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Impacts of lower incubation temperature and different lactic starter microbiota on the preparation of buffalo milk curd: Insights from organoleptic evaluation

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Abstract
The various starter cultures and their combinations were used to prepare buffalo milk curd samples with standardized buffalo milk. The sensory scores of buffalo milk curd samples prepared with various combinations of starter cultures by incubating at lower incubation temperature 37 °C and then curd samples were stored at refrigeration temperature. Buffalo milk curd samples were underwent organoleptic analysis. Samples made with buffalo milk curd made with a combination of *Lactococcus lactis* starter cultures and its subspecies incubated at a lower incubation temperature of 37 ºC had higher sensory scores on day 0 (8.88±0.01, 8.76±0.01, 8.84±0.01, 8.87±0.01) than the control and other treatments in terms of flavor, body and texture, color, and appearance. Buffalo milk curd made with *Lactococcus lactis* subsp. *cremoris* inoculation and incubated at 37 °C starting culture on day 0 had the lowest overall acceptability scores (7.52±0.01, 7.13±0.01, 8.03±0.01, 7.50±0.01) for flavor, body and texture, color, and appearance.

Keywords: Organoleptic evaluation, refrigeration, Starter cultures, lower curd incubation temperatures

Introduction
In order to initiate desirable changes during fermentation of fermented products, a starter culture is intentionally added. The processing, development of taste, and texture of fermented products is greatly influenced by starter cultures. The selection of starter cultures for dairy fermentation is directly influenced by many factors, including the production of undesirable metabolites, the history of safe use, and the ability to resist pathogens. The metabolic activities of starter cultures are heavily studied for their selection. This research is focused on finding starter cultures that are suitable for incubating buffalo milk curd at lower temperatures.

Curd is an extremely nutrient-dense diet. Curd is a good source of protein, vital vitamins, and minerals. Curd contains a lot of calcium and riboflavin. Buffalo milk produces more curd due to its larger total solids composition, especially its fat and protein level. Buffalo milk curd, with its white hue and firm, solid texture, satisfies the necessary curd qualities. Buffalo milk curd has a lower percentage of syneresis due to its higher fat and total solid content. Whey is less likely to be excluded from curd mass when buffalo milk has more fat and solids. Consequently, buffalo milk curd has a better texture.

The sensory scores of buffalo milk curd samples made with different combinations of starter cultures by incubation at lower temperatures and storing at refrigerator temperature are shown in Tables 3, 4, 5, 6, and Figure 1-4. Following the use of different starter cultures and their combinations, buffalo milk curd samples with 5% fat and 9% solids without fat were displayed in Table. 1.

The optimal starter culture combination with the best sensory attributes was identified by organoleptic evaluation of all buffalo milk curd samples, which were then compared to the control curd sample.

The panelists evaluate food through the senses of sight, smell, taste, and touch, as well as the properties (texture, flavor, taste, appearance, smell, etc.) of a product. For more than a decade, this analysis has been utilized to either reject or approve food products.

Lactic acid bacteria play a significant role in most fermented foods, including dairy products, making them the subject of extensive research among dairy products.

In India, curd is a popular fermented milk product. Both cottage and industrial scales are used to prepare curd. After the milk has been pasteurized, it is cooled to 35-40 °C and then inoculated with commercial starter cultures from the previous day before being transferred to earthenware pots for curdling overnight. To meet the current market trend of curd among consumers is of utmost importance. Most of the curd manufacturers who sell curd don't take any precautions to preserve the culture's dependability and purity. Milk fat, solids, not fat, and total solids content were among the notable differences in the milk used to make curd.

In view of the increasing nationwide demand for curd and the growing interest on the part of the organized dairies for large-scale manufacture, there is an urgent need for modernizing the technology for its commercial production. In this communication, investigation related to explore the best lactic starter culture suitable for the preparation of buffalo milk curd incubated at lower temperature and stored under refrigerated conditions through organoleptic analysis.

