JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2016. Vol. **10**(4), p. 3221-3230

Storage Stability and Chemical Constituents of Cultured Buttermilk Prepared by Incorporation of Paneer Whey

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http://dx.doi.org/10.22207/JPAM.10.4.100

(Received: 17 Octuber 2016; accepted: 11 Noveber 2016)

Paneer whey is a by-product obtained during manufacture of coagulated milk products and is generally considered as a waste. Its disposal as waste leads to heavy increase in BOD and COD of the dairy effluent. Moreover, it also leads to loss of valuable milk solids. Hence, efficient and economic utilization of paneer whey is the need of the hour. Looking to physic-chemical nature of paneer whey, its utilization in preparation of cultured milk appears to be most attractive. In this study the fermented whey added cultured buttermilk was analysed for their mineral content and storage stability. The mineral content like calcium, phosphorus, sodium, potassium and zinc content of RT_3 (40% added fermented whey), RT_4 (30% added fermented whey) and RT_5 (control, 30% added water) were 75.52, 106.85, 28.37, 90.78 and 0.39; 74.17, 100.36, 27.66, 88.52 and 0.43; and 64.40, 63.65, 19.88, 63.63 and 0.38 mg/100g respectively. In case of storage stability, the product was acceptable till 5 days of storage due to acceptable sensory qualities, physico-chemical characteristics and microbial qualities.

Keywords: Paneer whey, Cultured buttermilk, Proteolytic activity.

Whey is produced in very large amount and its utilization has been a continuing challenge for dairy industry. More than half of the solids in milk remain in the whey and the quantity of liquid whey produced is roughly ten times that of the products of interest (Canli, 2005).

The safe disposal of whey results in increased operating costs of the effluent treatment plants due to high consumption of electrical energy. The way of whey disposal as a waste leads to the loss of valuable milk nutrients. These in turn affect the profitability of dairy plant. Hence, utilization of this valuable whey (gutter-to-gold) leads to the financial advantage in dairying, as well as, it reduces the organic load and treatment costs on the effluent treatment plant by reducing the consumption of electrical energy (Mallik and Kulkarni, 2009).

Looking at the composition and characteristics of paneer whey, particularly its acidic nature and salty taste, the most appropriate product from among different dairy products for utilization of the this whey is cultured buttermilk. This appears to be the simplest and practicable approach to utilize paneer whey. The cultured buttermilk is sour, refreshing beverage used as staple food since ancient time. Cultured buttermilk especially being a live product, certain specific and non-specific shortcomings may be expected during storage. Stability of cultured buttermilk, similar to other food products, during storage is of vital importance and crucial for commercial success.

Therefore, various types of cultured buttermilk prepared in the laboratory were evaluated for their minerals content and storage stability.

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MATERIALS AND METHODS

Cultured buttermilk was prepared according to the method described by Ghanshyambhai *et al.* (2015). Fresh paneer was prepared by laboratory method and whey was collected. The whey and double toned milk were heated to 90°C for 5 min to kill the all living bacteria and cooled to 37°C. The whey and milk were inoculated with *Lb. helveticus* MTCC 5463 and *L. lactis* subspp. *Diacetylactis* NCDC 60 at the rate 2 per cent (1:1) and incubated at 37°C for 48 h. The fermented whey and dahi were mixed in to the two different ratios 60:40 (RT_3), 70:30 (RT_4) and control (RT_5) was prepared by using water in case of fermented whey in 70:30 (RT_5) (dahi:water) ratio. All the samples of buttermilk were analyzed for its minerals content and storage stability.

Estimation of selected minerals of cultured buttermilk

Preparation of ash solution

The cultured buttermilk sample was subjected to ashing. The ash was obtained by method described BIS method (BIS, 1981). The ash solution was prepared by AOAC method (AOAC, 2006).

Determination of Calcium

Calcium content of all samples were determined by the procedure described by BIS method (BIS, 1981).

Determination of phosphorous

Phosphorous content of all samples were determined by the procedure described by BIS method (BIS, 1981).

Determination of sodium and potassium

Sodium and potassium were determined by flame photometer (CL- 378, ELICO ltd) using ash solution (as above mention) after appropriate dilution.

Determination of zinc

The ash solution (as above mention) was used for the analysis of zinc by atomic absorption spectrophotometer (Perkin Elmer, Model: 3110) after appropriate dilution.

