Intl. J. Food. Ferment. 5(1): 27-38, June 2016

DOI: 10.5958/2321-712X.2016.00004.1

Formulation, Sensory and Microbial Aspects of Functional Fermented Dairy Product – Synbiotic Dahi

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Abstract

The present investigation was carried out for the formulation of new functional dairy product "synbiotic dahi" and to study its properties. To formulate synbiotic dahi in three different forms, heat treated cows' whole milk (95 °C/5 min. and subsequently cooled to 37 °C) was inoculated with probiotic culture *L. helveticus* MTCC5463 @2.0% v/v. Prebiotic inulin @2.0% along with other functional food ingredients like WPC (whey protein concentrate) @3.0%, NMCP (natural milk calcium powder) @200 mg/100ml was added to prepare a plain synbiotic dahi (blend A). To make synbiotic dahi sweeten, in one form of dahi added non nutritive sweetener sucralose @19.5 mg/100 ml (blend B) and into another form added cane sugar @9.0% w/v (blend C). These different rates of addition of ingredients were decided on the basis of sensory profile evaluation during preliminary studies carried out. These three blends once formulated were compared with control dahi (M) which was made by using cow milk, without any additives and fermented with the same probiotic culture. All the four dahi prepared were stored at 4 ±1 °C for the period of 28 days and evaluated for chemical composition and microbiological attributes at an interval of seven days. As all the three forms of synbiotic dahi prepared were acceptable throughout the storage period for all the sensory and biochemical parameters, without noticeable and undesirable changes, along with around 10^7 to 10^8 cfu/ml of live count for probiotic culture.

Keywords: Dahi, probiotics, inulin, WPC, calcium, sucralose, sweetener, synbiotic, prebiotics, health, functional foods

One of the most popular and perhaps oldest documented fermented milk product in Indian subcontinent is 'dahi' which resembles the popular western fermented milk product 'yoghurt', and is generally obtained through lactic fermentation of cow/buffalo/of mixed milk. Since primeval *vedic* times, people of all ages savor dahi and is considered to be one of the healthiest and nourishing dairy product in Asian sub-continent. Dahi is accredited with several nutritional and therapeutic properties (Laxminarayana, 1984; Prajapati and Nair, 2003). Dahi/yoghurt is more digestible than milk due to reduced protein particle size and increased the contents of soluble nitrogen, non-protein nitrogen and free amino acid during processing and manufacturing (Laxminarayana, 1984). Deeth and Tamime (1981) confirmed that yoghurt proteins are twice as digestible as milk proteins, which was evident from the fact that for yoghurt only 3 h are required to attain more than 70% digestion, compared with 6 h for milk. Contents of niacin, folic acid, vitamin $B_{12,}$ biotin *etc.* may increase during fermented milk manufacture, thus improving its nutritive value. Highest increased in folic acid *i.e.* 10 fold in yoghurt as well as in dahi observed (Deeth and Tamime, 1981). These products are valued for controlling the growth of undesirable bacteria and in curing intestinal diseases like constipation, dysentery and acute diarrhoea in children (Agarwal and Bhasin, 2002) and also effective in lowering the blood cholesterol (Kansal and Chawla, 1984).

Dahi at home or commercially, can be normally prepared from inoculating single/mixed microflora comprising *Lactobacilli, Lactococci,* and *Streptococci spp.* to milk (Laxminarayana 1984; Eleven and Prasad 1998). However, literature review clearly indicated that few attempts have been made to manufacture dahi with probiotic cultures (Mukhopadhyaya and Singh, 1973), and few with prebiotics and or with added ingredients like WPC and SMP.

The synbiotic concept combines efficacious probiotic strain with specific prebiotic compound in a single product (Ashwell, 2002). Synbiotic is defined as "a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplement in the gastrointestinal tract". Prebiotics especially, inulin and oligofructose are well documented to provide many health benefits and this in synbiotic products ought to be more fruitful in giving wide-ranging nutritional and health benefits (Roberfroid, 2005; Shah, 2009).