Materials and methods

Experiment trials using the starter culture combination *viz*. Dahi culture, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, *Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, *Lactococcus lactis* subsp. *cremoris* + *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, *Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *cremoris* + *Lactococcus lactis subsp. lactis biovar diacetylactis*, *Leuconostoc lactis*, *Ln.mesenteroides* subsp. *cremoris*, *Leuconostoc lactis* + *Ln.mesenteroides* subsp. *cremoris*, *Lactobacillus plantarum*, *Brevibacillus brevis*, *Lactobacillus paraplantarum*, *Streptococcus salivarius* subsp. *thermophilus*. Dairy starter cultures were obtained from the National Collection of Dairy Cultures, National Dairy Research Institute, Karnal (Haryana) were used in this research. Buffalo milk curd were prepared and incubated at 37 °C using five per cent fat and nine per cent SNF combination in buffalo milk.

Table 1: Experiment trials using the starter culture combination as given in Table 1

S. No.	Treatment Combinations
1	*Lactococcus lactis* subsp. *lactis*
2	*Lactococcus lactis* subsp. *cremoris*
3	*Lactococcus lactis* subsp. *lactis biovar diacetylactis*
4	*Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *lactis biovar diacetylactis*
5	*Lactococcus lactis* subsp. *cremoris* + *Lactococcus lactis* subsp. *lactis biovar diacetylactis*
6	*Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *cremoris* + *Lactococcus lactis* subsp. *lactis biovar diacetylactis*
7	*Leuconostoc lactis*
8	*Leuconostoc mesenteroides* subsp.*cremoris*
9	*Leucuconostoc lactis* + *Leuconostoc mesenteroides* subsp. *cremoris*
10	*Lactobacillus plantarum*
11	*Lactobacillus paraplantarum*
12	*Streptococcus salivarius* subsp. *thermophilus*

Organoleptic analysis of curd

Color appearance, flavor, body, texture, and overall acceptability were the sensory attributes evaluated.

A sensory panel of twelve panelists with experience in grading curd was presented with fresh buffalo milk samples. The panelists used hedonic ratings recommended by Hussain *et al.,* 2016 to assess the curds' flavour, body, texture, and appearance on a score card during the zero, first, and sixth day with slight modifications.

Guidelines were presented to the panel prior to the evaluation. The following hedonic ratings were included in the sensory score card. A panel of discriminative and communicative judges assessed the curd samples for sensory qualities like color and appearance, body and texture, flavor, and overall acceptability using a nine-point hedonic scale (9 for strongly like and 1 for disliking excessively). Just before serving, a single cup (100 ml) of each treatment was randomly selected from the refrigerator and given to each panelist for evaluation.

Results and Discussion

The sensory scores for buffalo milk curd samples prepared using B1Ll+Lc+Ld combination of starter cultures incubated at 37ºC were higher than the control and other treatments in terms of flavour, body and texture, colour and appearance, overall acceptability (8.88±0.01, 8.76±0.01, 8.84±0.01, 8.87±0.01) on day 0 and lowest flavour, body and texture, colour and appearance, overall acceptability scores (7.52±0.01, 7.13±0.01, 8.03±0.01, 7.50±0.01) were observed for buffalo milk curd sample prepared using *Lactococcus lactis* subsp. *cremoris* starter culture on day 0. (Table 3-6).

The flavour, body and texture, colour and appearance, overall acceptability scores (6.92±0.01, 6.67±0.01, 7.05±0.01, 7.04±0.01) were higher than the control and other treatments for the buffalo milk curd samples prepared using B1Ll+Lc+Ld (Table 2-5) starter culture combinations and incubated at 37 °C on day 6 of storage at 5.00±0.5 °C and whereas flavour, body and texture, colour and appearance, overall acceptability scores were lower than the control and other treatments for the buffalo milk curd sample prepared using by *Streptococcus salivarius* subsp. *thermophilus* inoculation (B1St) starter culture (3.89±0.01, 3.65±0.01, 6.16±0.01, 4.04±0.02) on day 6 of storage at 5.00±0.5 °C.

The curd samples made with *Lactococcus lactis* subsp. lactis, *Lactococcus lactis* subsp. cremoris, and *Lactococcus lactis* subsp. lactis biovar diacetylactis mixed cultures had the highest sensory score from the sensory panel on both days 0 and 6 of storage at refrigerator temperature. When compared to other samples, the B1Ll+Lc+Ld sample that was incubated at 37ºC had higher sensory scores. The curd's body and texture, which contained *Lactococcus lactis* subsp. lactis, *Lactococcus lactis* subsp. cremoris, and *Lactococcus lactis* subsp. lactis biovar diacetylactis starter culture, were characterized by a firm, homogenous gel, a characteristic white, smooth, and shiny surface, and the absence of syneresis. These outcomes concur with the research conducted by Joon et al. (2017) [5].

