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Research Article

MICROBIOLOGICAL, SENSORY AND ECONOMICAL ANALYSIS OF CULTURED LOW FAT SYNBIOTIC BUTTERMILK

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A B S T R A C T

Cultured low fat synbiotic buttermilk samples were prepared with probiotics such as *L.acidophilus* and *B.bifidum* and prebiotic substances like oligofructose and honey. The experimental samples were divided into six treatments from T1 to T6. All the samples prepared were subjected to microbiological analysis for standard plate count (SPC), lactic acid bacterial count, Lactobacillus acidophilus count, Bifidobacterium bifidum count, coliform count and yeast and mould count. The control and experimental samples showed no significant difference in coliform count, yeast and mould count but showed significant difference in standard plate count. The mean lactic acid bacterial (LAB) count in the control was 12.44 $\log_{10}$ cfu/ml while for treatments T1 to T6, the values were 12.42, 12.39, 12.35, 12.40, 12.34 and 12.44, respectively and differences between control and treatments were not statistically significant. *L. acidophilus* counts of ($\log_{10}$ cfu/ml) in cultured low fat synbiotic buttermilk (T1, T3, T5 and T6) were 8.43, 8.43, 8.39 and 8.29, respectively and the values were within the therapeutic minimum (10^6/ml) requirement on 0 day storage at refrigeration temperature. The counts ($\log_{10}$ cfu/ml) of *B.bifidum* in control and cultured low fat synbiotic buttermilk samples were 8.51, 8.51, 8.46 and 8.41 for T2, T4, T5 and T6, respectively and showed no significant difference. The costs of the experimental samples were priced higher due to the additional cost of probiotic and prebiotic substances.

INTRODUCTION

Traditionally, in India, buttermilk milk is considered as an important and inseparable food ingredients in the diet of predominant population since time immemorial. Milk is consumed in different forms such as whole milk, yoghurt, butter, ghee, buttermilk, skim milk, flavoured milk, cheese, ice cream etc. The milk and its products contain nutritionally essential components such as fat, proteins, carbohydrates, vitamins and minerals at varying levels and hence are always recommended as health drink for improving the consumer's health. Fermentation technique was used to preserve the milk during excess production. Various fermented milk products are also dominated in meeting the food demand of the country. The success of Anand model combined with operation flood has changed the face of dairying in India drastically from household activity to commercial enterprise leading to the establishment of many dairy plants and larger production of western dairy products such as ice cream, cheese etc., in addition to the traditional indigenous dairy products. Buttermilk is one among the fermented dairy products produced in India, known for its cooling, refreshing, thirst quenching, delicious, nutritive and digestive properties.

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According to Mathur (1991), preparation of buttermilk date backs to Paleolithic and Neolithic ages. It is a popularly known cultured milk beverage prepared with different variations in different parts of the country. Buttermilk prepared in the traditional way is considered beneficial to health as it contains probiotic microbes and generally referred as "Grandma's probiotic". The probiotic nature of buttermilk is beneficial to the gut and improves immunity when taken regularly. In addition, buttermilk also contains many vitamins, minerals like calcium, potassium and phosphorus which help in improving the health status of patients. In buttermilk production, *L.acidophilus and B.bifidum* are commonly used as probiotics which have beneficial effect on human health.

The concept of synbiotic was introduced by Gibson and Roberfroid (1995) who defined synbiotic as a food substance having the combination of both prebiotics and probiotics in which the prebiotics have synergistic effect on the function of probiotics resulting in improved immune and health status of the host. Prebiotics improved the survival of probiotics in intestine and facilitates their inoculation into the colon. The prebiotic induced growth and increases activity of positive endogenic intestinal flora (Gallaher and Khil, 1999).

