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Development, Sensory and Nutritional Evaluation of Probiotic Butter Milk with *Lactobacillus acidophilus*

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Abstract

Probiotics refer to live micro-organisms which provide health benefits to the host organism by improving the microflora of human digestive system. Probiotics are mainly lactic acid bacteria (LAB). In the present study, the culture of probiotic micro-organism *Lactobacillus acidophilus* (NCDC-16) was used to develop probiotic butter milk. The organoleptic acceptability was assessed using 9-point hedonic scale. Probiotic butter milk was evaluated for nutritional parameters. The sensory evaluation scores revealed that probiotic butter milk has significantly higher scores as compared to control. A significant increase in total lysine content and *in vitro* protein digestibility was observed in probiotic butter milk. Probiotic buttermilk exhibited 62.66, 39.39 and 46.45 per cent *in vitro* availability of calcium, iron and zinc, respectively which was significantly higher than control. Development and consumption of such complementary probiotic food can go a long way in improving the nutritional status of infants and children especially those suffering from malnutrition.

Key words: Probiotic, Lactic acid bacteria, Butter milk, Organoleptic acceptability, Infants, Children

Scientifically, it has been proved that breast milk is the perfect food for infant feeding during the first six months of life. It contains all the nutrients and immunological factors an infant requires to maintain optimal health and growth. It contains still-undiscovered substances that cannot be reproduced artificially and its overall nutrient composition is superior to any alternative including infant formula. However, at the age of six months, breast milk is no longer sufficient to meet the nutritional needs of growing infants therefore; nutritious complementary foods should be introduced at this time. In this context in recent years, efforts to improve the health and nutritional status of weaning age children have focused primarily on the production of nutritious low-cost weaning foods. Fermentation, a simple and convenient domestic technique is one such processing method known to be effective for improving the nutritive value of plant foods. Besides nutrients, these fermented foods are known to contain different micro-organisms including probiotics, such as *Lactobacillus acidophilus* and such foods are termed as a good reservoir of probiotic bacteria.

Probiotics refer to live micro-organisms which provide health benefits to the host organism by improving the microflora of human digestive system [1]. Probiotics are mainly lactic acid bacteria (LAB) belonging to the genera *Lactobacillus* and *Bifidobacterium*. LAB are gram-positive, anaerobic rod shaped which ;0pare derived from human GIT [2]. Literature indicates several potential health and nutritional benefits possible from probiotic foods.

Probiotic bacteria act on carbohydrates and convert them into lactic acid and short chain fatty acid which helps in lowering the pH of intestinal microflora. Probiotic bacteria also generate a number of volatile organic aroma compounds (acetaldehydes, diacetyl and acetic acid) which impart a characteristic flavor to dairy products [3]. Besides, some of lactic acid bacteria secrete substances having bacteriostatic effects, which prevent the growth of pathogenic micro-organisms [4]. Besides, probiotic organisms have also a role in improving metabolism, reducing constipation, lowering of cholesterol levels in blood, stimulation of immune system, increasing the phenol tolerance and detoxification of potential carcinogens [5]. Commercially Probiotics are available either in the form of food or food supplement and also in the form of tablets, capsules and ampoules [6].

Infant feeding and rearing practices have a major effect on short-term and long-term nutritional status of the children, as most of under-nutrition is associated with faltering practices that occur in weaning period. In the developing countries like India, the problem of malnutrition in infants is due to late initiation of supplementary foods and this condition becomes more critical when infants reach the age of 5-6 years. This leads to high mortality rates in infants and preschoolers. Since butter milk is popular fermented dairy beverage in India which is rich in lactic acid. Buttermilk would be considered a probiotic product only if it contains live or viable bacteria. Keeping this in mind, the present study was planned to develop nutritious probiotic butter milk using culture of *Lactobacillus acidophilus*

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and to assess its organoleptic acceptability and nutritional evaluation.

MATERIALS AND METHODS

Microbial culture

The culture of *probiotic* micro-organism *Lactobacillus acidophilus* (NCDC-16) was purchased from the Microbial Culture Collection Centre, National Dairy Research Institute, Karnal. The stock culture of *L. acidophilus* was added to 100ml sterilized milk to obtain 10^6 cells/ml, incubated at 37°C for 12h and this inoculum (5%) was used for preparation of probiotic curd.

