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Study of microbial changes in probiotic and synbiotic lassi during storage

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Abstract

Objective of the present investigation was to assess the microbial changes occurring in the formulated synbiotic lassi containing carrot juice. A (probiotic lassi), and B (synbiotic lassi) were initially evaluated on 0th day and later at an interval of 7 day period up to 28 days, wherein the samples were stored at refrigerated conditions 4 ± 1 °C. During refrigerated storage of 28 days, Lactobacillus count remained well above 10^8 cfu/ml in both the samples (A and B). Coliforms were found to be absent along with yeast and mold count throughout the storage study. These products can surge ahead in market as appealing functional fermented beverage for consumers giving health benefits due to presence of probiotic culture, FOS and other beneficial constituents from carrot juice.

Keywords: Microbial changes, probiotic lassi, synbiotic lassi

1. Introduction

Lassi is a, very popular traditional fermented milk product consumed usually as a summer drink in India, especially in northern India, whose origin is dating back to paleolithic and neolithic times in India (Mathur, 1991) [18]. It is a ready-to-serve beverage that occupies prominent place in the Indian diet (Tiwari, 1998) [32]. It is a blend of dahi, water, and sometimes even spices or fruits are added. It close to sweet stirred yoghurt (Fluid yoghurt). In Middle East including Iran and Lebanon, a similar salty yoghurt beverage named “Doogh” is popular (Kosikowski, 1978; Tamime and Robinson, 2007) [16, 31]. Similar products like *lassi* in African continent are also present *viz.*, Lben a Moroccan fermented product, Susa (made from camel's milk) and Maziwa Lal are traditional fermented milk's of Kenya (Tamime and Robinson, 2007) [31]. During 2002, commercial products resembling sweet *lassi* began appearing on the U.S. market, with names like drinking yoghurt, and yoghurt smoothie (Sabikhi, 2003) [24]. Yogurt-type drinks are the fastest-growing product category, and have registered a significant sales growth in Europe (Saxelin, 2008) [25]. There is an outburst of consumer's interest in the functional foods which possess health beneficial aspects due to an active food components (Hasler, 1998) [9]. Functional food refers to any food or food ingredient that may provide a health benefit beyond the traditional nutritional function (Clydesdale, 1997; Roberfroid, 1999; Milner, 1999) [2, 23]. The easiest way to position dairy products as “functional” is addition of probiotic cultures. Milk and milk products are considered as an ideal food medium for delivering probiotics (lactobacilli and bifidobacteria) and other functional ingredients which are not ordinarily found in dahi / yogurt as they are rich sources of protein, calcium and a variety of vitamins, minerals and bioactive compounds. (Danone Vitapole, 2000) [3].

The word “Probiotic” was derived from Greek word meaning pro-life and has had several meanings over the years. With time many definitions were given and amongst them recent one is that given by International Dairy Federation (IDF) in 2003 for “Oral probiotics” as “living micro-organisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition”. Prebiotics are food ingredients, which can be digested, only by bacteria in colon which are involved in selective stimulation of probiotics to benefit the health of the host. The classical definition of prebiotics given by Gibson and Roberfroid (1995) [8], defines them as “non-viable food components (usually oligosaccharides), which evade digestion by mammalian enzymes in the upper regions of the gastrointestinal tract, reach the colon in an intact state and are hydrolyzed by beneficial members of the indigenous microbiota.

FAO in 2007 defined prebiotic as non-viable food component that confers health benefit on the host associated with modulation of the microbiota”.

The synbiotic concept combines efficacious probiotic strains with specific prebiotic compounds in a single product. Synbiotic is defined as “a mixture of probiotics and prebiotics that favourably affects the host by improving the survival and implantation of live microbial dietary supplement in the GI tract” (Gibson and Roberfroid, 1995) [8]. Combined effects of these give more health benefits (Fooks and Gibson, 2002).

Keeping these points in view, an endeavour to develop synbiotic *lassi* with carrot juice which could be a refreshing and thirst quenching with health promoting properties was carried out. Hence this investigation was taken up with the following objectives. To evaluate the effect of some food ingredients / additives (carrot juice) on the growth of probiotic culture and the changes in probiotic population stored at 4 ± 1 °C (28 days at an interval of 7 days).

