International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 09 (C); September 2019; Page No.19895-19900
DOI: http://dx.doi.org/10.24327/ijcar.2019.3869.19900

Research Article

STORAGE STUDIES OF CULTURED LOW FAT SYNBIOTIC BUTTERMILK

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A R T I C L E I N F O

Article History:
Received 4th June, 2019
Received in revised form 25th July, 2019
Accepted 18th August, 2019
Published online 28th September, 2019

Key words:
(L.acidophilusand B. bifidum).

A B S T R A C T

A study has been made to develop functional buttermilk with prebiotics (honey and oligofructose) and probiotic cultures (L.acidophilusand B. bifidum). Various levels of prebiotics (2, 3, 4 and 5 per cent) were added in the buttermilk samples to assess the optimum inclusion level based on the sensory evaluation. Honey and oligofructose were added separately at the rate of two per cent level in the samples and were acceptable by the sensory panel. In buttermilk, mainly the growth and survival of probiotic L. acidophilus and B.bifidum alone and in combination either with prebiotic substances namely honey or oligofructose had been assessed during refrigerated storage for 21 days and the experimental buttermilk samples either with L.acidophilus or B.bifidum and both in combination had maintained the minimum level of probiotic bacterial cells 10^6–10^7 cells per gram so as to exert probiotic properties up to 15 days of storage without affecting sensory properties.

INTRODUCTION

Traditionally, in India, buttermilk occupies a major part of Indian's diet. Buttermilk has well known beneficial effect such as cooling, refreshing, thirst quenching, delicious, nutritive and digestive properties upon regular consumption. Keeping these beneficial properties in mind, a study has been made to develop functional buttermilk with prebiotics (honey and oligofructose) and probiotic cultures (*L.acidophilus* and *B.bifidum*). Different levels of prebiotics (2, 3, 4 and 5 per cent) were added in the buttermilk samples to assess the optimum inclusion level based on the sensory evaluation. Honey and oligofructose were added separately at the rate of two per cent level in the samples and were acceptable by the sensory panel. In buttermilk, mainly the growth and survival of probiotic *L. acidophilus* and *B.bifidum* alone and in combination either with prebiotic substances namely honey or oligofructose had been assessed during refrigerated storage for 21 days.

MATERIALS AND METHODS

Fresh skim milk collected from local market of Madurai was utilized in this study. Oligofructose obtained from the Bayleaf Wellness Pvt Ltd, Noida, India, was utilized for preparation of cultured low fat synbiotic buttermilk. Honey procured directly from honey hives, Madurai was utilized as prebiotics for preparation of cultured low fat synbiotic buttermilk. The starter culture, mixed dahi culture (Mesophilic type), freeze dried cultures of *Lactobacillus acidophilus* (NCDC 014) and *Bifidobacteriumbifidum* (NCDC 232) were purchased from National Collection of Dairy Cultures, NDRI, Karnal, Haryana was used for preparation of buttermilk. The cultures were reconstituted and maintained in sterile skim milk and sub-cultured at weekly intervals. The starter cultures were tested periodically for their purity and activity. The culture media like plate count agar, Bifidobacteriumagar, Lactobacillus MRS agar, Violet red bile agar and Potato dextrose agar were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India was used to enumerate microbes in the samples.

The buttermilk was prepared as per the procedure of Sukumar De (2004) with slight modifications. The control buttermilk and experimental cultured low fat synbiotic buttermilk samples were prepared by mixing of curd and water in a ratio of 1: 1 and to achieve a uniform texture, the samples were homogenized.