Preparation of probiotic curd

Took 100 ml fresh skimmed milk, autoclaved at 121°C, at 1.5 kg/cm^2 for 15 min and cooled to 40°C. Added *L. acidophilus* inoculum (5 %), incubated at 37°C for 6 hrs. and *L. acidophilus* (probiotic) curd was obtained.

Development of probiotic buttermilk

The probiotic curd prepared above was used to prepare buttermilk. Two types of buttermilks were prepared.

Ingredients	Control buttermilk	Probiotic buttermilk
Normal curd	150 g	-
Probiotic curd	-	150 g
Salt	To taste	To taste
Cumin powder	A pinch	A pinch
Water	100 ml	100 ml

Control and probiotic curd were mixed with water to obtain desirable consistency. Added salt, cumin powder and served chilled.

Organoleptic and nutritional evaluation of buttermilk

The control and probiotic butter milk was evaluated organoleptically by the panel of 10 judges using 9-point hedonic scale. The samples were analyzed nutritionally for the following parameters:

Crude protein: Crude protein was estimated by standard method of AOAC [7] using KEL PLUS automatic nitrogen estimation system.

Total lysine

Total lysine was estimated as per the method described by Miyahara and Jikoo [8].

In vitro protein digestibility

In vitro digestibility of protein was determined by the method of Akeson and Stahmann [9] as modified by Singh and Jambunathan [10].

Total calcium, iron and zinc

Calcium, iron and zinc in acid digested samples were determined by Atomic Absorption Spectrophotometer 2380, PERKIN-ELMER (USA) according to the method of Lindsey and Norwell [11].

Calcium and zinc availability (in vitro)

In vitro availability of Ca, and Zn: was determined by the method of Kim and Zemel [12].

Iron availability (in vitro)

Ionizable iron in the samples was extracted according to the procedure of Rao and Prabhavathi [13].

Statistical analysis

The data were subjected to statistical analysis for calculation of mean and standard error. The data were analyzed in complete randomized design for analysis of variance [14].

RESULTS AND DISCUSSION

Organoleptic evaluation of buttermilk

Buttermilk prepared from ordinary skim milk curd served as control. Probiotic buttermilk prepared from *Lactobacillus acidophilus* curd depicted significantly (P<0.05) higher mean score of colour (8.20), appearance (8.50), aroma (7.60), texture (7.70), taste (8.00) and overall acceptability score (8.00) as compared to control buttermilk (Table 1). Hence, probiotic buttermilk was 'liked very much' by the panelists, whereas Gupta *et at.* [15]; Sharma and Ghosh [16] observed that *Lactobacillus* yoghurt and regular yoghurt were found almost similar in terms of their organoleptic characteristics. The improved acceptability of probiotic butter milk was due to production of various flavor compounds like diacetyl [17-18].

Table 1 Organoleptic evaluation of buttermilk prepared from control and *L. acidophilus* curd

Butter milk	Colour	Appearance	Aroma	Texture	Taste	Overall acceptability
Control	7.60 ± 0.26	8.30 ± 0.21	7.50 ±0.22	7.50 ± 0.21	7.90 ± 0.21	7.76 ± 0.50
Probiotic	8.20 ± 0.24	8.50 ± 0.26	7.60±0.33	7.70 ± 0.15	8.00 ± 0.25	8.00 ± 0.34
't' value	1.76	2.75	1.50	0.70	3.88*	2.58

Values are mean ± SE of ten panelists
*Significant at 5% level
Buttermilk prepared from skimmed milk curd served as control whereas buttermilk prepared from *L. acidophilus* skimmed milk curd served as experimental

Protein, total lysine and in vitro protein digestibility of buttermilk

The (Table 2) reveals the data regarding protein, total lysine and *in vitro* protein digestibility contents of control buttermilk (prepared from control skim milk curd) and probiotic buttermilk (prepared from *L. acidophilus* skim milk curd). Protein content was 2.8 per cent in control and 2.9 per cent in probiotic buttermilk whereas lysine content was found to be increased from 0.30 g/g N in control and 0.58 g/g N in probiotic buttermilk. Increase in lysine content in probiotic butter milk might be due to the presence of fermenting microbes which cause increase in enzyme activity and protein hydrolysis [19]. Besides, during fermentation there is release of peptides and amino acids, which contributes to increase in lysine concentration [20].