Synbiotic product comes under categorization under functional foods as it contains a component (whether or not a nutrient) that benefits one or a limited number of functions in the human body in a targeted way that is relevant to either the state of well-being and health, or the reduction of the risk of a disease (Kalida and Gibosn, 2011), or if it has physiological or psychological impact beyond the traditional nutritional effect (Clydesdale, 1997). Dairy foods are rich sources of protein, calcium and a variety of vitamins, minerals and bioactive compounds. Hence, they provide an ideal food medium for delivering probiotics and other functional ingredients (Gawai and Prajapati, 2012). Probiotics are defined as living microbial feed supplements added to the diet, which have beneficial effects on the host by improving its intestinal microflora balance (Fuller, 1989). Probiotic bacteria are preferred over antibiotics by being preventive, non-invasive and free of any undesirable effects.

Increase in the consumption of several fermented milk products *viz*. yoghurt, dahi, acidophilus sour milk, cultured buttermilk, koumiss are attributed to their palatability, and nutritional and therapeutic attributes (Tamime and Deeth, 1980). Among all the aforesaid products, *Lactobacillus acidophilus* based milk and related probiotic food products occupy an important place. In comparison to *Bifidobacteria spp., L. acidophilus* is a friendly bacterium, which thrives in unfavourable environment, encourages intestinal microflora balance and promotes the health functioning of the intestinal system (Sandine *et al*., 1972). Acidophilus cultures are furthermore known to synthesize nutrients in the intestinal tract and counteract the pathogenic microorganisms. It may well be used for digestive maintenance and flora restoration after prolong courses of antibiotic treatment (Goldin and Gorbach, 2008).

Prebiotics are food ingredients, in particular certain non-digestible dietary oligosaccharides/fibres; that cannot be digested, other than by some privileged bacteria and are involved in selective stimulation of the growth and/or activity of good bacteria (probiotics) in the gastrointestinal tract to benefit the health of the host (Roberfroid, 2000).

The most successful applications of prebiotics are in several forms of food products inclusive of dairy products (fermented milks, milk drinks, cheeses, ice creams and desserts), bakery products, spreadable products, chocolate, meal replacers, bars and cereals, beverages or prebiotic drinks *etc*. (Kaur and Gupta, 2002; McDevitt and Rooyakkers, 2003). Inulin and oligofructose are extensively used in a variety of food products both, for their technological as well as nutritional attributes (Roberfroid, 2002; Tungland and Meyer, 2002).

Whey is a reliable source of high quality and biologically active proteins, carbohydrates and minerals. Whey proteins are recognized as being of very high nutritional quality. They are easily digested and the essential amino acid profile meets or exceeds all the nutritional requirements of the FAO/WHO (Hugunin, 1999). Additionally, whey and whey derived bioactive compounds have been studied for their ability to enhance general health and well being (Cross and Gill, 2000). Several physiological roles have been defined or suggested for whey proteins or peptides, which includes the immune enhancing, antioxidant and anticarcinogenic properties of whey proteins, with specific emphasis on the tripeptide glutathione (Ha and Zemel, 2003).

Calcium as a mineral plays an essential and diverse role in the body and is vital for the health. The majority (~ 99%) of calcium present in the body is found in bone, where it plays a structural function, which provides rigidity to the skeleton. Daily nutritional requirements of calcium can be fulfilled by consuming milk and dairy products, which are considered to be an excellent source of calcium and with higher bioavailability as compared with other plants foods (Theobald, 2005).

This brief review of the concept and details of ingredients described above could have been best matched for this formulation of "synbiotics dahi" which is depicted in present investigation. Synbioitc dahi formulation was planned with the use of probiotic culture along with addition of prebiotic inulin. Functional food ingredients *viz.* whey protein concentrate powder, natural milk calcium powder were also tried to add in this dahi to boost its nutritive, therapeutic as well as functionality and organoleptic appeal which can confer enumerable health benefits to the consumers. Sugar added in synbiotic dahi not merely for energy, sweet taste and pleasing appeal but also it acted as a thickening agent considering that it will exhibit preservation of ingredients from undesirable organisms (Dannone, 1999). However, another variant of synbiotic dahi was prepared considering importance of dietetic and diabetic foods (Binns, 2003) by replacing sugar with non nutritive/intense sweetener *i.e.* sucralose.