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

Items	Control	Treatments					
		T1	T2	T3	T4	T5	T6
Skim milk	Skim milk	Skim milk	Skim milk	Skim milk	Skim milk	Skim milk	Skim milk
Starter culture	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 material	-	Honey	Honey	Oligofructose	Oligofructose	Honey	Oligofructose

The prepared buttermilk was kept in cold storage (5-10^{0}C) for 21 days to assess the shelf life. The buttermilk samples developed were assessed at weekly intervals. The qualities considered during the study were organoleptic, physico chemical and microbiological. (Sailaja *et al*., 2014). The titratable acidity percentage of the buttermilk samples were analyzed as per the procedure given in IS: SP 18 (Part XI),

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1981. The pH of buttermilk samples were measured by electronic digital type pH meter (Hana No. H_1 8416, Germany) according to method No.981.12 of AOAC (2000).All the samples were subjected to microbiological analysis for standard plate count (IS: 5402, 1969),lactic acid bacterial count (ISI Handbook, 1981), *Lactobacillus acidophilus* count (De Man *et al*., 1960) and *Bifidobacteriumbifidum* count (Vinderola and Reinheimer, 1999). The bacterial colonies were enumerated with colony counter. The results have been expressed as total viable count per gram. The sensory evaluations of stored products were carried out by "9 point hedonic scale" to evaluate the sensory characters of buttermilk samples as per allotted parameters (Sonali *et al*., 2016). Fresh product at 0 day and the stored products (7, 15 and 21 days of storage at 5 ± 1°C) were brought to 10° C before giving for sensory evaluation by the panel of judges. The statistical analysis of data was carried out by factorial design CRD (Steel and Torrie, 1980). The values for microbial counts were log transformed before analysis.

RESULT AND DISCUSSION

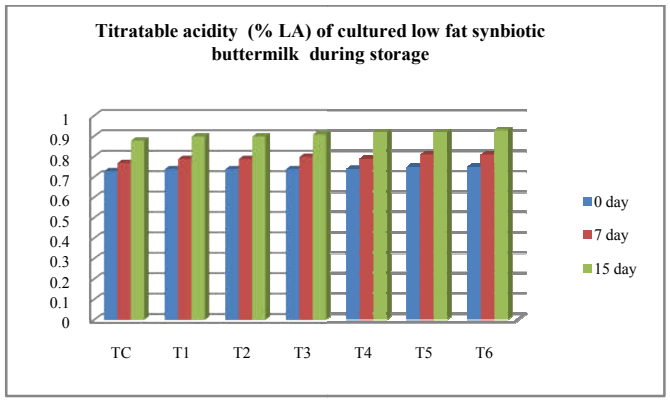
The titratable acidity (percentage of lactic acid) values for control and experimental low fat synbiotic buttermilk samples (mean ± SE) at 0, 7 and 15 days of storage are presented in Table 1**.**On day zero, the titratable acidity for control and experimental low fat synbiotic buttermilk samples (T1 to T6) 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. The corresponding values on day seven were 0.77, 0.79, 0.79, 0.80, 0.79, 0.81 and 0.81 respectively. Statistical analysis of the data showed significant difference ($P < 0.01$) between the control and treatments. On day 15, the titratable acidity for control and experimental low fat synbiotic buttermilk samples were 0.88, 0.90, 0.90, 0.91, 0.92, 0.92 and 0.93 respectively and the differences among the samples were statistically significant ($P < 0.01$).

The results also revealed that the titratable acidity of control as well as experimental samples increased as the days of storage advanced. During refrigerated storage period also, the added dahi culture and probiotic cultures or their enzymes might have increased the acidity in the control and experimental samples.

Table 1 Titratable acidity (% of LA) of cultured low fat synbiotic buttermilk during storage

Days of storage (n=6)	C	Treatments					
		T1	T2	T3	T4	T5	T6
0	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
7	0.77^{c} ± 0.02	0.79^{b} ± 0.03	0.79^{b} ± 0.01	0.80^{b} ± 0.02	0.79^{b} ± 0.03	0.81^{a} ± 0.03	0.81^{a} ± 0.02
15	0.88^{c} ± 0.03	0.90^{b} ± 0.01	0.90^{b} ± 0.02	0.91^{a} ± 0.02	0.92^{a} ± 0.04	0.92^{a} ± 0.03	0.93^{a} ± 0.03

