly nutritious food ingredients containing macro & micronutrients and moderate source of protein & calories. It could also be concluded that the lactic acid fermentation of these cereal by LAB starter culture containing *Lactobacillus acidophilus* & *Bifidobacterium bifidum* results in the sensorial characteristics *viz.* flavor, taste and texture of prepared probiotic cereal beverage. The shelf life of beverage is calculated (9 days) under refrigerator storage (4ºC). The process of preparation of cereal based probiotic beverage being a techno-economically feasible, justifies the suitability of cereals in probiotic based health or functional food for commercial exploitation.

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