Material and Methods

Fresh cow milk of Gir Breed (clean and antibiotic free) was collected from Livestock Research Station, Anand for preparing of synbiotic dahi. Details of ingredients used for study are listed below:

Whey Protein Concentrate (70% proteins) and Natural Milk Calcium Powder (NMCP) was supplied by Mahaan Proteins Ltd, Mathura, (UP). Inulin (trade name Raftiline) was supplied by Oraftii Ltd, Belgium. High quality food grade sucralose tablets (trade name of Zero, manufactured by M/S Alembic Ltd, Vetva, Ahmedabad, India) were locally purchased. Each tablet was contained 6.5 mg of sucralose. For fortification of synbiotic dahi with nutritive sweetener high quality sugar, free from impurities was purchased from local supplier in Anand town.

Probiotic culture and its maintenance

The culture used was *L. helveticus* MTCC 5463, obtained from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand. The culture was propagated in sterilized skim milk (10% T.S.) for 16 h and stored at 5 ± 2 °C. The culture was analyzed routinely for purity and was reactivated weekly to maintain viability during the course of the study.

Method for preparation of synbiotic dahi

The study was planned with the selection of the best rate of addition of ingredients for the manufacturing and development of synbiotic dahi. For this, preliminary trials were taken with different rates of addition of ingredients. Based on sensory evaluation of the products on 9 point hedonic scale, the products were screened by trained panel of judges and the final rate of addition of each ingredient was finalized.

Complete flow diagram for preparation of plain and synbiotic dahi is given in Fig. 1.

Fresh raw cow milk was filtered through muslin cloth. Milk was distributed in to different lots of desired size. As decided earlier, the cow milk was subjected to four different treatments/combinations designated as M, A, B and C for the manufacturing of dahi. Details of different treatment given are showed in table 1.

Addition of all different ingredients, except sucralose, was done at a desired level after warming the milk at 45 °C. Four lots of raw milk along with various ingredients in desired combinations (M, A, B and C) were subjected to heat treatment at 95 °C for 5 min. for dahi manufacturing. Only sucralose tablets were added aseptically after the heat treatment, just before the milk was subjected to cooling.

Table 1: Final combinations decided to develop the synbiotic dahi

Blends	Treatment
M	Control (milk fermented with *L. helveticus* MTCC 5463)
A	Milk + WPC + Inulin + NMCP (Calcium) + *L. helveticus* MTCC 5463
B	Milk + WPC + Inulin + NMCP + Sucralose + *L. helveticus* MTCC 5463
C	Milk + WPC+ Inulin + NMCP + Sugar + *L. helveticus* MTCC 5463

All the lots of milks were cooled to 37 °C and inoculated with culture *L. helveticus* MTCC 5463 @ 2% v/v. The contents were mixed thoroughly by stirring with stainless steel ladle. Inoculated milks in the quantity of around 30-35 ml were filled in sanitized plastic cups (previously treated by dipping in 100 ppm hypochlorite solution for 15 min., drained and dried). The cups were covered with UV light treated aluminium foil. The inoculated milk was incubated at 37±1 °C till acidity reached to 0.60-0.65% lactic acid. The acidity was measured periodically throughout the incubation period. When desired acidity was attained, the set curd was immediately transferred to refrigerator (3 to 4 °C) to promptly cease the further activity of the culture. Final acidity of products was taken after 3.0 – 3.5 h of cooling in refrigerator. The samples of synbiotic dahi were stored at 4±1 °C to study changes occurred during storage. The observations were taken at the interval of 7 days up to 28 days. Samples were evaluated on the basis of sensory (*i.e.* flavour, colour and appearance, body and texture and overall acceptability score), chemical composition of products and by analyzing for microbiological [*i.e. Lactobacillus* count (LC), yeast and mold count (YMC), coliform count (CC)] parameters.