Probiotic inhibited the growth of pathogenic bacteria in intestine (Gibson *et al*., 1997) by preventing the adhering of

pathogens to intestinal walls (Coconnier and Levin, 1997), decrease pH levels, produce hydrogen peroxide and bacteriocins like nisin and pediocin (Saavedra, 1995), They also competitively utilize the nutrients such as simple carbohydrates which are necessary for development of pathogens and thereby improved the health status of the host. Few dairy companies manufacturing buttermilk on commercial scale also follow their own method of processing. Hence, no detailed research studies have been conducted so far on the production of buttermilk and its value addition as for as the reported literature till date, The present study was conducted to prepare synbiotic buttermilk with honey and oligofructose as prebiotics and *L. acidophilus* and *B. bifidum* as probiotic and to analysis of its microbial, sensory properties and economics of production.

MATERIALS AND METHODS

Fresh cow milk collected from Arul Anandar College, Karumathur dairy farm and skim milk was prepared from that by removing of cream. Freeze dried mixed dahi culture (Mesophilic type NCDC 352), *Lactobacillus acidophilus* (NCDC 014) and *Bifidobacterium bifidum* (NCDC 232) purchased from National Collection of Dairy Cultures, NDRI, Karnal were used for preparation of buttermilk. The prebiotic oligofructose obtained from Kanisshka Flora Chem India, Chennai and honey procured directly from Honey hives, Madurai were utilized as prebiotics for preparation of cultured low fat synbiotic buttermilk. The media, reagents, chemicals and solvents used in this study were obtained from Hi Media, Mumbai, India. All the glassware (Borosil Pvt. Ltd.) used for this study were thoroughly washed, air dried and sterilized in hot air oven at 160 to 180^{0}C for 2 h prior to use.

The buttermilk was prepared as per the procedure of Sukumar De, (2004) with slight modifications. The details on composition of various control and experimental samples are presented in table 1. The prebiotics like honey and oligofructose were included in the buttermilk at the rate of 2 percent level. Skim milk was pasteurized at 85^{O}C for 30 minute and the cooled to room temperature (37ºC) after which inoculation of dahi culture and probiotic culture (at the rate of 2 per cent) were added based on the treatments and incubated at 37ºC so as to reach the pH 4.5, after the formation of curd it was cooled under refrigeration temperature of 5^{o}C. Cultured buttermilk was prepared according to the method described by Maheta *et al.* (2015). The control buttermilk and experimental cultured low fat synbiotic buttermilk samples were prepared by mixing of curd and water in a ratio of 1: 3 and to achieve a uniform texture it was homogenized.

All the samples prepared were subjected to microbiological analysis of standard plate count (SPC), lactic acid bacterial count, *Lactobacillus acidophilus* count, *Bifidobacterium bifidum* count, coliform count and yeast and mould count. The results of the microbial counts were expressed as $\log_{10}$ cfu/ml. Normal saline was used for serial dilution of samples. One ml of buttermilk sample was transferred aseptically into 9 ml of sterile saline. Serial dilutions were prepared up to 10^{-6} dilution factor. 1ml of appropriate dilution (10^{-4} to 10^{-6}) was used for enumeration of bacteria and $10^{-1,}$ 10^{-2} dilution each for coliform count and yeast and mould count. Quantification of standard plate count (SPC) was carried out as per the procedure of IS: 5402, 1969 using Plate count agar. The Lactic acid bacterial count of buttermilk and stored buttermilk drinks was determined as per the method described in ISI Handbook (1981). The *Lactobacillus acidophilus* count of the drink was measured based on the method described by De Man *et al*. (1960). The count of *Bifidobacterium bifidum* was determined as per the procedure of Vinderola and Reinheimer, (1999). The coliform count of the cultured buttermilk samples were analysed as per the procedure of IS: 5401, 1969 using violet red bile agar and the yeast and mould count of buttermilk samples were estimated as per the procedure of IS: 5403, 1969 using potato dextrose agar.

The economics of production of buttermilk samples were estimated based on the cost of the ingredients using linear programming model. The statistical analysis of data was carried out by applying completely randomized design (CRD) (Steel and Torrie, 1980). The values for microbial counts in log were transformed before analysis.

