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# The Pharma Innovation



ISSN (E): 2277- 7695 ISSN (P): 2349-8242 NAAS Rating: 5.03 TPI 2020; 9(1): 423-426 © 2020 TPI www.thepharmajournal.com Received: 15-11-2019 Accepted: 19-12-2019

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### Standardize the processing technology for preparation of cereal milk fortification with garden cress (*Lepidium sativum*) seed and pumpkin (*Cucurbita*) seed powder

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#### Abstract

The present investigation was carried to standardize the processing technology for preparation of cereal milk with millet (*Panicum*) and barley (*Hordeum vulgare*) as cereal by fortification with garden cress seed and pumpkin seed powder into it fermenting with *Lactobacilli* and *Bifidobacteria* cultures to the development of nondairy probiotic products in its effort to utilize the abundant natural resources by producing high quality functional products Probiotics represent probably the functional food, as a live microbial supplement which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition with the objectives, to study the sensory characteristics of probiotic beverages using above grains and to study the physicochemical and proximate composition of accepted beverages.

Keywords: Lepidium sativum, pumpkin, Cucurbita, powder

#### Introduction

The cereal products often ferment spontaneously, resulting in improved shelf life and better nutritional properties compares with raw materials. Single or mixed cereals are used as a substrate in the production of fermented foods and the final product can vary according to the microbial population and microbial condition. Fermentation procedures have been used to develop new foods with enhanced health properties (Blandino *et al.*, 2003)<sup>[5]</sup>.

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  $\beta$ -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) <sup>[8, 19]</sup>. Beta-glucan is also considered a prebiotic as it can support the growth of some beneficial bacteria in the colon (Stark and Madar, 1994) <sup>[17]</sup>. 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) <sup>[14, 18]</sup>.

Cereals contain water-soluble fiber (such as  $\beta$ -glucan and arabinoxylan), oligosaccharides (such as galacto and fructo oligosaccharides) and resistant starch, and thus have been suggested to full fill the prebiotic concept.(Anderson *et al.*, 2001). 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. Hence the present investigation is undertaken with following distinct objectives as, to study the physicochemical characteristics of grains i.e. Sorghum and Barley, to standardize the processing technology for preparation of cereal and millet milk by fortification with garden cress seed and pumpkin seed powder into it, to study the sensory characteristics of probiotic beverages using above grains and to study the physicochemical and proximate composition of accepted beverages.

#### **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 equipment's

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) <sup>[9]</sup> 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)<sup>[6]</sup>.

## 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 °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)<sup>[10]</sup>.

#### 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  $37^{\circ}$  C for 24 hours in incubator. It is having microbial count nearly about  $32 \times 10^7$  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.A.C.C., 2000)<sup>[1]</sup>.

#### **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



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)<sup>[2]</sup>.

#### Total soluble solid

The content of total soluble solid (T.S.S.) of Probiotic

#### 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 ° 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)<sup>[2]</sup>.

#### Microbial examination of probiotic cereal beverage

Microbial examination is the perfect quality assessment protocol performed in food products quality analysis.

#### Statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range tests (P < 0.05) to determine a significant difference among samples. The data were analyzed according to user's guide of statistical analysis system (SAS 1996)<sup>[16]</sup>.

#### **Result and Discussion**

It could be observed that highest fat content was recorded in pumpkin seeds (27.83%) whereas lowest value was recorded for barley (2.6%). Highest ash content was recorded for garden cress seeds (4.65%) whereas lowest ash was observed in sorghum (1.18%). Highest value of crude fibre was recorded in pumpkin seeds (12.3%) whereas lowest value was recorded in sorghum (1.3%). It could be observed that cereal grains had higher carbohydrate content than the pumpkin seeds and garden cress seeds. Highest value was recorded in sorghum (65.72%) whereas lowest was recorded in pumpkin seeds (22.21%).

These values of chemical properties recorded in the present study are similar to the values reported earlier by Hulse *et al.*, (1980) <sup>[13]</sup>. Similar results were obtained by Belitz *et al.*, (2009) <sup>[4]</sup>; Mohammed, (2004) <sup>[15]</sup>; Doke, (2014) <sup>[7]</sup>. In present investigation sincere efforts were made to standardize the addition levels of LAB starter culture *viz. Lactobacillus acidophilus* and *Bifidobacterium bifidum* in cereal beverage. Final probiotic cereal beverage was prepared from 100 g of composite flour (Control, A, B and C), 1 lit tap water, 5 per cent (w/w) sugar and LAB starter culture (3 and 4%) containing equal proportions of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. A starter culture was added at the rate of 3 percent with high viable microbial counts and when added to certain foods, it accelerates fermentation leading to a final product with a desirable alteration in the aroma, texture

and flavor profile (Holzapfel, 2002)<sup>[11-12]</sup>.

The composite flour slurry was mixed and boiled for 5 min then it was cooled. Then sugar was added and mixes properly. Starter culture inoculum was added to this and allowed to ferment at 37°C for 4 hours. After fermentation add pumpkin seed powder and garden cress seed. The cereal suspension formed was mixed properly and filtered. Filtration was done to give a smooth mouth feel as well as to allow its uniform sediment distribution. The probiotic cereal beverage was evaluated for sensorial and physicochemical properties. The results pertaining to different aspects of the investigation are presented and discussed under subsequent sections.

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

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.

Overall acceptability is based on multiple organoleptic quality parameters i.e. color, flavor, taste, texture etc. and shows the accumulative perception and acceptance by the panelists. The maximum score (i.e. 8.8) for overall acceptability was observed in sample A having 70 per cent barley and 30 per cent sorghum and 3% *Lactobacillus acidophilus* and *Bifidobacterium bifidum* starter culture while the minimum score (i.e. 8.3) was observed in control sample B. The addition of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* starter culture provides aromatic and pleasing flavor, and improves overall acceptability and shelf life of whole grain cereal beverage.

#### 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 most acceptable probiotic beverage i.e. sample "A" having 70 ml barley slurry, 30 ml sorghum slurry, 4 g garden cress seed, 4 g pumpkin seed powder and 3 per cent starter culture with fermentation period of 4 hours was found to be most desirable in terms of sensorial quality profile of health food. The shelf life of beverage is calculated (9 days) under refrigerator storage (4 °C). 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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