Receiving raw cow milk

⇓

Filtration

⇓

Warming up to 45 °C

⇓

Addition of selected ingredients (In case of synbiotic dahi and without ingredients in controlled dahi)

⇓

Heat treatment (95 °C for 5 min)

⇓

Cooling (37 °C)

⇓

Inoculation of culture (*L. heveticus* MTCC 5463 @ 2 %)

⇓

Filling in plastic cups and covered with aluminium foil

⇓

Incubation (37 °C)

⇓

Until acidity reaches 0.65 % L. A.

⇓

Shifting in refrigerator

⇓

Cooling (3 ± 1 °C)

⇓

Storage of dahi (3 ± 1 °C)

Fig. 1. Flow chart for the preparation of synbiotic dahi

Preparation of samples for microbiological analysis of dahi

After sufficient cooling (around 3.5 h) of dahi on the 0 day, a set of two cups from each lot, were removed for conducting *Lactobacillus* count (LC), coliform count (CC) and yeast and mould count (YMC). Similarly, thereafter the dahi samples were analyzed microbiologically at an interval of 7 days, up to 28 days of storage. The products were subjected to the sensory evaluation by an expert trained panel of five judges for colour and appearance, flavour, body and texture and overall acceptability criteria at the decided intervals. Fresh product at 0 day and the stored products at 7, 14, 21 and 28 days storage at 4±1 °C were brought to 10 °C before giving for judging.

The statistical analyzes were done by using completely randomized design as suggested by Steel and Torrie (1980). The square root transformation was applied for analysis of YMC. For lactic acid count and sensory analyses, average reading of three replications was subjected to factorial experiment in a completely randomized design. Co-relations were determined within and among different parameters *i.e.* with sensory and microbiological parameters.

Results and Discussions

On the basis of results obtained from preliminary trials, it was decided to incorporate the chosen ingredients at the following levels: WPC @3.0% (w/v), inulin @2.0% (w/v), NMCP @200 mg (w/v) to fortify calcium (ca. 54.5 mg/100ml), sugar @9.0% (w/v) and sucralose @19.5 mg (ca. 3.0 tablets), to formulate new functional fermented food - synbiotic dahi. Altogether, three combinations/blends of synbiotic dahi (A, B and C) along with control (M) were prepared as given in table 1. The probiotic culture *L. helveticus* MTCC 5463 was used to culture all the four lots (A, B, C and M) separately, @ 2.0% (v/v). The photograph of all blends of synbiotic *dahi* along with control dahi is given in figure 2.

Chemical composition

The average chemical composition of developed functional food - synbiotic dahi is given in table 2. The cow milk used to manufacture control dahi was recorded with average chemical composition as moisture 87.42, T.S. 12.58, fat 4.38, protein 3.4, ash 0.56 and carbohydrate (by difference) 4.24%. The control sample of dahi (M) indicated almost identical chemical profile to that of cow milk as no additives were added to this blend. In case of blends A and B, the T.S. was increased to nearly 4.40% due to supplementation of whey protein, inulin and NMCP. The protein content of the blends (A, B and C) was 5.5%. The carbohydrate content was also increased to nearly 2.0% for blends A and B, and 8.89% for blend C.

All the three fresh synbiotic dahi samples were significantly superior in compare to the control sample M, for overall acceptability scores. Overall acceptability score at the end of 28 days storage for control dahi was significantly lower from rest of the three synbiotic dahi samples. Based on overall acceptability, it was noticed that treatment B and C were non-significant throughout the entire storage period, which indicates that addition of either sucralose or sugar could not be differentiated in sensory evaluation of the products. This clearly provides the avenue to incorporate non-nutritive sweetener like sucralose in health and dietetic foods, without compromising consumers' acceptance for sensory pleasure of such products.

Fig. 2. The photograph of all blends of synbiotic dahi along with control dahi

Changes in Lactobacilli counts

Changes in *Lactobacilli* counts of control dahi (M) as well as synbiotic dahi (A, B and C) during storage at 4±1 °C are shown in figure. 3. The table 3 indicates mean values for the same.