RESULTS AND DISCUSSION

The standard plate count showed an increasing trend in the cultured low fat synbiotic buttermilk samples, as compared to the control and exhibit a significant difference ($p<0.01$) among them. The reason might be due to the addition of probiotic cultures that perhaps influenced the standard plate count of cultured low fat synbiotic buttermilk samples. The result is in agreement with the report of Deepika Shree *et al*. (2017) who found that the Psyllium husk as prebiotic substance added at the rate of 02 to 04 per cent level in the preparation of traditional buttermilk contained a total plate count 2.9 x10^8 cfu/ml during the first day.

Coliform count of cultured low fat synbiotic buttermilk samples showed no significant difference among them and was within the limits of Indian standard 9617 (1980).

Table 1 Quantity of ingredients for the preparation of different treatments of cultured low fat synbiotic buttermilk

Items	Control (ml)	Treatments (ml)					
		T1	T2	T3	T4	T5	T6
Skim milk	Skim milk 1000 ml	Skim milk 1000 ml	Skim milk 1000 ml	Skim milk 1000 ml	Skim milk 1000 ml	Skim milk 1000 ml	Skim milk 1000 ml
Starter culture	Dahi culture 20 ml	Dahi culture 20 ml	Dahi culture 20 ml	Dahi culture 20 ml	Dahi culture 20 ml	Dahi culture 20 ml	Dahi culture 20 ml
Probiotic bacteria	-	*L.acidophilus* 20 ml	*B.bifidum* 20 ml	*L.acidophilus* 20 ml	*B.bifidum* 20 ml	*L.acidophilus* +*B.bifidum* Each 20 ml	*L.acidophilus* + *B.bifidum* Each 20 ml
Prebiotic material	-	Honey 20 ml	Honey 20 ml	Oligofructose 20g	Oligofructose 20g	Honey 20 ml	Oligofructose 20g

Table 2 Microbial analysis of cultured low fat synbiotic buttermilk

Type	Treatments (log_{10} cfu/ml)						
	C	T1	T2	T3	T4	T5	T6
Standard plate count (Mean ± SE)	4.23 [a] ± 0.03	4.42 [b] ± 0.02	4.41 [b] ± 0.01	4.42 [b] ± 0.02	4.43 [b] ± 0.02	4.72 [c] ± 0.03	4.83 [c] ± 0.06
Coliform count (Mean ± SE)	7.33 ± 0.56	7.17 ± 0.60	6.50 ± 0.62	7.00 ± 0.58	7.17 ± 0.60	6.50 ± 0.62	7.00 ± 0.58
Yeast and mould count (Mean ± SE)	69.50 ± 3.45	66.67 ± 2.86	68.17 ± 3.49	67.33 ± 2.96	67.67 ± 2.95	66.83 ± 3.81	68.67 ± 2.44
Lactic acid bacterial count (Mean ± SE)	12.44± 0.04	12.42± 0.03	12.39± 0.03	12.35± 0.04	12.40± 0.04	12.34± 0.04	12.44± 0.03
L.acidophilus count (Mean ± SE)	------	8.43 ± 0.03	------	8.43 ± 0.03	------	8.39 ± 0.03	8.29 ± 0.05
B.bifidum count(Mean ± SE)	-------	------	8.51 ± 0.03	------	8.51 ± 0.03	8.46 ± 0.03	8.41 ± 0.03

Means bearing (n=6) with different superscripts differ significantly (P<0.01)