2. Materials & Methods

Present work was carried out in the Department of Dairy Microbiology, SMC College of Dairy Science, Anand. The materials, which were used during the course of study along with their sources, and course of work is delineated hereunder.

2.1 Probiotic culture and its maintenance

The culture used in the present study was *L. helveticus* MTCC5463 obtained from the culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand. The culture @ 2 % v/v was propagated in sterilized skim milk (10 % T.S., Sagar skim milk powder, Dudhsagar) for 16 h and in sterile whey for 12 h and stored at 5 ± 2 °C. The transfer was given every week during the course of the study.

2.2 Lassi Manufacture: The fresh cow milk was collected from Livestock Research Station, Anand for manufacturing of synbiotic and probiotic *lassi*. *Lassi* was manufactured as per the procedure adopted by Patidar and Prajapati (1998) with minor modification. Oraftii Ltd, Belgium, supplied the Fructo Oligosaccharide (FOS) having trade name Raftiline. Fructo oligosaccharide (FOS) was added @ 5 % (w/v) for formulating the synbiotic *lassi* (Sample B), while only probiotic culture was added to milk to prepare probiotic *lassi* (Sample A). Sugar (@ 10 % w/v in both samples A&B) of high quality, free from any impurity was purchased from local supplier to be used as sweetener during *lassi* manufacture. Freshly extracted carrot juice @15 % v/v was added to sample B and these carrots were procured from local market.

2.3 Microbiological analysis of synbiotic Lassi

2.3.1 Preparation of samples for microbiological analysis of Lassi

On the 0 day, 11 ml of freshly prepared *lassi* samples (Both of A & B) after thoroughly mixing were taken in 99 ml phosphate buffer for the enumeration of probiotic count (LAB), coliform count (CC) and yeast and mould count (YMC). Serial dilution was carried out till appropriate dilution. Similarly, thereafter, at an interval of 7 days, upto 28 days of storage.

2.3.2 Preparation of Agar plates for Lactobacilli count (Probiotic Count)

Probiotic count of inoculated *lassi* samples was determined as per the method described by De man *et al.* (1960).

2.3.3 Preparation of agar plates for coliform count

Coliform count was done as per standard methods for examination of Dairy products (Indian Standards, IS: 5401, 1969).

2.3.4 Preparation of agar plates for yeast and mold count:

According to Indian Standards, IS: 5403, (1969) procedure was followed for the enumeration of yeast and mold.

3. Results & Discussion

3.1 Lactobacilli Count

The changes in the *lactobacilli* count are shown in figure-1 and the statistical analysis showing mean values are given in table- 1. The *lactobacilli* count of both the products was well above $8.5 \log \text{ cfu/ g}$ in the fresh products as well as throughout the refrigerated storage period (28 days). The initial count was 33 and 43×10^7 (8.52 and $8.64 \log \text{ cfu/ g}$) in plain probiotic *lassi* (A) and synbiotic *lassi* with carrot juice (B), respectively which is statistically non-significant.

The statistical analysis for storage of *lassi* sample A up to 14th day, at an interval of 7 days indicated that there were highly significant ($P \leq 0.01$) changes and from, 14th to 21st day changes were non-significant. Later till 28th day the changes were highly significant ($P \leq 0.01$). While in sample B the changes were highly significant ($P \leq 0.01$) till 07th day and showed non-significant change there after till 28th day. Initially probiotic count in blend B was higher than blend A till 7th day, this may be due to the presence of prebiotic substances present in carrot juice as well as the FOS that we have added.

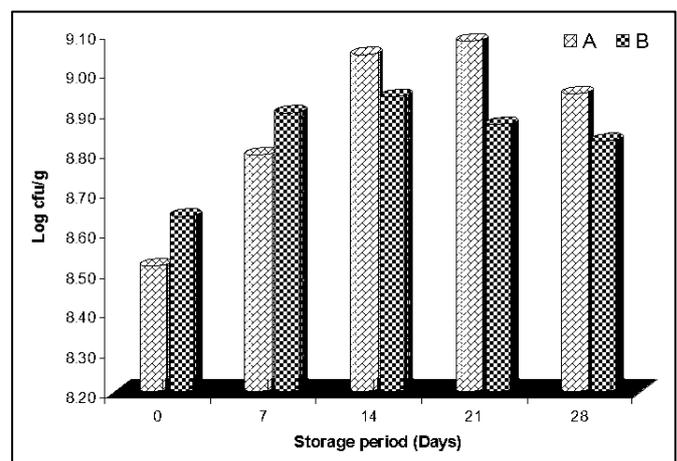


Fig 1: Changes in *lactobacilli* counts of plain probiotic *lassi* (A) and synbiotic *lassi* added with carrot juice (B) during refrigerated storage (4 ± 1 °C).