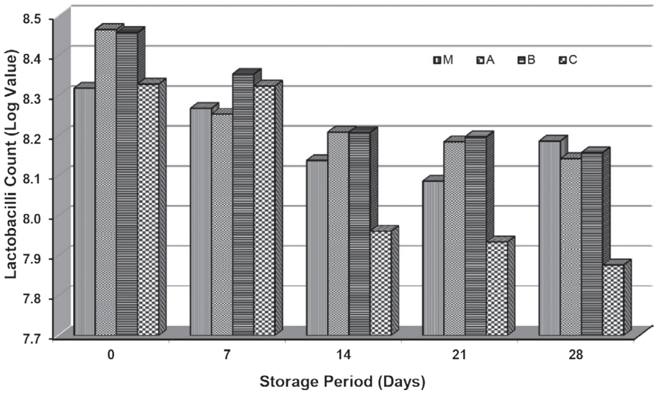

Fig. 3: Changes in lactobacilli counts of synbiotic dahi during storage

All the four freshly made (0 day) dahi samples had lactobacilli count varying from 20 to 29 × 10^7 cfu/ml (log values 8.3182 to 8.4650), which were statistically not significant ($p<0.05$). The statistical analysis for storage of dahi up to 28 days, at an interval of 7 days indicated that the three treatments and its comparison with control was also non significant. During refrigerated storage of the samples (0 to 28 days), in case of sample M, A and B, there was slight decline in lactobacillus counts. However, in case of blend C the decline was more prominent (from 21 to 7.5x10^7 cfu/ml) due to presence of sugar. Nevertheless, in the synbiotic dahi samples A and B and the control (M), the count of *L. helveticus* MTCC5463 was remained >10^8 cfu/ml even after the end of storage period of 28 days. The interaction (D x T) between storage period (D) and the treatments (T) was also non significant. This indicates that there was not much difference in lactobacilli counts during storage period and for individual treatment also, it had no influence on lactobacilli counts.

As suggested by different workers Lourens-Hattingh and Viljoen (2001) and Shah *et al*., (2001), the viable count of probiotic culture that should be in the range of 10^6-10^9 cfu/ml for therapeutic benefits. This criterion was satisfied in all the three synbiotic dahi samples and count were more than said after storage for 28 days. This could be an added advantage as some times during storage, the probiotic count drops below the required level (Shah *et al*., 2001). Iniguez *et al*., (2001) also reported similar results that counts of probiotic lactic acid bacteria in fermented milk prepared by mixing of buffalo and cow milk (70:30) remained >10^7 cfu/g in all the samples after low temperature storage at 4 °C for 10 days.

Few studies are available on refrigerated storage off dahi made with probiotic cultures. However, Dave *et al*., (1993) reported that lactic counts in the dahi with different levels of total solid and made by means of thermophilic cultures remained either unaffected or showed slight increase during first 5 days storage, which significantly reduced at 12 and 18 days of storage. Yadav *et al*., (2007) prepared synbioitc products with mixed dahi cultures NCDC167, *Lactococcus lactis* ssp *diacetylactis* NCDC60 and two probiotic strains; *Lactobacillus acidophilus* NCDC14 and *Lb. casei* NCDC19 and determined count from 24 h to 8 d storage at 7 °C. They reported that the viable counts of lactococci and lactobacilli decreased during storage but remained >106 cfu/ml after storage period with detection of oligosaccharides due to transgalactosidal and lactose hydrolysis activities of β-galactosidase.

