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Research Article

STUDY ON THE PHYSICO- CHEMICAL PROPERTIES OF CULTURED LOW FAT SYNBIOTIC BUTTERMILK

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A B S T R A C T

Buttermilk is one among the most important dairy products regularly included in the diets of Indian subcontinent. Further, the supplementation of buttermilk with optimum combination of prebiotics and probiotics helps to improve its nutritional qualities and deliciousness. In view of the above facts, a study was made to develop functional buttermilk with prebiotic substances viz., honey and oligofructose and probiotic cultures viz., L. acidophilus and B. bifidum. The titratable acidity (percentage of lactic acid) values for control and treatments were in between 0.73 and 0.75. The pH of 4.5 was taken as the cut-off point in the fermentation process of buttermilk as this pH was reported to be the optimum level for production of good quality buttermilk. Mostly, all the samples were maintained a pH of 4.5 and showed no significant difference. The total solids, protein, fat showed a significant increase in the synbiotic buttermilk samples when compared to the control. The ash content of the synbiotic buttermilk samples showed a little increase as compared to the control. The titratable acidity and the pH were maintained in all the synbiotic buttermilk samples as that of control. Further, it ic concluded that functional buttermilk samples can be prepared with prebiotic substance (at two per cent levels) honey and oligofructose and probiotic cultures L.acidophilus and B. Bifidum with increased total solids, protein, fat and ash.

INTRODUCTION

Historically, the value of milk and milk products had been appreciated because of its medicinal and nutritional properties. Further, the fermented milk products have been very popular in the Vedic and medieval era. Billions of bacteria inhabit the human digestive system and these bacteria are referred to as the gut flora. The gut flora consists of more than 400 different species, or types, of beneficial/harmful bacteria. One way of maintaining a balance between the beneficial and harmful bacteria in the gut is to consume a source of probiotics (beneficial bacteria) that can be introduced into the digestive system through food. Probiotic is a functional food that is essential for good health.

According to the FAO/WHO (2001), prebiotics are "non digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria". Recently, scientists defined a dietary prebiotic as ''a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal macrobiotics, thus conferring benefit(s) upon host health''. They have an advantage over probiotic supplements in that there is no concern about oligosaccharides being destroyed while in storage or in route to the intestine through the stomach acid and digestive enzymes. Prebiotics consist mainly of oligosaccharides, sugar molecules of three to six chains and soluble fiber.

The preparation of buttermilk date backs to Palaeolithic and Neolithic age Mathur (1991). Buttermilk prepared in the traditional way is considered beneficial to health as it contains probiotic microbes and is sometimes referred to 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.

Synbiotics refer to combination of probiotics and prebiotics and in a form of synergism. The main reason for using a synbiotic is that a true probiotic, without its prebiotic food, does not survive well in the digestive system. Establishing synbiotic concept in dairy foods will provide a new lease of life for the consumers. Roberfroid (2007), reported that prebiotics are the food ingredients non digestible (dietary fibre) in the stomach but are selectively fermentable and selectively stimulate the growth, composition, and activities of microflora in gastrointestinal tract of the host and probiotics

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are live microbial feed supplements which enhance the consumer health by improving the balance of microflora in the gut when ingested live in sufficient members, Fuller (1989).

In India, it was considered as household product and so the required technology for commercial manufacture of buttermilk has not been systematically standardized (Patel, 2009).

The experimental cultured low fat synbiotic buttermilk samples will possess functional properties than the traditional buttermilk. Such kind of buttermilk possesses therapeutic and health promoting properties to the consumers. The low cost nature of cultured low fat buttermilk can be available for the all section of people and upon regular consumption improves the gut health.

In view of the above facts, a study was made to develop functional buttermilk with prebiotic substances viz., honey and oligofructose and probiotic cultures viz., *L. acidophilus* and *B. bifidum*.