Means bearing different superscripts differ significantly (P<0.01)

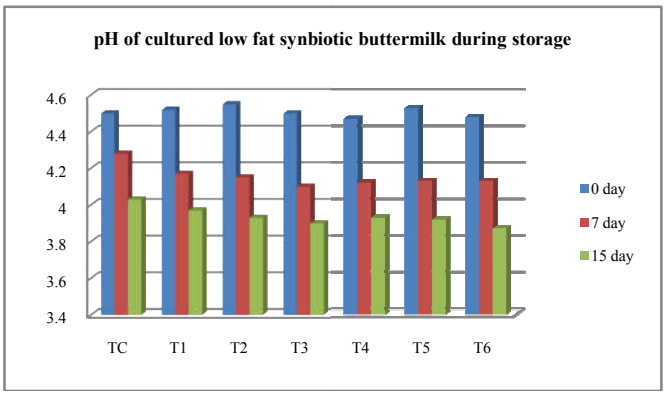


The results pertaining to the pH values for control and experimental low fat buttermilk samples (mean ± SE) at 0, 7 and 15 days of storage are presented in Table 2.The pH of 4.5 was taken as the cutoff point in the fermentation process of buttermilk as this pH was reported to be the optimum level for production of good quality buttermilk. The result was in agreement with the findings of Fornelli *et al*. (2014) for effect of inulin and oligofructose on the pH of symbiotic dairy beverages prepared by using *Lactobacillus paracasei* and found that the pH of the product lowered progressively up to 21 days of storage under refrigerated condition.

Table 2 pH of cultured low fat synbiotic buttermilk during storage

Days of storage (n=6)	C	Treatments					
		T1	T2	T3	T4	T5	T6
0	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
7	4.28^{b} ±0.03	4.17^{b} ±0.04	4.15^{b} ±0.05	4.10^{b} ±0.04	4.12^{b} ±0.05	4.13^{b} ±0.04	4.13^{b} ±0.05
15	4.03^{a} ±0.02	3.97^{b} ±0.03	3.93^{b} ±0.03	3.90^{b} ±0.04	3.93^{b} ±0.04	3.92^{b} ±0.03	3.87^{b} ±0.03

Means bearing different superscripts differ significantly (P<0.01)



On day 0, the pH for control and experimental low fat synbiotic buttermilk samples were 4.50, 4.52, 4.55, 4.50, 4.47, 4.53 and 4.48 respectively. On day 7, the pH for control and T1 to T6 treatment samples were 4.28, 4.17, 4.15, 4.10, 4.12, 4.13 and 4.13 respectively and analysis of the data showed a significant difference ($P < 0.05$) between the control and experimental samples. On day 15, the pH for control and experimental low fat synbiotic buttermilk samples were 4.03, 3.97, 3.93, 3.90, 3.93, 3.92 and 3.87respectively. Statistical analysis of the data showed significant difference ($P < 0.01$) between the control and experimental low fat synbiotic buttermilk samples.

Growth and survival of *L. acidophilus*($\log_{10}$cfu/ml) in the cultured low fat synbiotic buttermilkduring storage at refrigeration temperature is presented in Table 3.Honey and

oligofructose incorporated low fat synbiotic buttermilk samples with *L. acidophilus* and *B.bifidum* had significantly (P<0.01) higher *L. acidophilus* count in the treatments T5 and T6 as compared to the treatments T1 and T3 on 0 day.