Storage study

The buttermilk samples packed in PET bottles and stored at 7±1ºC were evaluated for their sensory (9-point hedonic score card was used for sensory evaluation of buttermilk) and physico-chemical characteristics i.e. acidity and proteolytic activity. In case of microbial analysis total lactic counts (by using lactic agar), yeast and mould counts (by using potato dextrose agar) and coliform counts (by using violet red bile agar) were done during the storage study by using BIS methods (BIS, 1969).

Determination of acidity

Titratable acidity of the double toned milk was measured as per the BIS method (BIS, 1981).

Determination of proteolytic activity

Proteolytic activity was measured by OPA (O-phthaldialdehyde) method (Quantitative method) described by Hati *et al.* (2015).

Statistical analysis

Statistical analysis of data was carried out by applying completely randomized design (CRD) (Steel and Torrie, 1980). The data obtained during storage study of cultured buttermilk were subjected to statistical analysis using Factorial completely randomized design (FCRD).

RESULTS AND DISCUSSION

The average values of total solids for prepared cultured buttermilk RT_3 (60:40), RT_4 (70:30) and RT_5 (control) were 6.97, 7.57 and 6.83 per cent respectively, whereas fat values were 1.34, 1.32 and 1.02 per cent, respectively. Similarly the protein and ash contents were 2.28, 2.24 and 2.13; and 0.75, 0.73 and 0.57 per cent respectively for RT_3, RT_4 and RT_5 buttermilk. The lactose content was 2.60, 3.28 and 3.11 per cent for RT_3, RT_4 and RT_5 samples respectively.

Minerals content of buttermilk were determined as method described in methods and materials. The results of analysis for selected minerals like, calcium, phosphorous, sodium, potassium, zinc and iron of different types cultured buttermilk are presented in Table 1.

From the data it can be seen that 60:40 (RT_3) contained more calcium, phosphorus, sodium and potassium compared to 70:30 (RT_4) and control (RT_5). The three types of cultured buttermilk differed significantly ($P<0.05$) from each other for all mineral content.

Minerals such as calcium, copper, iron, manganese, magnesium, phosphorus, sodium, and zinc that have different roles in the body including enzyme functions, bone formation, water balance maintenance, and oxygen transport are found in milk.

The bovine milk contains 50 mg of sodium; 145 mg of potassium; 120 mg of calcium; 13 mg of magnesium; 95 mg of phosphorous; and 0.39 mg of zinc per 100 ml (Fox and MacSweeney, 1998). While the concentration of salts in buffalo milk are 182.6 mg of calcium, 18.04 mg of magnesium, 43.85 mg of sodium, 106.6 mg of potassium and 82.39 mg of phosphorus per 100 ml. It is also reported that 39.75 and 39.2 mg of calcium; 8.30 and 7.8 mg of magnesium; 41.59 and 47 of sodium; 100 and 143 mg of potassium; and 26.20 and 38.4 mg of phosphorous per 100 ml are present in dissolved phase in buffalo and cow milk respectively (Sindhu and Arora,2011).

Paneer whey contains approximately 350 mg/l of sodium; 300 mg/l of potassium; 480 mg/l of calcium; and 280 mg/l zinc (Goyal and Gandhi, 2009).

Thus the incorporation of higher rate of fermented paneer whey in RT_3 (40 per cent) have contributed to higher calcium, phosphorus, sodium and potassium content in comparison with RT_4 (containing 30 per cent fermented paneer whey). On the contrary concentration of zinc was higher in RT_4. This is because most of the zinc in milk is in the skim milk fraction, of which 95% is associated with casein micelles, with a small proportion (5%) associated with citrate (Cashman, 2011). RT_3 contained more amount of paneer whey and less proportion of dahi resulting in low casein content compared to RT_4.

The control cultured buttermilk RT_5 prepared by blending dahi with water in 70:30 (w/w) had lower content of all minerals compared to RT_3 andRT_4. This due to the fact that RT_3 contained total solids from dahi only and hence lacked the contribution made by fermented whey to mineral content.

During acid precipitation, more highly ionized calcium was produced which leads to higher calcium content in paneer whey (Goyal and Gandhi (2009). This was conceptualized by Wong *et al*. (1978) and later confirmed by other research workers like Padmavati *et al.* (2007). A significant effect of fermentation by probiotics *Lb. helveticus* MTCC 5463 was seen in amount of soluble calcium that increased from 46.65 in milk to 153.25 mg/100 g product (Goswami 2012).