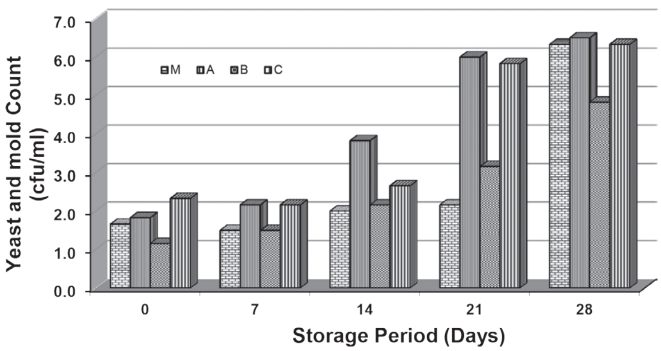

Fig. 4. Changes in yeast and mold counts of synbiotic dahi during storage

A few workers have reported either the positive or no influence of fortification of WPC on the growth of probiotic cultures. Martin-Daina *et al*., (2004) studied the supplementation of WPC @3% in fermented product containing *S. thermophilus* ST-20Y, *L. acidophilus* LA-5 and *B. lactis* BB-12, and recorded the highest counts of the probiotic strains *L. acidophilus* LA-5 (3.3×10^5 cfu/g) and *B. lactis* BB-12 (5.5×10^7 cfu/g) after 21 days of storage at 4 °C. Cantunes *et al*., (2005) manufactured probiotic product containing *L. acidophilus*, *B. longum*, *L. bulgaricus* and *S. thermophilus* with addition of milk and SMP and or WPC @2%. At the end of storage period of 21 days, they concluded that the viability of probiotic microorganisms did not lower down to less the 10^5 cfu/ml. In these studies, it was also reported that the addition of WPC had no influence on the total viable count, and in the present investigation too; WPC fortification did not have any positive impact on the count of the probiotic culture used.

On the contrary, Martin-Daina *et al*., (2003) observed that enrichment of goats' milk with WPC @3%

Table 2: Average chemical composition of functional food - synbiotic dahi

Bends	Moisture (%)	T.S. (%)	Fat (%)	Protein (%)	Carbohydrate (By diff %)	Ash (%)
Milk	87.42	12.58	4.38	3.4	4.24	0.56
M	87.37	12.63	4.43	3.4	4.20	0.60
A	83.04	16.96	4.40	5.5	6.35	0.71
B	82.98	17.02	4.35	5.5	6.49	0.69
C	74.20	25.80	4.40	5.5	15.09	0.81

Table 3: Changes in lactobacilli count (cfu/ml) during storage of synbiotic dahi

Storage period (Days)	M	A	B	C	Period mean
0	8.31	8.46	8.45	8.32	8.39
7	8.26	8.25	8.35	8.32	8.29
14	8.13	8.20	8.20	7.96	8.12
21	8.08	8.18	8.19	7.93	8.10
28	8.18	8.14	8.15	7.87	8.09
Treatment mean	8.19	8.25	8.27	8.08	
Total 492.13	C.V. % 3.96				General mean 8.20

NS - Non significant (P<0.05)

Table 4: Changes in yeast and mold count (cfu/ml) during storage of synbiotic dahi

Storage period (Days)	M	A	B	C	Period mean
0	1.43	1.50	1.23	1.58	1.44
7	1.09	1.62	1.36	1.52	1.40
14	1.51	2.00	1.52	1.76	1.70
21	1.56	2.54	1.75	2.46	2.08
28	2.61	2.64	2.30	2.60	2.54
Treatment mean	1.64	2.06	1.63	1.98	
Total 110.03	C.V. % 27.23				General mean 1.83

*Square root transformed values; NS - Non significant, * significant, ** highly significant (P<0.01)

and fermentation with *S. thermophilus* ST-20Y, *L. acidophilus* LA-5 and *B. lactis* BB-12 cultures reduced fermentation time by 2 h due to the increase in viable counts of *S. thermophilus* and *B. bifidum* by 0.3 and 0.7 log units, respectively. Kailasapathy and Supriadi (1996) also reported the beneficial effect of WPC addition on the survival of *L. acidophilus* in lactose hydrolysed yoghurt during refrigerated storage. It was observed that sufficiently high numbers of *L. acidophilus* remained viable during the refrigerated storage (approximately 5°C/21 days) of yoghurt. Bozanic and Tratnik (2001) investigated the quality of fermented bifidus milk from cows' and goats' milk, with (skim milk powder and WPC powder) and without supplements during storage. The growth of *B. bifidum* BB-12 during fermentation was better in WPC supplemented samples. On the 9th day of storage the viable counts in fermented cows' milk samples was 1.1×10^8 cfu/ml and in fermented goats' milk slightly higher (2.3×10^8 cfu/ml). The authors also reported that WPC supplementation had no influence on survival of *bifidobacteria* during storage. The varied influence of WPC on probiotic cultures in the above experiments clearly indicates that the effect is culture dependent.