Table 3 Growth and survival of L. acidophilus (log_{10} cfu/ml)in cultured low fat symbiotic buttermilk during storage

Groups (n=6)	0 day*	7 day*	15 day*
T1	$8.43^{Aa}\pm 0.03$	$7.83^{Ba}\pm 0.08$	$7.28^{Ba}\pm 0.05$
T3	$8.39^{Aa}\pm 0.03$	$7.89^{Ba}\pm 0.11$	$7.33^{Ba}\pm 0.12$
T5	$8.45^{Aa}\pm 0.03$	$7.79^{Ba}\pm 0.13$	$7.39^{Ba}\pm 0.13$
T6	$8.89^{Ba}\pm 0.13$	$7.76^{Bb}\pm 0.07$	$7.32^{Ba}\pm 0.12$

*Significant at1%level (P < 0.01)
Means bearing different with superscripts between treatments differ significantly (P < 0.01)

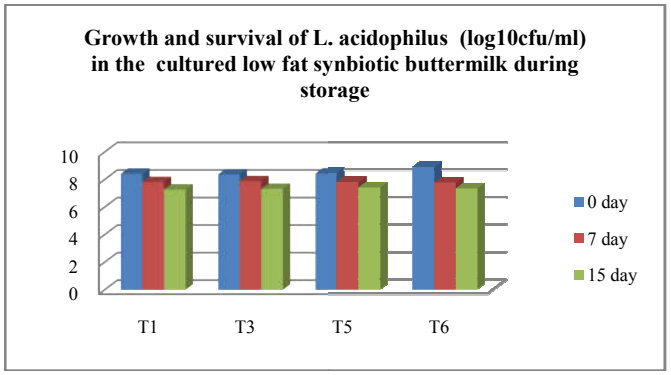
On 7th day of storage, the honey and oligofructose incorporated low fat synbiotic buttermilk samples either with *L. acidophilus* alone or in combination with *B.bifidum* had significantly (P<0.01) higher *L. acidophilus* count in the treatments T1 and T3 as compared to the treatments T5 and T6.On 15th day of storage, in all the treatments T1, T3, T5 and T6, the *L. acidophilus* count was maintained almost at the same level irrespective of added prebiotics. In general, the *L. acidophilus* count progressively decreased as the refrigerated storage period of the treatment samples increased (Table 3).

Shah, (2000) suggested that the minimum live count of probiotic bacteria should be 10^6 cfu/ml in a probioticproduct to exert therapeutic properties and the results obtained in this study, had more than 10^6 cfu/ml *L. acidophilus* count. The addition of an appropriate strain of *L. acidophilus* to cultured buttermilk or yogurt after fermentation at a level of approximately 1 x 10^7 cfu/g could result in numbers of viable *L. acidophilus* in excess of 1 x 10^6cfu/g after 28 days of storage at 5 and 7°C, respectively (Nighswonger, 1996).

A minimum range of 10^6–10^7 plate microorganisms per gram or milliliter should be present in food product in order to meet the requirements of a probiotic food, as by the Japanese fermented milk and lactic acid bacteria drinks association (Ishibashi and Shimanura, 1993) and the present results are in agreement with this report as the *B.bifidum* count was maintained above 10^6–10^7 cfu/ml.

Dong (2015) prepared symbiotic cultured buttermilk by combining CHN22 (Chr-Hansen starter culture containing multiple mixed strains of *Lactococcuscremoris*, *Lactococcuslactis,* *Leuconstoccremoris*, *Lactococcusdiacetylactis)* (0.015 per cent, w/w), *L. acidophilus* (LA-5) and *Bifidobacterium*spp. (BB-12) (0.1 per cent, w/w), and inulin (0.8 per cent, w/w). He reported that the counts of *L. acidophilus* and *Bifidobacterium*spp. were initially above 10^7cfu/ml and remained at10^6cfu/ml over 12-week stored at 4°C.

The present results with regard to probiotic bacterial count of the experimental buttermilk samples are in agreement with the results of Nighswonger, (1996), Ishibashi and Shimanura, (1993) and Dong (2015).