As per the recommended dietary allowance (RDA) for Indians 600 mg of calcium, 17 mg of iron, 12 mg of zinc; 600 mg of calcium, 35 mg of iron and 10 mg of zinc; and 600 mg of calcium and 9 to 13 mg of iron and 5 to 8 mg of zinc are advised per day for men, women and children (1 to 9 years). For boy and girls between 10 to 17 years of age 800 mg of calcium, 21 to 32 mg of iron and 9 to 12 mg are recommended per day (NIN, 2011).

The bio-availabilities to the host of such minerals as calcium, zinc, iron, manganese, copper, and phosphorus may also be enhanced upon consumption of fermented dairy products and improve the digestibility of the proteins. Milk fermentation results in a complete solubilisation of calcium, magnesium, and phosphorus and a

Table 1. Selected minerals content of cultured buttermilk

Types of buttermilk	Minerals (mg/ 100g)				
	Calcium	Phosphorous	Sodium	Potassium	Zinc
RT_3 (60:40)	75.52^b	106.85^c	28.37^c	90.78^c	0.39^b
RT_4 (70:30)	74.17^a	100.36^b	27.66^b	88.52^b	0.43^c
RT_5 (control)	64.40^c	63.65^a	19.88^a	63.63^a	0.38^a
SEm±	0.27	0.27	0.25	0.79	0.004
CD	0.84	0.83	0.77	2.47	0.01
CV %	0.86	0.67	2.20	2.21	2.00

* First digit in the ratio stands for per cent of dahi and second digit for per cent of fermented paneer whey on w/w basis.
RT_5: Control (dahi: water in 70:30 w/w ratio)
#The values are mean of five replications.
***Values within columns (treatments) with same lowercase superscript did not differ significantly (P<0.05) from each other.

partial solubilisation of trace minerals. During milk fermentation, the composition of the minerals remains unchanged (Padmavati *et al.*, 2007).

So the optimized cultured buttermilk can provide a good source of minerals to contribute to the dietary requirement suggested by NIN (2011).

Storage studies

Cultured buttermilk especially being a live product, certain specific and non-specific shortcomings may be expected during storage. Stability of cultured buttermilk, similar to other food products, during storage is of vital importance and crucial for commercial success. Therefore, various types of cultured buttermilk prepared in the laboratory were evaluated for their storage stability.

Changes in physico-chemical properties of cultured buttermilk during storage

The samples of cultured buttermilk were evaluated at regular interval of 1 day for changes in physico-chemical properties i.e. titratable acidity and proteolytic activity (by using method as mention above) and the results are depicted in Table 2.

Changes in acidity

Acidity of prepared cultured buttermilk samples were measured by method described in method and materials. From the data in Table 2 it may seen that the initial acidity of the cultured buttermilk prepared by addition of fermented paneer whey i.e. RT_3 (60:40) and RT_4 (70:30) and the control, RT_5 were 0.68, 0.68 and 0.60 per cent lactic acid, which increased to 0.80, 0.77 and 0.70 per cent lactic acid, respectively on 5th day of storage.

The changes in acidity among the treatments as well as for storage period was significant ($P<0.05$). Similarly, interaction between types of buttermilk and storage period was also statistically significant for changes in acidity of cultured buttermilk over the storage period.

The acidity of cultured buttermilk prepared by addition of water instead of fermented paneer whey was significantly less compared to RT_3 and RT_4 throughout the storage period. On increase in rate of paneer whey addition from 30 to 40 per cent, acidity of the cultured buttermilk also increased significantly ($P<0.05$). The changes in acidity of cultured buttermilk prepared with fermented paneer whey i.e. for RT_3 and RT_4 remained almost parallel all throughout the storage period.

Yadav *et al*. (2007) evaluated changes during storage of probiotic dahi. The pH decreased

Table. 2: Changes in physico-chemical properties of cultured buttermilk during 5 days of storage

Types of buttermilk	Storage period (Days) Titratable acidity (% lactic acid)					
	1	2	3	4	5	Average of treatments
RT_3 (60:40)	0.68	0.71	0.74	0.76	0.80	0.74
RT_4 (70:30)	0.68	0.70	0.72	0.75	0.77	0.73
RT_5 (control)	0.60	0.63	0.65	0.68	0.70	0.65
Average of periods	0.65	0.68	0.70	0.73	0.75	
CD(0.05) T=0.01; P=0.02; TxP=0.08; CV%=0.10						
Proteolytic activity (absorbance at 340 nm)						
RT_3 (60:40)	0.638	0.708	0.761	0.919	1.081	0.821
RT_4 (70:30)	0.596	0.635	0.699	0.762	0.912	0.721
RT_5 (control)	0.421	0.453	0.480	0.612	0.763	0.546
Average of periods	0.552	0.599	0.647	0.764	0.919	
CD(0.05) T=0.004; P=0.003; TxP=0.002; CV%=0.03						

*First digit in the ratio stands for per cent of dahi and second digit for per cent of fermented paneer whey on w/w basis.