Supplementation of inulin is usually shown to promote growth of probiotics like intestinal lactobacilli and especially bifidobacteria (Roberfroid, 2005). However, the influence may again be strain specific. Bozanic *et al*., (2004) and Kurien *et al*., (2005) in individual studies investigated the supplementation of inulin @3% and 1.5% in probiotic product containing *L. acidophilus* strains and observed that addition of inulin improved the growth and acid production along with high sensory quality during refrigerated storage. Zuleta *et al*., (2004) prepared fermented milk containing inulin and starch as additives for lactic acid bacteria (*L. plantarum* D34, *Lactobacillus spp*. SLH6, and *S. thermophilus* ST4) and established that growth of all strains was promoted by inulin. On the contrary, Ozer *et al*., (2005) studied the effect of supplementation of lactulose and inulin as prebiotics on the growth of *L. acidophilus* LA-5 and *B. bifidum* BB-12 in Acidophilus-Bifidus (AB) yoghurt and noted that inulin and lactulose did not affect the growth of yoghurt starter bacteria as well as *L. acidophilus* LA-5, but stimulated the growth of *B. bifidum* BB-12 to a great extent. In the present study too, it was also observed that fortification of inulin had no influence on growth of the probiotic culture *L. helveticus* MTCC 5463. Therefore, the blends A, B and C of synbiotic dahi, which were made using the same probiotic culture, can be used for deriving such and many more therapeutic benefits.

Sugar addition @ 9.0% had no negative influence on synbiotic dahi (blend C) in fresh samples (0 day) on the probiotic count, but it showed slight reduction in the lactobacilli count after 28 days storage period when compared with blend A and B as well as with the control (M). However, the viable count remained well above the minimum level required (10^7 cfu/ml) for probiotic foods claims.

Shah and Ravula (2000) made probiotic yoghurts (*S. thermophilus*, *L. delbrueckii ssp. bulgaricus, L. acidophilus* and *Bifidobacterium spp.*) with 0, 4, 8, 12 or 16% sucrose. There were no major differences in the initial counts of yoghurt and probiotic bacteria in all the five batches prepared. However, the counts of all the four groups of organisms declined during storage, in the batches containing 12% and 16% sugar. Thus, sugar addition at higher may leads to deleterious effect on the growth of yoghurt and survival of probiotic bacteria during storage. Kar and Mishra (1999) prepared sweetened wheyghurt drink using different concentrations of sugar (0, 6, 8, 10, 12 and 16%) and observed that as the level of sugar addition increased, there was very slight change in the titratable acidity, total viable count and antibacterial activity of the product. That change was continued up to 10% level of sucrose addition. Sucrose added @12% level caused undesirable changes, which were statistically significant ($P<0.05$). Sivadha *et al*., (2003) manufactured misti yoghurt and bio-yoghurt using probiotic cultures and inulin (raftiline) along with addition of natural sweetener fructose at different levels *i.e.* 8, 10, 12, 14% and observed that fructose addition lowers the probiotic count during storage.

Coliform Counts

The presence of coliform bacteria in dairy products is suggestive of insanitary conditions or inappropriate practices followed during production, processing and storage (Speck, 1984). It was found that during entire course of study, the coliforms in all the four fresh blends initially, as well as during all the intervals at refrigerated storage periods was absent in 1 ml dahi samples. Absence of coliform count indicates that the hygienicity was maintained during manufacturing and storage of dahi samples. The results were in confirmation with the work carried out by Kale *et al*., (2011), Nahar *et al*., (2007) and Dave *et al*., (1993). They found that the coliform count was <10 cfu/ml for dahi prepared from buffalo milk in laboratory condition.