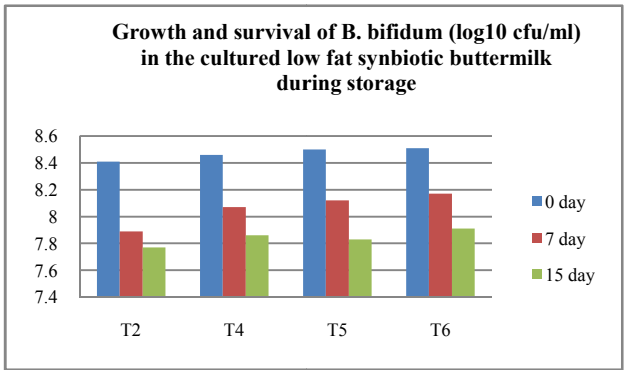


Growth and survival of *B. bifidum* (log_{10}cfu/ml) in the cultured low fat synbiotic buttermilk samples during storage at refrigeration temperature are presented in Table 4.Honey and oligofructose incorporated low fat synbiotic buttermilk samples with *L. acidophilus* and *B.bifidum* had significantly (P<0.01) higher *B.bifidum* count in the treatments T5 and T6 on 0 day as compared to the treatments T2 and T4.

Table 4 Growth and survival of *B. bifidum*(log_{10} cfu/ml) in cultured low fat synbiotic buttermilk during storage

Groups (n=6)	0 day*	7 day*	15 day*
T2	$8.41^{Ba}\pm 0.13$	$7.89^{Bb}\pm 0.05$	$7.77^{Ba}\pm 0.05$
T4	$8.46^{Aa}\pm 0.03$	$8.07^{Bb}\pm 0.13$	$7.86^{Ba}\pm 0.12$
T5	$8.50^{Aa}\pm 0.03$	$8.12^{Bb}\pm 0.03$	$7.83^{Ba}\pm 0.13$
T6	$8.51^{Aa}\pm 0.03$	$8.17^{Ba}\pm 0.11$	$7.91^{Bb}\pm 0.12$

*Significantat (P < 0.01) level
Means bearing different superscripts between treatments differ significantly with in the period of time (P < 0.01)



On 7th day of storage, the honey and oligofructose incorporated low fat synbiotic buttermilk samples either with *B.bifidum* alone or combination with *L. acidophilus* had significantly (P<0.01) higher *B.bifidum* count in the treatmentsT2 and T4 as compared to the treatments T5 and T6.On 15th day of storage, in all the treatments T2, T4, T5 and T6, showed no significant difference in *B.bifidum* count irrespective of added prebiotics honey and oligofructose. As the refrigerated storage period increased up to 15 days, the *B.bifidum*count decreased in the treatments (Table 4).

The overall average sensory evaluation scores of the control and cultured low fat synbiotic buttermilk samples are given in Table 5. Refrigeration storage of control and cultured low fat synbiotic buttermilk sample up to 7 days did not affect the flavour score. The maximum average flavour score of 8.17 was observed in control sample while minimum score of 7.83was noticed in treatments T1, T3 and T5 respectively.

Continued storage of control and cultured low fat synbiotic buttermilk samples at refrigeration condition up to 15 days had resulted in lowered average sensory scores of 4, 4.33, 4.17,

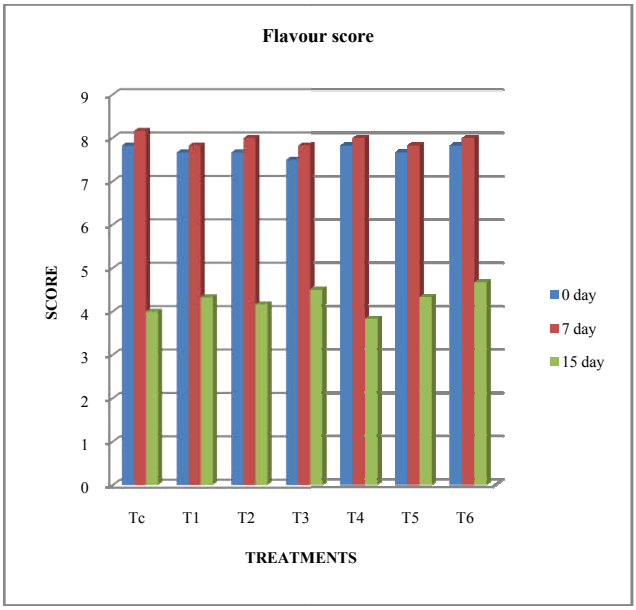
4.50, 3.83, 4.33 and 4.67 for control and treatments T1 to T6, respectively. Statistical analysis of sensory scores revealed no significant difference between control and treatments and within the treatments. The lowered sensory perception of the flavour scores of all the treatments including control obtained on day 15 revealed that storage of buttermilk samples up to 15 days had reduced its flavor even if the samples are stored under refrigeration condition.