RT_5: Control (dahi : water in 70:30 w/w ratio)

The values are mean of five replications. Observations are taken at 1 day interval.

** Values within rows (periods) and columns (treatments) with same lowercase superscript did not differ significantly ($P<0.05$) from each other.

and titratable acidity increased significantly from 4.93 and 0.89 per cent lactic acid on first day to 4.84 and 1.12 per cent lactic acid respectively on the 5th day during the storage period. On the eighth day, the pH and titratable acidity were 4.64 and 1.32, respectively, which were acceptable to the assessors.

Shukla *et al*. (2013) developed probiotic beverage from whey and pineapple juice. They found that the acidity increased during the refrigerated storage from 0.55 to 0.89 per cent after 28 days and the increase in acidity was more prominent in case of storage at ambient temperature wherein the acidity reached 0.89 per cent after 120 h of storage.

The results suggest that the trend change in acidity was in accordance with the values reported in the literature. Over acidification during storage is an occasional problem, but lack of flavor caused by reduction of diacetyl to acetoin is a more frequently occurring problem (Walstra *et al*., 2005). This increased acidity may be due to an increase in metabolites and other biochemical changes caused by lactobacilli and lactococci, even at low temperatures.

Changes in proteolytic activity

The data for changes in the proteolytic activity of the cultured butter milk during storage are presented in Table 2. and measured as method described in method and materials.

Table. 3: Changes in sensory attributes of cultured buttermilk during 5 days of storage

Types of buttermilk	Storage period (Days) Flavor					
	1	2	3	4	5	Average of treatments
RT_3 (60:40)	7.75	7.60	7.23	7.12	6.85	7.31^b
RT_4 (70:30)	8.12	7.94	7.60	7.20	6.95	7.56^c
RT_5 (control)	6.89	6.71	6.51	6.50	6.03	6.53^a
Average of periods	7.59^e	7.42^d	7.11^c	6.94^b	6.61^a	
CD(0.05) T=0.19; P=0.13; TxP=0.10; CV%=1.22						
Colour and appearance						
RT_3 (60:40)	8.04	7.95	7.85	7.85	7.80	7.90^b
RT_4 (70:30)	8.13	8.00	8.00	8.00	8.00	8.03^b
RT_5 (control)	7.73	7.69	7.70	7.60	7.20	7.59^a
Average of periods	7.97^b	7.88^b	7.85^{ab}	7.82^{ab}	7.67^a	
CD(0.05) T=0.29; P=0.20; TxP=0.14; CV%=1.84						
Body and Texture						
RT_3 (60:40)	8.03	8.00	7.65	7.40	7.00	7.62^b
RT_4 (70:30)	8.34	8.30	7.98	7.77	7.42	7.96^b
RT_5 (control)	6.78	6.70	6.55	6.10	6.08	6.45^a
Average of periods	7.72^d	7.67^{cd}	7.39^c	7.09^{ab}	6.83^a	
CD(0.05) T=0.40; P=0.28; TxP=0.20; CV%=2.57						
Overall acceptability						
RT_3 (60:40)	7.94	7.85	7.24	7.03	6.57	7.32^b
RT_4 (70:30)	8.19	8.08	7.43	7.12	6.68	7.50^c
RT_5 (control)	7.14	7.04	6.94	6.57	5.98	6.74^a
Average of periods	7.75^e	7.65^d	7.20^c	6.91^b	6.41^a	
CD(0.05) T=0.06; P=0.04; TxP=0.03; CV%=0.41						

*First digit in the ratio stands for per cent of dahi and second digit for per cent of fermented paneer whey on w/w basis.

RT_5: Control (dahi : water in 70:30 w/w ratio)

The values are mean of five replications. Observations are taken at 1 day interval.** Values within columns (treatments) and rows (periods) with same superscript did not differ significantly (P<0.05) from each other.