In a work carried out by Patidar and Prajapati (1998), *L. acidophilus* and *S. thermophilus* counts in *lassi* remained above 10^7 throughout the refrigerated storage period (7 ± 1 °C) of 15th days and the workers also reported that the *lactobacilli* count remained at satisfactory level even on 27th day of storage. Study carried out by Korbekandi *et al.*, 2008, also reported that the probiotic count remained above 10^7 cfu/ml , even after refrigerated storage period (5 ± 1 °C) of 21 days in probiotic yoghurt; which is in support of the present findings.

Table 1: Statistical analysis for changes in *lactobacilli* count of plain probiotic *lassi* (A) and synbiotic *lassi* added with carrot juice (B) during refrigerated storage (4 ± 1 °C).

Days	0	07	14	21	28	Treatment mean
A	8.52	8.79	9.05	9.08	8.95	8.88
B	8.64	8.90	8.94	8.87	8.83	8.84
Period mean	8.58	8.85	8.99	8.98	8.89	
Total	265.69		General mean: 8.86			

Different workers have reported similar trends in the counts of lactobacilli in fermented milk products during storage study (Kailasapathy *et al.* 2008; Mortazavian *et al.*, 2007; Hingu, 2009; Sharma *et al.*, 2016; Choi *et al.*, 2016) [14, 21, 10, 28, 1].

As suggested by several workers (Shah, 2000; Lourens-Hattingh and Viljoen, 2001; Shah *et al.*, 2001) [26, 27, 17], the viable count of probiotic culture that should be available to the consumers' for therapeutic benefits should be in the range of 10^6 to 10^9 in the product, which was available in both the samples *viz.*, probiotic *lassi* and synbiotic *lassi* samples, even after stipulated refrigerated storage (4 ± 1 °C) for 28 days.

3.2 Coliform Counts

In the present study coliforms were absent in both the samples in 10 g quantities throughout 28 days of refrigerated storage period. The presence of coliform bacteria in dairy products is indicative of unhygienic conditions or practices followed during manufacture, and storage (Speck, 1984) [29]. The confirmation of presence of faecal coliforms in the products, further, indicates probable and alarming presence of potent human pathogens too. In the present work to adjudge mainly the extent of sanitary practices followed during production and storage or otherwise, the coliform count of plain probiotic *lassi* and synbiotic *lassi* with carrot juice was carried out. These results were comparable with the results of different workers who have reported similar trends in the coliform counts in fermented milk products during storage study (Momin, 2009; Hingu, 2009; Gawai and Shah, 2016; Sharma *et al.*, 2016) [10, 28, 7].

3.3 Yeast and Mold Counts

Yeast and mould count was performed to check the aerial contamination of the product during manufacturing and as well as during the stipulated refrigerated storage period. In the present study yeast and mold counts were found absent in both the samples throughout the 28 days storage period. These results also coincided with the observations reported by Vahedi *et al.*, 2008, who also reported absence of yeast and mold count in the yogurt drink added with apple. Young and Nelson (1978) reported that the growth of yeast and mould count was marginal at refrigerated storage up to sell date of the market samples in sweet acidophilus milk.

The high level of yeast and mould count in fermented dairy products indicates poor aerial sanitation, insanitary conditions during manufacture, contaminated packaging material, use of contaminated culture as well as luxuriant growth of this group under acidic nature of the products during storage (Tamime and Robinson, 1999).

4. Conclusion

Present study has proved that synbiotic *lassi* as well as probiotic *lassi* were having satisfactory shelf-life of 28 days at 4 ± 1 °C. This product could be a potential functional food mainly because they contained viable cell of probiotic

lactobacilli well above 10^7 cfu/gm and 15% carrot juice v/v that had many reported health benefits. Current study has shown a positive influence of carrot juice on the survival of probiotic lactobacilli.

5. Acknowledgement

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