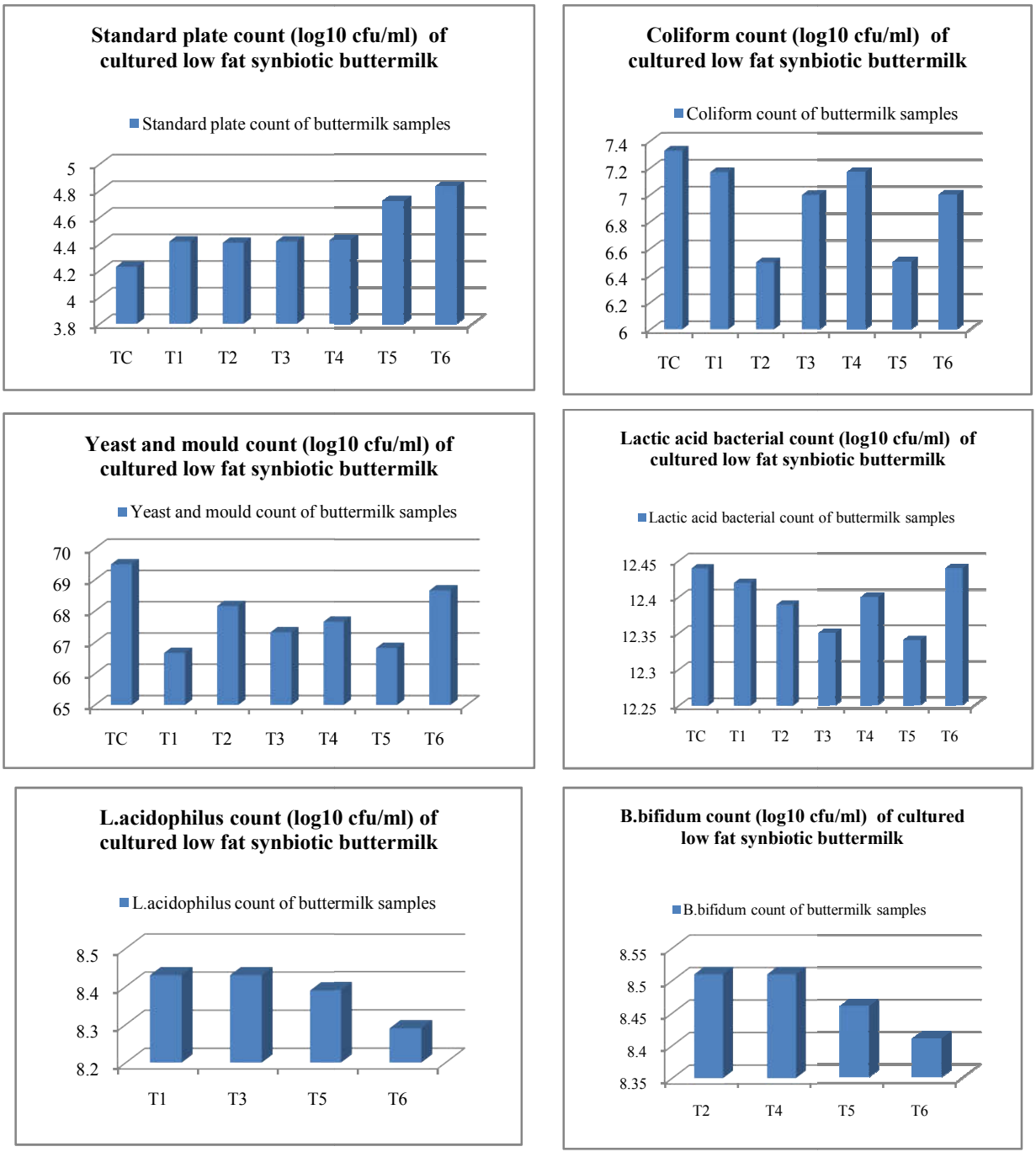

Figure 1 Microbial count of cultured low fat synbiotic buttermilk samples

The mean yeast and mould count (log_{10} cfu/ml) in the control and the treatments were 69.50, 66.67, 68.17, 67.33, 67.67, 66.83 and 68.67, respectively. There was an increase in the yeast and mould count of control as compared to the experimental buttermilk samples, but the differences were not significant and within the limits of Indian standard 9617 (1980).

The mean lactic acid bacterial (LAB) count in the control was 12.44 log_{10} cfu/ml and for the treatments were 12.42, 12.39, 12.35, 12.40, 12.34 and 12.44, respectively for treatments T1 to T6. Lactic acid bacterial count of cultured low fat synbiotic buttermilk samples showed no significant difference between control and treatments. A minimum range of 10^6–10^7 plate microorganisms per gram or millilitre should be present in food product in order to meet the requirements of a probiotic food, as reported by the Japanese fermented milk and lactic acid bacteria drinks association (Ishibashi and Shimanura, 1993).