The initial proteolytic activity expressed as absorbance at 340 nm of the cultured buttermilk prepared by addition of fermented paneer whey i.e. RT_3 (60:40) and RT_4 (70:30) and the control, RT_5 were 0.638, 0.596 and 0.421 on first day and it increased to 1.081, 0.912 and 0.768 respectively on 5th day of storage in PET bottles at 7 ± 1ºC. The proteolytic activity increased gradually up to 3rd day and thereafter it increased drastically in all the three types of cultured buttermilk.

Biologically active peptides are generated during milk fermentation by proteolytic enzymes produced by various LAB such as *L. helveticus*, *L. lactis* subsp. *cremoris* FT4 and *L. delbrueckii* subspp. *Bulgaricus* SS1 (Gobbetti *et al*., 2002). These biologically active peptides include hypotensive peptides which inhibit angiotensin I-converting enzyme (ACE), opioid agonist and antagonist peptides, and mineral binding, immunomodulatory, antibacterial, and antithrombotic peptides (Pihlanto and Korhonen, 2003).

Yadav *et al*. (2007) assessed the extent of proteolysis of dahi prepared using *Lb. acidophilus* NCDC 14, *Lb. casei* NCDC 19 and *L. lactis* subspp *lactis biovar diacetyllactis* NCDC 60 during storage at 7ºC. They found that soluble nitrogen increased with storage time. They stated that during storage, hydrolysis of individual casein fractions was measured as a percentage of the concentration of these fractions on day 1 (fresh dahi) of storage and small amounts of $\pm s_1$-casein and $\pm s_1$-I-casein were hydrolysed during storage. They reported that saw much of the degradation occurred during day 8 of storage. They correlated the hydrolysis of these casein fractions with the rheological properties of dahi made with lactobacilli as it had a creamy layer and good firmness in the early stages of storage, but after long storage, the dahi lost its firmness, which may be due to protein hydrolysis by a particular type of adjunct. They stated that *Lactobacilli* with higher intracellular peptidase activity produced a high level of casein hydrolysis in dahi, leading to smaller peptide

Table. 4: Microbial changes during 5 days of storage

Treatments	Storage period (Days) Total lactic count (log cfu/g)					
	1	2	3	4	5	Average of treatments
RT_3(60:40)	10.59	10.63	10.66	10.60	10.54	10.60^a
RT_4(70:30)	10.48	10.50	10.54	10.51	10.49	10.50^b
RT_5(control)	10.38	10.36	10.42	10.39	10.38	10.45^c
Average of periods	10.46^a	10.50^b	10.54^c	10.50^b	10.47^a	
CD(0.05) T=0.02; P=0.02; TxP=0.01; CV%=0.14						
Coliform count						
RT_3(60:40)	Nil	Nil	Nil	Nil	Nil	Nil
RT_4(70:30)	Nil	Nil	Nil	Nil	Nil	Nil
RT_5(control)	Nil	Nil	Nil	Nil	Nil	Nil
Average of periods	Nil	Nil	Nil	Nil	Nil	Nil
Yeast and mould count						
RT_3(60:40)	Nil	Nil	Nil	Nil	Nil	Nil
RT_4(70:30)	Nil	Nil	Nil	Nil	Nil	Nil
RT_5(control)	Nil	Nil	Nil	Nil	Nil	Nil
Average of periods	Nil	Nil	Nil	Nil	Nil	Nil
CD(0.05)						

*First digit in the ratio stands for per cent of dahi and second digit for per cent of fermented paneer whey on w/w basis.

RT_5: Control (dahi : water in 70:30 w/w ratio)

The values are mean of five replications. Observations are taken at 1 day interval.** Values within columns (treatments) and rows (periods) with same superscript did not differ significantly (P<0.05) from each other.

fragments and free amino acids.

Kapila *et al*. (2009) evaluated the proteolytic activity of whey and whey supplemented with whey protein concentrate (WPC) fermented by *Lactobacillus helveticus* NCDC 292 (1 per cent, 37ºC/48 h) under *in vitro* conditions. When the whey and whey supplemented with WPC were fermented with *L. helveticus* for a period of 48 h using 1% inoculum size, the increase in the release of peptide was nearly 10 and 18 fold in fermented whey (FW) and fermented whey supplemented with WPC (FWW), respectively.

Hati *et al*. (2015) have confirmed the proteolyic activity of *Lb. helveticus* MTCC 5463 in skim milk at 37ºC.