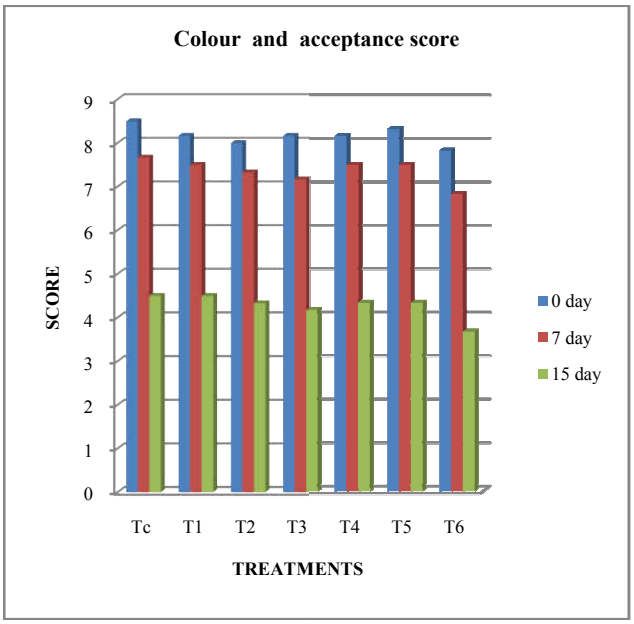
Table 5 Sensory evaluation scores of cultured low fat synbiotic buttermilk samples during storage

Days of storage (n=6)	C	Treatments					
		T1	T2	T3	T4	T5	T6
Flavor Score(NS)							
0 day	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
7 day	8.17 ± 0.17	7.83 ± 0.17	8.00 ± 0.26	7.83 ± 0.31	8.00 ± 0.26	7.83 ± 0.31	8.00 ± 0.26
15 day	4.00 ± 0.37	4.33 ± 0.33	4.17 ± 0.31	4.50 ± 0.22	3.83 ± 0.31	4.33 ± 0.21	4.67 ± 0.21
Colour and appearance score(NS)							
0 day	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
7 day	7.67 ± 0.21	7.50 ± 0.22	7.33 ± 0.33	7.17 ± 0.31	7.50 ± 0.22	7.50 ± 0.34	6.83 ± 0.17
15 day	4.50 ± 0.22	4.50 ± 0.22	4.33 ± 0.33	4.17 ± 0.31	4.33 ± 0.21	4.33 ± 0.33	3.67 ± 0.21
Body and texture Score (NS)							
0 day	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
7 day	6.83 ± 0.31	6.67 ± 0.21	6.17 ± 0.17	6.17 ± 0.17	6.50 ± 0.22	6.17 ± 0.17	6.33 ± 0.21
15 day	3.83 ± 0.31	3.67 ± 0.21	3.17 ± 0.17	3.17 ± 0.17	3.50 ± 0.22	3.17 ± 0.17	3.33 ± 0.21
Overall acceptability Score (NS)							
0 day	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
7 day	7.55 ± 0.14	7.33 ± 0.09	7.16 ± 0.19	7.05 ± 0.13	7.33 ± 0.17	7.11 ± 0.14	7.05 ± 0.10
15 day	4.11 ± 0.14	4.16 ± 0.11	3.89 ± 0.19	3.95 ± 0.13	3.89 ± 0.11	3.95 ± 0.20	3.89 ± 0.11