L. acidophilus counts of (log_{10} cfu/ml) in cultured low fat synbiotic buttermilk (T1, T3, T5 and T6) were 8.43, 8.43, 8.39 and 8.29, respectively and the estimates were within the therapeutic minimum (10^6/ml) requirement on 0 day as reported by Robinson (1989). The present result is also similar to that of Dong (2015), who prepared synbiotic cultured buttermilk containing inulin as a prebiotic and the probiotics *Lactobacillus acidophilus* and *Bifidobacterium species* and opined that the survivability of *L. acidophilus* was above 7.5 log_{10} cfu/ml on first of preparation of synbiotic cultured buttermilk.

The count (log_{10} cfu/ml) of *B.bifidum* in control and cultured low fat synbiotic buttermilk samples were 8.51, 8.51, 8.46 and 8.41 for T2, T4, T5 and T6, respectively and the differences were not statistically significant. Dias *et al*. (2013) prepared milk serum-based drink consisting of 50 per cent milk serum containing 10 per cent saccharose, 25 per cent powdered milk, 15 per cent yacon pulp, and cultures of *L. acidophilus-La 5E* and *B.bifidum BB12* and found that the *B.bifidum* population was at 2.0×10^9 cfu/ml. Addition of probiotics in combination with prebiotics showed a marked increase in the total *B. bifidum* count of T4 and T6, respectively.

Table 3 Sensory Evaluation Score of Cultured Low Fat Synbiotic Buttermilk Samples

Characters	TC	Treatments					
		T1	T2	T3	T4	T5	T6
Flavour Score	7.83 ± 0.31	7.67 ± 0.21	7.67 ± 0.33	7.50 ± 0.34	7.83 ± 0.31	7.67 ± 0.33	7.83 ± 0.31
Colour and appearance Score	8.50 ± 0.22	8.17 ± 0.31	8.00 ± 0.37	8.17 ± 0.40	8.17 ± 0.17	8.33 ± 0.33	7.83 ± 0.31
Body and texture Score	7.83 ± 0.40	7.33 ± 0.42	7.00 ± 0.37	7.17 ± 0.40	7.17 ± 0.17	7.33 ± 0.33	7.00 ± 0.37
Overall acceptability Score	8.05 ± 0.28	7.72 ± 0.18	6.16 ± 1.26	7.61 ± 0.30	7.66 ± 0.21	7.78 ± 0.20	7.66 ± 0.19

The sensory evaluation of synbiotic buttermilk samples was carried out using nine point hedonic scale as per the recommendation of Sonali *et al*. (2016). The overall average sensory scores of control and different cultured low fat synbiotic buttermilk samples are given in table 3. The flavor scores obtained by different samples showed no significant difference between control and treatments and within the treatments. The (mean ± SE) flavor scores for control and treatments T1 to T6 were 7.83 ± 0.31, 7.67 ± 0.21, 7.67 ± 0.33, 7.50 ± 0.34, 7.83 ± 0.31, 7.67 ± 0.33 and 7.83 ± 0.31, respectively. The scores of body and texture also showed no significant difference between control and treatments and within the treatments. The average body and texture scores obtained by control and treatments T1 to T6 were 7.83 ± 0.31, 7.67 ± 0.21, 7.67 ± 0.33, 7.50 ± 0.34, 7.83 ± 0.31, 7.67 ± 0.33 and 7.83 ± 0.31, respectively.

The color and appearance scores of control and T1 to T6 treatment buttermilk samples showed no significant difference and the mean ± SE of scores were 8.50 ± 0.22, 8.17 ± 0.31, 8.00 ± 0.37, 8.17 ± 0.40, 8.17 ± 0.17, 8.33 ± 0.33 and 7.83 ± 0.31, respectively. The overall average acceptability scores of control and treatment buttermilk samples showed no significant difference between and within the treatments. The overall average acceptability scores for control and T1 to T6 treatments were 8.05 ± 0.28, 7.72 ± 0.18, 6.16 ± 1.26, 7.61 ± 0.30, 7.66 ± 0.21, 7.78 ± 0.20 and 7.66 ± 0.19, respectively.