Yeast and Mold Count

Yeast and mold perhaps, are one of the most

Table 5: Correlation matrix among sensory, chemical and microbiological attributes

	Flavour	Colour & Appearance	Body & Texture	Overall Acceptability	Lactic Count	Yeast & Mold Count
Flavour	—					
Colour & Appearance	0.7574**	—				
Body & Texture	0.8140**	0.8327**	—			
Overall Acceptability	0.9507**	0.8284**	0.9026**	—		
Lactic Count	-0.6250**	-0.2304	-0.5334**	-0.6001**		
Yeast & Mold Count	-0.2675	0.1428	-0.0384	-0.1800	-0.5956**	—

Critical value (1-tail, 0.05) = + / - 0.37911

Critical value (2-tail, 0.01) = + / - 0.44260

important groups of microbes present among several other groups of spoilage microflora in fermented milk products, including dahi. Yeasts and molds are mainly contaminated to the dahi samples through air (Tamime and Robinson, 1999).

Changes in yeast and mold count of control dahi (M) as well as synbiotic dahi (A, B and C) during storage are shown in Fig. 4. The statistical analysis for yeast and mold count is given in table 4, which indicates mean values.

It was observed that yeast and mold count for all the four blends of dahi i.e. M, A, B and C, were very low (<10 cfu/ml) during the entire storage period of 28 days. This is the indication of good aerial sanitation as well as hygienic conditions during manufacturing and storage of synbiotic dahi. The synbiotic dahi blends (A, B and C along with control M) were having yeast and mold count < 3.0 cfu/ml on the 0 day and at the end of 28 days storage period, it was < 7 cfu/ml. Even though, the magnitude of change recorded was very low. The YMC when compared among the treatments were significant, and among various storage periods, it was highly significant. However the interaction between treatment and storage period was non-significant.

Dave *et al*., (1993) studied the laboratory made buffalo milk dahi and found that the Y & M count was less than 100 cfu/ml, which was within specified limit of BIS (max. 100 cfu/ml). On the other hand, market survey in various cities of India revealed that the Y & M count was varying to a great extent, from 100 to 61,00,000 cfu/ml (Dave *et al*., 1993; Sarkar *et al*., 1996). The high level of yeast and mold count in fermented dairy products indicates poor aerial sanitation, insanitary conditions during manufacture, contaminated use of 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).

Correlation studies for sensory and microbial attributes

Sensory and microbiological attributes were statistically correlated. It was observed that all the sensory attributes very well and positively correlated with each other. Co-relation among sensory attributes and microbial counts is given in table 5.

It can be seen from the statistical analysis that flavour score was positively and significantly correlated ($p<0.01$) with all the sensory attributes, including colour and appearance ($r= 0.75$), body and texture ($r= 0.81$) and overall acceptability score ($r= 0.95$). In developing the synbiotic dahi, the correlations between flavour and other sensory parameters indicates the direct and positive influence of these factors on consumers' preference.

Flavour score did not exhibit any significant correlation with microbes (lactobacilli and YM count) associated with synbiotic dahi samples. Colour and appearance was positively and significantly correlated ($p<0.01$) with body and texture (r= 0.83) and with overall acceptability (r= 0.82). The colour and appearance scores revealed highest and positive correlation (r= 0.83) with body and texture, indicating that the synbiotic dahi fortified with ingredients also had better colour and appearance. It was also observed that colour and appearance did not show signs of any significant correlation with microbiological parameters. Similarly, body and texture showed a positive and significant correlation ($p<0.01$) with overall acceptability (r= 0.90), pH (r= 0.83).

Conclusion

The synbiotic dahi added with nutritive ingredients which were formulated, they confirmed stability during storage from both microbial and sensory parameters point of view. As all the three synbiotic dahi preparations were acceptable throughout the refrigerated storage of 28 days for all the sensory parameters (colour, flavour, body and texture and overall acceptability), without noticeable/undesirable changes, along with around 10^7 to 10^8 cfu/ml of live count for probiotic culture. They can withstand steadfastly in the market, if introduced, and may confer nutritional and therapeutic benefits to the consumers of all ages and genders. Introduction of such kind of proprietary product may open new door for the well-being of human and for fermented milk industry. At the same time, it requires further research and trial for *in vivo* assessment of these products for whether actual benefits are being conferring human or not.

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