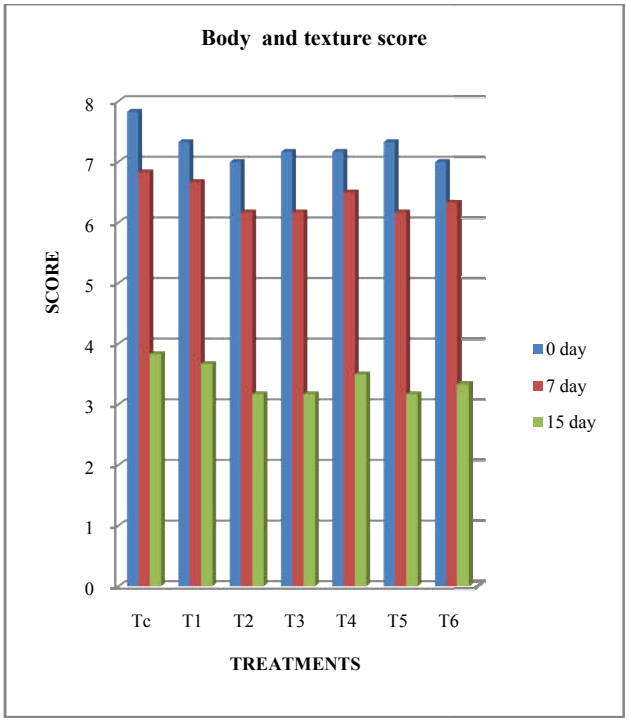
Refrigeration storage of control and cultured low fat synbiotic buttermilk sample up to seven days had resulted in reduced colour and appearance scores of control and treatments but the differences between control and treatment samples were not statistically significant. The maximum colour and appearance score of 7.67 was observed in the control while minimum score of 6.83 was observed for treatment T6. The colour and appearance scores of control and treatments T1 to T6 had reduced further to the levels of 4.50, 4.50, 4.33, 4.17, 4.33, 4.33 and 3.67 respectively during storage up to 15 days at refrigerated condition. However, the differences among control and treatment samples were not statistically significant. In the present study, a decreasing trend in the sensory evaluation of the colour and appearance scores of all the samples was observed over increase in the storage days. Therefore, the results indicated that the experimental samples and control had not been accepted by the sensory panel when they were stored up to 15 days.

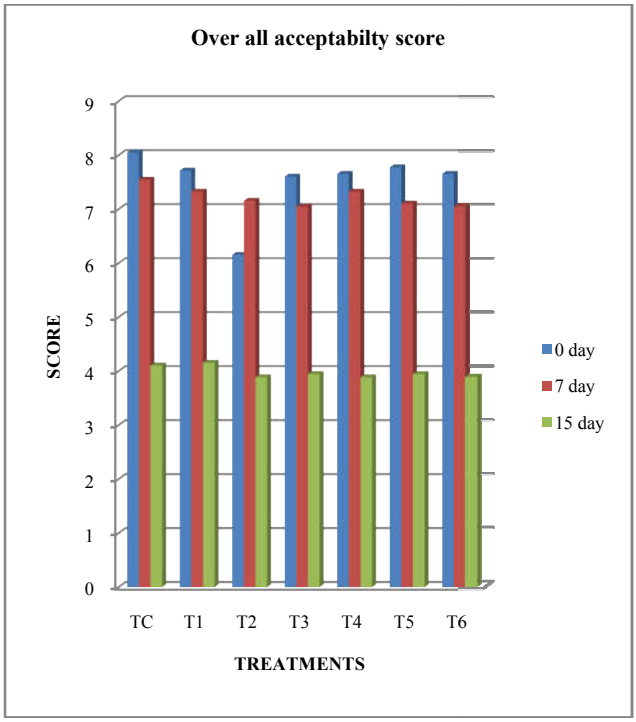
Refrigeration storage of control and cultured low fat synbiotic buttermilk sample up to seven days did not alter the body and texture scores and the differences among control and treatment samples were not statistically significant. The maximum average body and texture score of 6.83 was observed in control sample while the minimum score of 6.17was observed in the cultured low fat synbiotic buttermilk samples of treatments T2, T3 and T5. Continued storage of the control and treatment samples up to 15 days had drastically reduced the body and texture scores however, the differences between control and treatment samples were not statistically significant. The averages for body and texture scores of the control and cultured low fat synbiotic buttermilk samples were found to be 3.83, 3.67, 3.17, 3.17, 3.50, 3.17 and 3.33, respectively. Similar to the other sensory scores, body and texture scores of control and all the treatment samples were very low on day 15 as compared to day 0 and 7. The lowered body and texture scores obtained by the buttermilk samples at day 15 revealed that storage of buttermilk under refrigerated conditions up to 15 days had deteriorated the body and texture resulting in unacceptable sensory scores.





