Effect of *Lactobacillus acidophilus* and *Bifidobacterium lactis* on the Quality Characteristics of Paneer Spread

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Abstract
This study analyzed the effects of incorporating the probiotics Bifidobacterium lactis and Lactobacillus acidophilus into paneer spread on the physical and sensory parameters of this product during storage. Incorporation of the probiotic strain did not affect the yield of the obtained paneer. The counts of L. acidophilus and B. lactis were approximately 6 log CFU/g during the pre-determined storage period. The paneer samples that contained a probiotic strain showed increased hardness, cohesiveness, gumminess, chewiness and springiness after storage as compared with the samples that did not contain any probiotic. There were no differences observed in the spreadibility of the paneer containing or not containing a probiotic. All of the paneer samples had a homogeneous texture; however, the paneer containing L. acidophilus and B. lactis had a more acidic flavor. These results have shown that there is a feasibility of increasing the health benefits of paneer spreads using L. acidophilus and B. lactis because they did not negatively affect the quality characteristics of the product and indicate that paneer spread is an effective food matrix for maintaining the viability of these probiotics during storage.

IndexTerms - Paneer spread, Lactobacillus spp., Bifidobacterium spp., Probiotics.

I. INTRODUCTION

Indian market has seen a remarkable growth in milk production during the last decades due to the success of the world's largest and successful integrated dairy development program -Operation Flood. It helped India to produce 104.8 million metric tonnes (MMT) of milk in 2008 accounting for 15% of the world's total milk production [1, 2]. It is estimated that 5% of milk produced in India is converted to paneer [3, 4]. Paneer is defined as a non- fermented, creamy dairy product that is generally prepared by heat-induced coagulation followed by acid-precipitation of whey proteins from cow, buffalo or goat milk [5]. According to PFA, it shall not contain more than 70% of the moisture, and the milk fat should not be less than 50% of the dry matter [6]. Paneer is a low cost and a rich source of fat, vitamins, minerals like calcium and phosphorus and animal protein for vegetarians. The chemical composition of the products like paneer spread from paneer depends on types of milk (buffalo, cow and admixed milk) used for its preparation. Buffalo milk is preferred over cow milk due to its properties like high total solids content, superior colour texture and viscosity. Production of paneer involves precipitation of casein along with entrapped fat and water-soluble components of milk like whey, lactose, minerals, proteins, vitamins by addition of coagulate to milk at near- boiling temperatures. Since in paneer the moisture content is high and salt in low amount with initial pH above 6.0 [7], it provides a favorable environment for the survival of probiotic bacteria. Hence, dairy products are reported to be suitable vehicles of probiotics that provide health benefits to the consumers [8, 9].

The growing public awareness regarding health issues has led to the increase in the demand for foods with some health-promoting effects, such as probiotic foods [10,11]. Probiotics are viable microorganisms that are beneficial to the host when administered in adequate amount [12]. They protect human hosts from infections that occur on the internal mucosal surfaces of the gastrointestinal tract and do not allow pathogens to adhere [13, 14]. A food to be called as probiotic product must have a microbial count of ≥6 log CFU per milliliters or gram until the end of their shelf-life period to impart their protective benefits [15, 16]. The factors that affect the viability of probiotic during the storage are acidity, nutrient content, pH, inhibitory metabolites (like organic acids and bacteriocins) and water activity [17, 18].

Most commonly used probiotic bacteria include *Bifidobacterium animalis subsp. lactis* (*B. lactis*) and *Lactobacillus acidophilus* (*L. acidophilus*) which are widely used as major ingredients of functional dairy products [19]. When these probiotics were added to bovine fermented milks, yogurts, dairy beverages [20, 11 &21] and in ewe cheese [22], *B. lactis* and *L. acidophilus* showed required viability and did not impart any negative effects on the nutritional and sensory aspects of products during their storage.

Keeping above points in focus, this study aims: 1) to manufacture paneer spread containing the well-known probiotic strains *B. lacti* and *L. acidophilus* [22, 23]; 2) to analyze the survival of each of the test probiotics when introduced into the paneer spread during their storage; 3) to evaluate the effects of the incorporated probiotic strain on physical and sensory parameters of the obtained paneer during their storage.

II. MATERIALS AND METHODS

Raw materials

The cow milk was used to manufacture the paneer which was obtained from Sahiwal breeds and was pasteurized at 65 °C for 30 min.