From the data, it can be seen that the proteolytic activity increased day by day. After 5 days of storage, it was highest in RT_3 amongst all the samples. Various research workers have confirmed that various strains of *Lb. helveticus* have remarkable proteolytic activity especially in whey and whey based media. Similar results are also reflected in the present study.

Changes in sensory attributes of cultured buttermilk during storage

The samples of cultured buttermilk were evaluated at regular interval of 1 day for flavor, colour and appearance, body and texture and overall acceptability using 9- point hedonic scale. The average values of results obtained for each sensory attributes along with statistical analysis are presented in the Table 3.

The examination of the data for flavour, colour and appearance, body and texture and overall acceptability scores of cultured buttermilk indicated that the changes in all four sensory scores among the treatments were significant (P<0.05). The data also suggested that the storage period had significant effect on all four sensory scores of the cultured buttermilks. The interaction effect between types of buttermilk and storage period was also statistically significant for changes in all four sensory score.

From the studies on changes in sensory characteristics of cultured buttermilk stored in PET bottles at 7±1ºC, it was inferred that up to 4th day scores of all attributes especially that of flavour and overall acceptability were above 7.0 for the experimental samples RT_3 and RT_4 prepared by blending dahi with 40 and 30 per cent of fermented whey respectively. On the 5th day the score for flavour and overall acceptability of all types of cultured buttermilk were below 7.0 (the score 7.0 stands for "like moderately" on Hedonic scale) due to the formation of bitter peptide giving bitter taste. This is very well correlated with increased proteolytic activities found in these samples. So after 5 days of storage, the studies of cultured buttermilk were discontinued.

Yadav *et al*. (2007) evaluated the storage changes in probiotic dahi stored at 7°C up to 8 days. On fresh day falvour score was 21.6 out of 25 and on day 8, they saw little deterioration of flavour and the score was 19.6 out of 25. However, after 8 days of storage, the samples were disliked by the panel, which reported slight bitterness, caused probably due to proteolysis. They justified the findings by reasoning that during storage, the degradation of ²-casein due to the action of chymosin and plasmin, results in the formation of smaller peptides that may be responsible for bitterness in dahi. In case of appearance, on first day it was 4.5 out of 5 and on day 8, they saw little changes in appearance and the score was 4.2 out of 5.

Ghanshyambhai *et al.* (2015) found that flavour scores of culture buttermilk prepared by the addition of unfermented paneer whey and fermented paneer whey (*S. thermophiles* MTCC 5460 and *Lb delbrueckii* subspp *bulgaricus*) to curd decreased from 7.08 and 8.60 on first day to 4.77 and 6.01, respectively , in case of colour and appearance scores they saw that the scores was decreased from 7.83 and 7.91 first day to 7.32 and 7.41 respectively and for body and texture scores also decreased from 7.58 and 8.13 first day to 5.97 and 6.36 respectively at the end of the storage (5th day) period.

In present study also a similar trend in decrease of flavour, colour and appearance, body and texture and overall acceptability scores were observed.

Thus, from the sensory point of view both the RT_3 (60:40) and RT_4 (70:30) were acceptable up to 4 days of storage at refrigeration temperature (7± 1ºC).

Microbial changes during storage study

Most of the milk products have a highly perishable nature. This perishability of dairy products is mostly ruled by microbiological quality of that product. Cultured buttermilk packed in PET bottles and evaluated for its microbiological quality during storage at refrigerated temperature (7±1°C).

The microbiological status viz., total lactic count, yeast and mould count and coliform count of cultured buttermilk samples are presented in Table 4.

Total lactic count is the collective enumeration of the total lactic acid bacteria (*Streptococci* and *Lactococci*). It represents the overall microbiological quality of the product, after production and during its storage period. The changes in microbial population of cultured buttermilk expressed as total lactic count (Log cfu/g) of cultured buttermilk during storage are presented in Table 4.

It can be seen that initial total lactic count of RT_3 (60:40), RT_4 (70:30) and RT_5 (control) increased from 10.59, 10.48 and 10.38 Log cfu/g to10.66, 10.54 and 10.42 Log cfu/g of cultured buttermilk respectively on 3rd day of storage day. Thereafter, the total lactic count decreased up to last day of storage study (5th day).