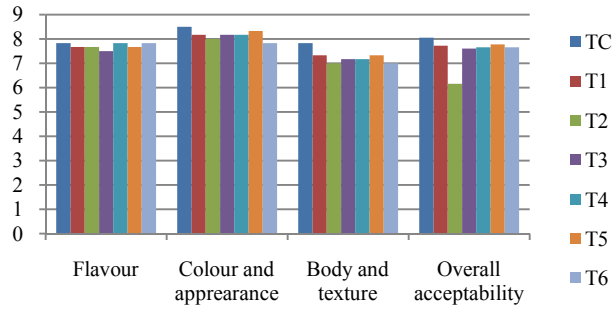

Fig 2 Sensory Evaluation Score of Cultured Low Fat Buttermilk Samples

The above results revealed that addition of prebiotics viz., honey and oligofructose and probiotics *L.acidophilus* and *B.bifidum* did not alter the overall acceptability scores. Hence, all the experimental samples were at par with control with regard to sensory evaluation by the panellists.

Zeynab *et al*. (2010) reported that synbiotic acidophilus milk prepared by fermenting milk with combinations of all cultures (*L. acidophilus*, *B.bifidum* and *L. casei*) along with honey or 10 per cent inulin significantly increased the colour, flavour, texture and overall acceptability. Deepak and Sheweta (2016) found that buttermilk fortified with partially hydrolyzed guar gum has markedly improved its sensory characteristics as compared to control sample. The fibre fortified buttermilk samples had significant changes in body, mouth feel and overall acceptability without affecting the color and appearance and flavor of buttermilk. The results obtained in the present study are on par with the findings of with Zeynab *et al*. (2010) and Deepak and Sheweta (2016).

Table 4 Cost of production of low fat synbiotic buttermilk

Ingredients (cost in rupees)	C	T1	T2	T3	T4	T5	T6
Skim milk Qty (L)	1	1	1	1	1	1	1
Price (Rs)	40	40	40	40	40	40	40
Dahi culture Qty (ml)	20	20	20	20	20	20	20
Price (Rs)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
L. acidophilus culture Qty (ml)	0	20	0	20	0	20	20
Price (Rs)	0	0.5	0	0.5	0	0.5	0.5
B. bifidum culture Qty (ml)	0	0	20	0	20	20	20
Price (Rs)	0	0	0.5	0	0.5	0.5	0.5
Honey Qty (ml)	0	20	20	0	0	20	0
Price (Rs)	0	8.00	8.00	0	0	8.00	0
Oligofructose Qty (gm)	0	0	0	20	20	0	20
Price (Rs)	0	0	0	8.00	8.00	0	8.00
Total Cost (Rs)	40.50	49.00	49.00	49.00	49.00	49.50	49.50
Difference in price (Rs)	0	8.50	8.50	8.50	8.50	9.00	9.00
Increase in price (%)	0	19.85	19.85	19.85	19.85	20.05	20.05

The cost of preparation of one litre of cultured low fat synbiotic buttermilk samples are presented in Table 4. The results indicated that probiotic cultures and prebiotic

substances incorporated buttermilk samples showed an increase in the cost of production as compared to the control. In general, any food product prepared with active and functional ingredients naturally fetch higher price than the normal food product. Similarly, in our experimental buttermilk samples, functional ingredients such as probiotic and prebiotic material have increased the cost of production. The cost difference between control and control experimental has ~~is~~ ranged from Rs. 9.0 to 9.50 only.

CONCLUSION

From the above microbial evaluation studies, it was concluded that all the cultured low fat synbiotic buttermilk samples and control showed no significant difference in coliform count, yeast and mould count but showed significant difference in standard plate count between control and treatments and within treatments. The preparation costs of experimental samples were higher due to the additional cost of probiotic and functional food. Considering the beneficial effect of cultured low fat synbiotic buttermilk samples, consumers always prefer to such functional foods rather than the routine foods. Moreover, in the present days consumer's due to health consciousness make them preferences towards the purchase of functional foods are obviously increasing. Such kind of foods will fetch an importance in the market.

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