Refrigerated storage of control and cultured low fat synbiotic buttermilk sample up to seven days did not alter the overall acceptability scores much in control and treatment samples. The overall acceptability score of the control and cultured low fat synbiotic buttermilk samples stored up to 7 days were 7.55, 7.33, 7.16, 7.05, 7.33, 7.11 and 7.05, respectively. The maximum overall acceptability scoreof 7.55 was noticed for control samples while the minimum average score of 7.05was noticed in treatments T3 and T6.





Refrigerated storage of control and cultured low fat synbiotic buttermilk sample up to 15 days had reduced the overall acceptability scores of all the samples. However the differences among the samples were not statistically significant. The overall average acceptability scores of the control and cultured low fat synbiotic buttermilk samples were 4.11, 4.16, 3.89, 3.95, 3.89, 3.95 and 3.89, respectively. In general, the overall acceptability scores of all the samples including control were very less on day 15.

From the results of sensory evaluation studies, it may be inferred that refrigerated storage of all the cultured low fat synbiotic buttermilk samples and control up to 15 days had reduced the overall acceptance of the buttermilk samples. But, up to seven days of storage at refrigerated condition, the experimental cultured low fat synbiotic buttermilk samples scored better in overall acceptability as compared to the control. Thus, it may be recommended that the low fat synbiotic buttermilk prepared with prebiotics and probiotics can be stored up to 7 days under refrigerated conditions and storage thereafter reduced the overall acceptability of the drink due to spoilage of the experimental cultured low fat synbiotic buttermilk and control samples.

Similar to the present finding, Binjan*et al*. (2017) reported that different buttermilk samples namely moringa leaf buttermilk, moringa pod buttermilk, Zeynab*et al*. (2010) for synbiotic acidophilus milk and Deepak and Sheweta (2016) for buttermilk fortified with soluble fiber (partially hydrolyzed guar gum).On 21st day of storage, the control and experimental buttermilk samples had a change in consistency, flavor and taste. But the sensory panel rejected the samples stored on 21st day. Sensory evaluation is the most important step in the development of new food product; hence, these samples were rejected for further analysis.

Conclusion

Buttermilk samples prepared either with probiotic *L. acidophilus* and *B.bifidum* alone and in combination and either with prebiotic substance honey or oligofructose had been assessed during refrigerated storage for 21 days and the experimental buttermilk samples either with *L.acidophilus* or *B.bifidum* and both in combination had maintained the minimum level of probiotic bacterial cells 10^6–10^7 cells per gram so as to exert probiotic properties up to 15 days of storage without affecting sensory properties.

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How to cite this article:

Malarkannan, S.P and Ramanathan, A (2019) 'Storage Studies of Cultured Low fat Synbiotic Buttermilk', *International Journal of Current Advanced Research,* 08(09), pp. 19895-19900. DOI: http://dx.doi.org/10.24327/ijcar.2019.3869.19900

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