Momin (2009) carried out the storage study of mushli added herbal probiotic lassi as well control (without herb) probiotic lassi prepared using *S. thermophilus* (MD8) and probiotic *Lb. helveticus* MTCC 5463. It was observed that the total viable *Streptococci* counts on first day were 1.29 x 10^9 and 1.70 x 10^9 cfu/ml in fresh herbal probiotic lassi and control lassi, respectively. At the end of storage, control had 2.19 x 10^8 and herbal product had 1.55 x 10^8 cfu/ml live *Streptococci*. While total viable *Lactobacilli* count changed from 6.60 x 10^7 to 1.02 x10^7cfu/ml in herbal probiotic lassi during storage.

Shah (2011) developed synbiotic lassi with honey and whey drink with inulin and orange juice using *L. helveticus* MTCC 5463 as probiotic culture in both products. She found that the count of *L. helveticus* MTCC 5463 remained at level of more than 8 Log cfu/ml at the end of storage period (28th). Similarly for whey based product, the count before storage was 8.72 Log cfu/ml and declined to 8.39 Log cfu/ml on 28th day.

Shukla *et al*. (2013) developed probiotic beberage from whey and pineapple juice. They found that the initial total viable count of the beverage was 3.8×10^7 cfu/ml, which decreased to 1.8×10^7 cfu/ml at refrigerated storage and the viability of *Lactobacillus acidophilus* population decreased, but the viable count of the probiotic beverage did not fall below 10^6 cfu/ml. They found that during storage at 30±1°C, the total viable count first increased to 9.5×10^8 cfu/ml (in 48 hr) and then gradually declined to 2.9×10^7 cfu/ml after 120 hr.

Sharma (2010) carried out the storage study by using *S. thermophilus* and *L. helveticus* cultures up to 28 days for lassi added with 5 per cent honey (synbiotic lassi) as well as control without honey (probiotic lassi) stored at 5± 2°C. It was observed that total viable lactobacilli count was 8.96 to 8.42 Log cfu/ g in control lassi while in synbiotic lassi it varied from 9.22 to 8.75 Log cfu/g, respectively at end of storage.

Burns *et al*. (2010) checked the suitability of the buttermilk for fermentation with a proteolytic strain of *Lb. helveticus* to enhance its value by the production of a functional peptide enriched powder. They assessed the *Lactobacilli* counts of *Lb. helveticus* 209 grown in reconstituted buttermilk, at different concentrations, after 24 h of incubation at 43°C. They found that as the buttermilk concentration increased the count was also increased. At 4 per cent buttermilk concentration (w/v), it was 7.0 Log cfu/ml whereas, at 25 per cent buttermilk concentration (w/v), it was 8.7 Log cfu/ml.

Yadav *et al*. (2007) assessed the extent of bacterial counts of dahi prepared using *Lb. acidophilus* NCDC 14, *Lb. casei* NCDC 19 and *L. lactis* subspp *lactis biovar diacetyllactis* NCDC 60 during storage at 7ºC. The bacterial counts of dahi during storage increased on day 2 of storage and counts of lactococci in fresh samples and after 2 days of storage were 1.17 × 10^7 and 4.62 × 10^9 cfu/ml, respectively, and the counts of lactobacilli were 3.51 × 10^7 and 2.45 × 10^9 cfu/ml, respectively. However, from 2 days onwards, i.e. on the fourth, sixth and eighth day of storage, the counts for both lactobacilli and lactococci decreased.

Similar trend as observed in the present study for total lactic count was also reported by Yadav *et al*. (2007). Decreased in count after 3rd day can be related to various factors like to increase in the acidity (concentration of lactic acid) of the product, exhausting of nutrients, oxygen content as well as temperature of storage.

Thus, the results indicated that storage condition (7±1ºC) and time (up to 3 days) were favouring the growth of lactic acid bacteria initially but long storage did not favor their growth.

Cultured buttermilk samples stored at refrigeration temperature (7±1°C) were found to be free from coliform and yeast and mould count during the entire storage period of the study. This indicates hygienic manufacture and purity of cultures used.

CONCLUSION

Results of this study entailed to conclude that cultured buttermilk with acceptable sensory and physico-chemical characteristics can be prepared by blending 60 per cent dahi with 40 per cent of fermented whey by using starter culture *Lb. helveticus* MTCC 5463 and *L. lactis* subspp. *diacetylactis* NCDC 60 (1:1). The product has good source of minerals, acceptable sensory qualities and shelf life up to 4 days when stored in PET bottles at 7 ± 1ºC.

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