In vitro protein digestibility results showed that fermentation resulted in significant increase in digestibility value of the probiotic butter milk with a value of 88.50% as compared to digestibility value of 50.02% for control butter milk (P<0.05).

Increase in protein digestibility of probiotic curd is mainly because of the fact that micro flora may produce some proteolytic enzymes during fermentation which are responsible for breakdown of protein to peptides and amino acids [21]. Similar increase in lysine content and protein digestibility of fermented foods has been reported by various workers [22-24].

Table 2 Protein, total lysine and *in vitro* protein digestibility of buttermilk (on dry matter basis)

Buttermilk	Protein (%)	Total lysine (g/g N)	*In vitro* protein digestibility (%)
Control	2.8±0.05	0.30±0.01	50.02±0.58
Probiotic	2.9±0.07	0.58±0.02	88.50±0.28
't' value	0.19	0.23*	13.88*

*Significant at 5 per cent level
Buttermilk prepared from skimmed milk curd served as control whereas buttermilk prepared from *L. acidophilus* skimmed milk curd served as experimental

Total minerals and in vitro availability of minerals

Data depicted in (Table 3) reveals the total mineral contents of control and probiotic buttermilk. Control buttermilk (prepared from skim milk curd) contained 120.02, 0.25 and 0.20 mg/100g total calcium, iron and zinc contents, respectively which differed non-significantly (P<0.05) from probiotic buttermilk as *L. acidophilus* fermentation also did not cause any significant change in total mineral contents.

Table 3 Total mineral content and *In vitro* availability (%) of buttermilk*(mg/100g, on dry matter basis)

Butter milk	Total Calcium (mg/100g)	Available Calcium (%)	Total Iron (mg/100g)	Available Iron (%)	Total Zinc (mg/100g)	Available zinc (%)
Control	120.02	42.31 ± 0.66	0.25	22.06 ± 3.5	0.20	39.83 ± 0.57
Probiotic	121.03	62.66 ± 0.33	0.26	39.39 ± 12.1	0.21	46.45 ± 1.73
't' value	NS	28.26*	NS	43.94*	NS	19.78*

*Significant at 5% level
*Buttermilk prepared from skimmed milk curd served as control whereas buttermilk prepared from *L. acidophilus* skimmed milk curd served as experimental

The data regarding *in vitro* availability of calcium, iron and zinc contents of control as well as probiotic buttermilk given in Table III reveals that control buttermilk contained 42.31, 22.06 and 39.83 per cent *in vitro* availability of calcium, iron and zinc, respectively. These values were found to be significantly (P<0.05) lower compared to probiotic buttermilk. Probiotic buttermilk exhibited 62.66, 39.39 and 46.45 per cent *in vitro* availability of calcium, iron and zinc, respectively.

Various processing treatments involved had a cumulative effect for bringing about improvement in mineral extractability/ availability of minerals. Divalent cations i.e. calcium, iron and zinc etc. are generally present in bound form with phytic acid and a protein-phytate mineral complex is formed. Reduction in phytic acid during germination and fermentation possibly through hydrolysis by inherent phytase in sprouts and fermented micro flora may release these metallic ions in free form and therefore, may account for increased *in vitro* availability of minerals in fermented products [25]. Probiotic fermentation has been shown to improve protein and starch digestibility, reduce the level of antinutrient and enhance the bioavailability of minerals [26]. Besides, during fermentation, lactic acid bacteria increase the nutritional value of food by synthesizing various vitamins and minerals [27].

CONCLUSION

Probiotic buttermilk containing 10^6 cells/ml *L. acidophilus* was prepared using 5% inoculum. The product was organoleptically acceptable and a significant increase in total lysine and *in vitro* availability of protein and minerals was found. This clearly indicates that lactic acid bacteria play a significant role in improving the acceptability and nutritional value of butter milk. Hence development and consumption of such complementary probiotic food can go a long way in improving the nutritional status of children especially those suffering from malnutrition. Consumption of probiotic foods can provide protection against GIT infections as well as raise the nutritional status. Hence, there is a great scope to more research work in this area so as to develop commercially viable health foods.

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