MATERIALS AND METHODS

Fresh skim milk collected from local market of Madurai was utilised in this study for preparation of buttermilk. Prebiotics like Oligofructose obtained from the Bayleaf Wellness Private Ltd, Noida, India and honey procured directly from honey hives, Madurai were utilized for preparation of cultured low fat synbiotic buttermilk. The dahi culture of mixed Mesophilic) strain and probiotic of freeze dried cultures of *Lactobacillus acidophilus* NCDC 014 and *Bifidobacterium bifidum* (NCDC 232) were obtained from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal, Haryana, India.

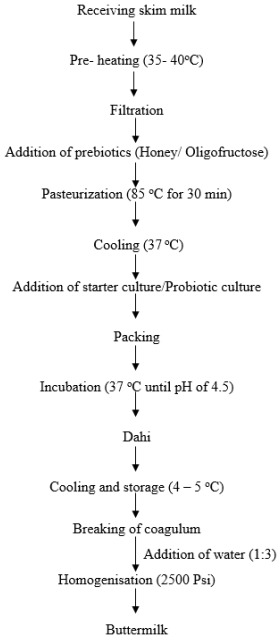

Fig 1 Flow chart for preparation of cultured low fat synbiotic buttermilk

Table1 Ingredients used in the preparation of different treatments of cultured low fat synbiotic buttermilk

Items	Control	T1	T2	T3	T4	T5	T6
Skim milk	Skim milk	Skim milk	Skim milk	Skim milk	Skim milk	Skim milk	Skim milk
Starter cultures	Dahi culture	Dahi culture	Dahi culture	Dahi culture	Dahi culture	Dahi culture	Dahi culture
Probiotic bacteria	-	*L.acidophilus*	*B.bifidum*	*L.acidophilus*	*B.bifidum*	*L.acidophilus +B.bifidum*	*L.acidophilus + B.bifidum*
Prebiotic materials	-	Honey	Honey	Oligofructose	Oligofructose	Honey	Oligofructose

Method of Preparation

The dahi samples were prepared by slight modification of procedure described by Sukumar De (2004). The measured quantity of skim milk was taken and filtered through muslin cloth. Milk was preheated heated to 35°C to which prebiotic like honey or oligofructose were added at 2 per cent as per the treatment schedule. Then pasteurised at 85°C for 30 min and then cooled to 37°C. To which dahi culture and probiotic cultures were added as per the treatment.

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 at the ratio of 1:3 and then homogenized to achieve a uniform texture.

The titratable acidity percentage of the buttermilk samples were analysed as per the procedure given in IS: SP 18 (Part XI), 1981. pH of the samples were measured by Electronic digital type pH meter Hanna No. H_1 8416 according to method No.981.12 of AOAC (2000).The moisture content was measured as per the procedure described in IS: 1479, part II, (1961). The total solids content were estimated based on the calculation, total solid (g %) $=(\text{weight of dish} + \text{Dry buttermilk})/\text{weight of sample} \times 100$. The fat percentage of cultured low fat buttermilk samples were determined as per the procedure given in IS: 1224, (Part- I), 1981 and the protein content of product was estimated by Kjeldahl method as described in IS: 9617, (1980). The method was modified and the cultured buttermilk sample was taken in place of milk. The ash percentage of buttermilk samples were estimated by incineration of the sample placed in the muffle furnace at 600 °C for 6 h as per the procedure of IS: 1479, Part II, 1961.The statistical analysis of data was carried out by applying completely randomized design (CRD) (Steel and Torrie, 1980).