Bacterial strains

Lyophilized cultures of *L. acidophilus* and *B. lactis* were initially cultured in de Man, Rogosa, and Sharpe (MRS) broth for 24 h at 37 °C and in Bifidobacterium selective media, respectively. Successive heat treatments were performed according to **Minervini et al., 2012** in a water bath at 65 °C for 30 min to induce heat adaptation. The cell cultures (20 mL) were centrifuged at 1200 ×g for about 15 min and the supernatants were discarded, and then 20 mL of sterile distilled water was added to the pellet obtained. The cells were then plated on selective growth medium to check their recovery after heat treatment. The counts of the *L. acidophilus* and *B. lactis* cells ranged from 7.5 and 7.0 log of colony forming units per milliliter (log cfu/mL).

Production of Paneer Spread

Three different types of Paneer spread was produced from each type of milk, as follows: Cow milk-C1 — paneer spread without probiotic cells; C2 — paneer spread containing *L. acidophilus*; and C3 — paneer spread containing *B. lactis* cells. The paneer spreads were prepared using the procedure shown in fig.1.

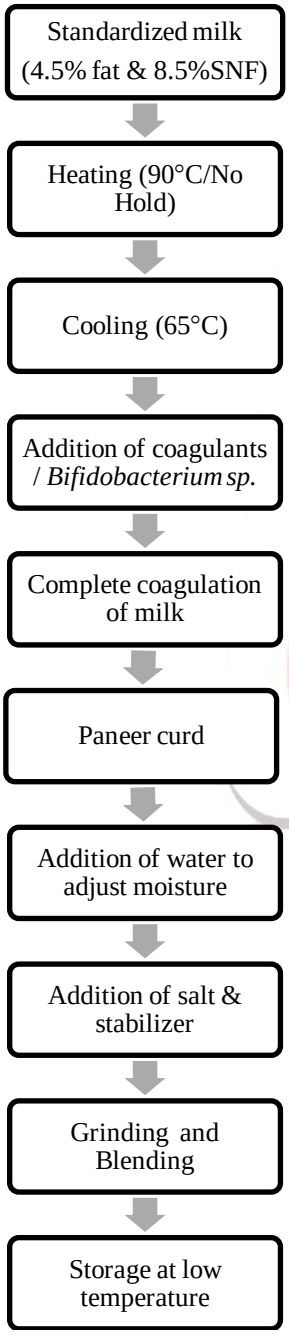

Figure1. Procedure for production of Paneer

These samples were then further analyzed for physical and sensory characteristics. The paneer spread samples were first assessed on day 1 and then after 3 days of storage at 7 °C because it has been suggested that paneer has a shelf-life of 3 days of storage

under refrigeration [25]. Samples of 30 g each from all the three types of paneer spreads were aseptically collected from different parts of the spread for microbiological analysis. For the analysis of the texture, at least 0.5 cm of the rind was discarded, and the samples were carefully collected along a line passing from the center to the exterior. The remaining sample was used for physical and sensory analyses.

Microbiological analysis and viability analysis of the probiotics during storage

The paneer spread samples were analyzed for total count (TC), coliform count, yeast and mould count using various agar medium (Hi-media Laboratories Pvt. Ltd. Bombay). The total count, coliform count and Yeast and mould count was estimated using plate count agar (PCA) medium, dehydrated violet red bile agar (VRBA) medium and malt extract agar (MEA) medium using the procedure given in [26]. The MEA pH was adjusted to 3.5 by adding sterilized lactic acid (10 %) solution aseptically to the molten medium immediately before pouring into the plates

The *L. acidophilus* and *B. lactis* counts in the paneer samples were determined using viable-cell count procedure given by **Oliveira et al., 2014**. In this procedure, 30g paneer spread from 1 day and 3 days old samples was homogenized in 200 mL of peptone water using homogenizer and the homogenates were serially diluted (10^{-4}–10^{-6}) using the same diluents. Followed by, taking a 1-mL aliquot of each dilution and culturing into MRS and bifidobacterium selective agar (for counting L. acidophilus and B. lactis, respectively) using the pour-plate technique for inoculation method and the plates were incubated for 3 days at 37 °C in incubator.