Table 2 Quantity of the ingredients added for different treatments of cultured low fat synbiotic buttermilk

Ingredients	Control	T1	T2	T3	T4	T5	T6
Skim Milk (ml)	1000	1000	1000	1000	1000	1000	1000
Dahi Starter (%)	2	2	2	2	2	2	2
Lactobacillus acidophilus (%)	-	2	-	2	-	2	2
Bifidobacterium bifidum (%)	-	-	2	-	2	2	2
Honey (%)	-	2	2	-	-	2	
Oligofructose (%)	-	-		2	2	-	2

RESULT AND DISCUSSION

The data with mean ± SE for various parameters of analysis of cultured low fat synbiotic buttermilk are presented in table 3 and figure 2. Buttermilk has characteristically sour taste. Increased acidity of buttermilk is primarily due to lactic acid produced by lactic acid bacteria; while fermenting lactose, the primary sugar in milk (Negussie *et al.,* 2016).The titratable acidity (percentage of lactic acid) values for control and treatments T1 to T6 (mean± SE) were 0.73 ± 0.01, 0.74 ± 0.02, 0.74 ± 0.02, 0.74 ± 0.03, 0.74 ± 0.01, 0.75 ± 0.01 and 0.75 ± 0.02, respectively. Statistical analysis of the results showed no significant difference among them. The titratable acidity of the all experimental buttermilk samples were at par with the

control and are in agreement with the results reported by Antunes *et al*. (2005). Further, Vasavada and White (1979) investigated eight brands of cultured buttermilk from retail outlets in the city of Athens for the titratable acidity and the estimates varied between 0.79 and 0.85 in fresh samples and from 0.84 to 0.88 in stored samples. The titratable acidity estimates obtained in this study are in agreement with that of Vasavada and White (1979) and Sonali *et al*. (2016). Further, the acidity levels in control and experimental buttermilk samples obtained in the present study were well within the stipulated Indian standard (1980) range of 0.6 to 0.8 (as lactic acid percent by mass) for dahi.

The pH of 4.5 was taken as the cut off point in the fermentation process of buttermilk as this pH was reported to be the optimum level for production of good quality buttermilk. The (mean ± SE) pH values obtained for control and treatments T1 to T6 were 4.50 ± 0.04, 4.52 ± 0.06, 4.55 ± 0.04, 4.50 ± 0.05, 4.47± 0.03, 4.53 ± 0.03 and 4.48 ± 0.06 respectively. Statistical analysis of the results pertaining to pH of the control and experimental buttermilk samples showed no significant difference among them. In general, milk curdling and gelation are normally initiated at the pH value below 5.5 as the casein micelles destabilize and the protein matrix irreversibly precipitates. The pH estimates of experimental buttermilk samples prepared in the study were at par with the control buttermilk samples. The statistical analysis of the pH data showed that there is no significant difference between control and experimental buttermilk samples. Deepika Shree *et al*. (2017) reported that the Psyllium husk added buttermilk contained a pH of 4.82 had better sensory properties. Thus, the pH values of control and experimental samples obtained in the present study are in agreement with the result of Antunes *et al*. (2005), Sodini *et al.* (2006) and Deepika Shree *et al*. (2017).

Table 3 Physico-chemical analysis of cultured low fat synbiotic buttermilk

Parameters		Treatments						
		TC	T1	T2	T3	T4	T5	T6
Titratable acidity (% LA)	Mean ± SE	0.73 ± 0.01	0.74 ± 0.02	0.74 ± 0.02	0.74 ± 0.03	0.74 ± 0.01	0.75 ± 0.01	0.75 ± 0.02
pH	Mean ± SE	4.50 ±0.04	4.52 ±0.06	4.55 ±0.04	4.50 ±0.05	4.47 ±0.03	4.53 ±0.03	4.48 ±0.06
Total solids (g%)	Mean ± SE	11.32^{a} ± 0.04	11.50^{b} ± 0.04	11.55^{c} ± 0.04	11.55^{c} ± 0.04	11.57^{c} ± 0.03	11.62^{c} ± 0.05	11.67^{c} ± 0.03
Fat (%)	Mean ± SE	0.45^{a} ±0.03	0.52^{a} ±0.03	0.57^{b} ±0.03	0.57^{b} ±0.03	0.55^{b} ±0.04	0.60^{b} ±0.04	0.62^{b} ±0.03
Protein (g %)	Mean ± SE	3.78^{a} ±0.01	3.83^{b} ±0.02	3.83^{b} ±0.01	3.84^{b} ±0.01	3.84^{b} ±0.01	3.83^{b} ±0.02	3.84^{b} ±0.01
Ash (mg %)	Mean ± SE	0.73 ±0.01	0.74 ±0.02	0.74 ±0.01	0.74 ±0.01	0.75 ±0.02	0.74 ±0.02	0.75 ±0.02