Textural analysis

The textural properties of the samples were evaluated using a Texture Analyzer (Model - TA-XT2) which had components like 25 kg load cell for two-stage liner compression of paneer spread samples and a cone shaped probe was attached to the moving cross-head. Various textural parameters were determined that included hardness, cohesiveness, gumminess, chewiness and springiness. The hardness/ firmness is the maximum peak force (F) during the first compression cycle (first bite) and the unit is Newton. The gumminess is the negative peak force (F) during the second compression cycle (second bite) and the unit is Newton. The spreadibility is the area of positive curve (Area 1:2) and the adhesiveness is the area of negative curve (Area 3:4). The unit of spreadibility and adhesiveness is Newton/s.

III. Results and Discussion

Microbiological analysis of paneer spread samples and viability of the probiotic during storage

When the microbial analysis all of the paneer spread samples were done the bacterial count was found to be < 50000/g and <90/g of coliform. There were no colonies of *Staphylococcus*, *Salmonella spp.* and *L. monocytogenes*. These results indicated that the paneer spread produced had a satisfactory quality in terms of microbial count according to Indian Standard Institution (1983). The counts of *L. acidophilus and B. lactis* at 1 and 3 days of storage were approximately 6 log CFU/g in C2 and B2 (day 1: 6.02 ± 0.6 log cfu/g and 6.06± 0.6 log cfu/g; day 3: 6.18 ± 0.9 log cfu/g and 6.28± 0.9 log cfu/g) and C3 and B3 (day 1: 6.14 ± 0.4 log cfu/g and 6.20± 0.4 log cfu/g ; day 3: 6.23 ± 0.6 log cfu/g and 6.31± 0.6 log cfu/g), the initial counts in all of the paneer samples were slightly higher (±0.3 log cfu/g) than those at the end of the storage period. Maintaining a viable count of approximately 6 cfu/g during a 3-day storage period is important because it is the minimal count of probiotics that a probiotic food should have to provide their potential benefits to the host [28, 14].

Texture analysis

The texture analysis of different samples has shown that this product has a soft texture, cohesive body with sponginess and springiness and fragile structure. It is non-elastic and can be easily deformed. After 3 days of storage, an increased hardness was observed in all of the samples; also, the paneer containing one of the probiotics had higher value for this parameter compared with that of the sample lacking a probiotic. The increased hardness can be due to the increased cross-linking between proteins that occurred during storage forming a network and leading to compact structure [29, 30]. The difference in the hardness of the paneer samples containing a probiotic compared to the control samples could be because of the changes imparted by bacterial metabolites. In a previous study it was proposed that increase in acidity during storage could be due to *L. acidophilus* done on this has shown that cheese with *L. acidophilus* [31]. It was found that samples C2 and C3 were higher in acidity after storage of 3 days as compared to C1 which could be attributed to presence of probiotics. Also, since *lactobacilli* and *bifidobacteria* produce exopolysaccharides (EPSs) which impart increased levels of chewiness and gumminess to the paneer spread. Texture and viscosity of dairy products is improved by EPSs because they lead to changes at the structural level [32].

Table1. Textural parameters of Paneer spread not containing (C1), containing *L. acidophilus* (C2) and *B. lactis* (C3) after 1 and 3 days of refrigerated storage

Parameters	**Days of storage**	**Paneer Samples**		
		C1	C2	C3
Hardness (N)	1	13.10	13.60	13.62
	3	13.30	13.88	13.90
Cohesiveness	1	0.66	0.72	0.72

	3	0.71	0.75	0.77
Springiness (mm)	1 3	7.90 8.01	8.00 8.20	7.89 8.16
Gumminess (N)	1 3	9.60 9.70	9.90 10.02	9.87 10.02
Chewiness (N-mm)	1 3	72.00 73.00	73.00 75.01	73.20 75.03

IV. Conclusion

This study has demonstrated that incorporation of probiotic strains like *L. acidophilus* and *B. lactis* into paneer spreads had no effect on physical properties like hardness and colour but there was an increase in the acidity and pH value. The increase in these aspects is due to production of lactic acid as both these strains belong to Lactic Acid Bacteria (LAB) group. The results have shown that these strains remain viable upto satisfactory counts in the matrix of paneer spread to provide health benefits to consumers and they did not impart any negative effect on the quality of paneer spread. Studies can be done in a direction to preserve the viability of probiotics for longer duration that does not affect the properties of paneer spread at the same time help these organisms to thrive.

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