Means bearing different superscripts differ significantly (P<0.05)

The average total solids content (g percentage) of control and treatments T1 to T6 synbiotic buttermilk samples were 11.32 ± 0.04, 11.50 ± 0.04, 11.55 ± 0.04, 11.55 ± 0.04, 11.57± 0.03, 11.62 ± 0.05 and 11.67 ± 0.03 respectively. Statistical analysis revealed significant difference (p<0.01) between control and treatment samples and the total solids percentage of control samples were nearer to the T1 treatment samples as compared to other treatments T2 to T6. The control butter milk sample has a total solids content of 11.32 percent. But the total solid contents of experimental butter milk samples of treatments T1 to T6 were higher than the control which might be attributed to the addition of probiotic bacteria like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and prebiotic substances like honey and oligofructose in dahi culture. Therefore, incorporation of these essential ingredients into buttermilk contributes to a higher total solids content of the resultant cultured buttermilk. Meshram (2015) reported that the total solids in buttermilk added with various fruit beverages like mango, orange, banana ranged from 11.3 to 18.3 per cent. Sailaja *et al*., (2014) reported that a total solids percentage of14.33for the cinnamon flavored buttermilk. Our present results pertaining to the total solids of control and experimental buttermilk samples are in agreement with the results of Deepika Shree *et al*. (2017), Meshram (2015) and Sailaja *et al.* (2014) respectively.

The (mean ± SE) fat percentage values for control and treatments T1 to T6 were 0.45 ± 0.03, 0.52 ± 0.03, 0.57 ± 0.03, 0.57 ± 0.03, 0.55 ± 0.04, 0.60 ± 0.04 and 0.62 ± 0.03, respectively and the differences in fat percentage were statistically significant (P < 0.05). In general, the fat percentage of treatment samples T1 to T6 was higher than the control samples. Binjan *et al*. (2017) reported that higher fat content in buttermilk varying between 0.5 and 1.5 percentages. Antunes *et al*. (2009) found that the average fat content of buttermilk samples of several flavours (natural, strawberry, vanilla, graviola, cupuacu, pineapple with mint) sweetened with sucrose and sucralose were 0.21 and 0.23 per cent. Deepika Shree *et al*. (2017) reported that the Psyllium husk added at the rate of 2 to 4 per cent level in buttermilk contained the fat content of 0.50 per cent. The fat contents of control and experimental buttermilk samples obtained in the present study were well within the ranges reported by the above researchers.

The estimates for protein content (mean ± SE) of the control and experimental cultured low fat synbiotic buttermilk samples T1 to T6 were 3.78 ± 0.01, 3.83 ± 0.02, 3.83 ± 0.01, 3.84 ± 0.01, 3.84 ± 0.01, 3.83 ± 0.02 and 3.84 ± 0.01, respectively. Statistical analysis revealed significant difference (P<0.05) between the estimates of control and treatment samples. The treatments T1 to T6 had a little increase in the protein content as compared to the control but the increase was meager. The increase in the protein content of treated buttermilk samples over the control might be due to the addition of probiotic cultures along with medium such as *L.acidophilus and B.bifidum* separately and together in the treatments T1 to T6. However, the differences in the protein contents of treatment samples fromT2 to T6 were not statistically significant. Deepika Shree *et al*. (2017) reported that the Psyllium husk added at the rate of 2 to 4 per cent level in buttermilk contained the protein content of 3.76 per cent. Dong (2015) found that the protein content of synbiotic buttermilk and control buttermilk were 3.30 and 3.29, respectively. Sailaja *et al*. (2014) reported a protein percentage of 3.75 in cinnamon flavored buttermilk. Thus, the results with regard to the protein contents buttermilk samples prepared in the present study were in agreement with the findings of Deepika Shree *et al*. (2017), Dong (2015), and Sailaja *et al*. (2014).

The mean ash estimates (mg percentage) for control and treatments T1 to T6 were 0.73 ± 0.01, 0.74 ± 0.02, 0.74 ± 0.01, 0.74 ± 0.01, 0.75 ± 0.02, 0.74 ± 0.02 and 0.75 ± 0.02, respectively. Statistical analysis revealed non-significant difference between control and treatment samples. The ash contents of all the treatment samples were nearer to the estimates of control samples. The results obtained in the present study reveals that the addition of probiotic starter cultures and prebiotic substances such as honey and oligofructose did not influence the ash content of the experimental samples. Sailaja *et al*. (2014) reported an ash content of 0.54for the cinnamon flavored buttermilk. Libudzisz

and Stepaniak (2003) opined that traditional buttermilk is the liquid left after butter making from fermented cream or milk, with naturally occurring lactic acid bacteria contained 0.6–0.75 g/100 ml ash. The present results with regard to the ash content of control and experimental buttermilk samples are in accordance with the values reported by Sailaja *et al*. (2014), Libudzisz and Stepaniak (2003) and Sodini *et al*. (2006).

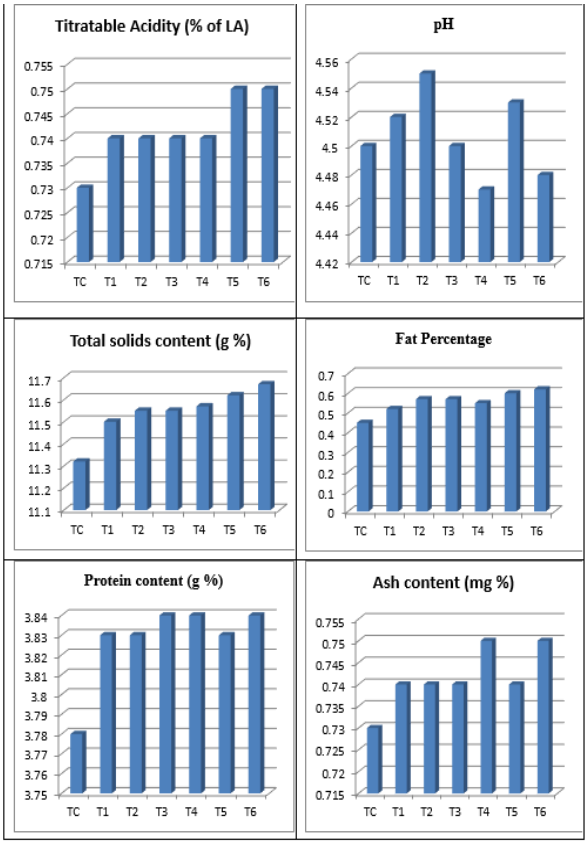

Figure 2 Physico - chemical characters of cultured low fat synbiotic buttermilk

CONCLUSSION

This investigation was made to prepare functional buttermilk samples with prebiotic substance (at two per cent levels) honey and oligofructose and probiotic cultures *L.acidophilus* and *B. Bifidum.* The total solids, protein, fat showed a significant increase in the cultured low fat synbiotic buttermilk samples when compared to the control. The ash content of the synbiotic buttermilk samples showed a little increase as compared to the control. The titratable acidity and the pH were maintained in all the synbiotic buttermilk samples as that of control.

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