Some starter cultures have lower sensory scores. This may due to the heterofermentative nature of the bacterial culture, which is known to create trace levels of formic acid and CO_2, which give the product a strong taste. Ghosh and Rajorhia (1990) [2-3] discovered that some dahi samples had low flavor ratings prepared with heterofermentative bacterial starter culture. Compared to the other samples, the B1Ll+Lc+Ld sample that was incubated at 37 ºC displayed higher sensory ratings.

Conclusion

The best lactic starter culture suitable for the preparation of Buffalo milk curd incubated at lower temperature of 37 °C and stored under refrigerated conditions was explored through organoleptic evaluation. The flavour, body and texture, overall acceptability scores were higher than the control and other treatments for the curd samples prepared using *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures starter culure combinations and incubated at 37 °C on day 6 of storage at 5.00±0.5 °C. On days 0 and 6 of refrigerated storage, the curd samples made with *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, and *Lactococcus lactis* subsp. *lactis biovar diacetylactis* mixed cultures received the highest sensory score from the sensory panel.

Table 2: List of treatments for the buffalo milk curd with various starter culture combinations incubated at 37 °C are as follows

Code	Description
B1C	Control buffalo curd prepared by Dahi culture incubated at 37 °C
B1Ll	Buffalo milk curd prepared by *Lactococcus lactis* subsp. *lactis* inoculation and incubated at 37 °C
B1Lc	Buffalo milk curd prepared by *Lactococcus lactis* subsp. *cremoris* inoculation and incubated at 37 °C
B1Ld	Buffalo milk curd prepared by *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* inoculation and incubated at 37 °C
B1Ll+Ld	Buffalo milk curd prepared by *L. lactis* subsp. *lactis* + *L. lactis* subsp. *lactis* biovar *diacetylactis* inoculation and incubated at 37 °C
B1Lc+Ld	Buffalo milk curd prepared by *L. lactis* subsp. *cremoris* + *L. lactis* subsp. *lactis* biovar *diacetylactis* inoculation and incubated at 37 °C
B1Ll+Lc+Ld	Buffalo milk curd prepared by *L. lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris* + *L. lactis* subsp. *lactis* biovar *diacetylactis* inoculation and incubated at 37 °C
B1Lnl	Buffalo milk curd prepared by *Leuconostoc lactis* inoculation and incubated at 37 °C
B1Lnm	Buffalo milk curd prepared by *Leuconostoc mesenteroides* subsp. *cremoris* inoculation and incubated at 37 °C
B1Lnl+CLnm	Buffalo milk curd prepared by *Leuconostoc lactis* + *Leuconostoc mesenteroides* subsp. *cremoris* inoculation and incubated at 37 °C
B1Lp	Buffalo milk curd prepared by *Lactobacillus plantarum* inoculation and incubated at 37 °C
B1Bb	Buffalo milk curd prepared by *Brevibacillus brevis* inoculation and incubated at 37 °C
B1Lpp	Buffalo milk curd prepared by *Lactobacillus paraplantarum* inoculation and incubated at 37 °C
B1St	Buffalo milk curd prepared by *Streptococcus salivarius* subsp. *thermophilus* inoculation and incubated at 37 °C

Table 3: Flavour scores of buffalo milk curd with various starter cultures incubated at 37 °C during refrigerated storage

Storage period in Days	Starter cultures							
	B1C	B1Ll	B1Lc*	B1Ld	B1Ll+Ld	B1Lc+Ld	B1Ll+Lc+Ld	B1St
0	8.74^{cA}±0.10	8.76^{bA}±0.10	7.52^{eA}±0.10	8.77^{bA}±0.10	8.88^{aA}±0.10	7.57^{dA}±0.10	8.88^{aA}±0.10	7.57^{dA}±0.10
2	8.05^{dB} ±0.10	8.04^{dB}±0.10	7.07^{eB}±0.10	8.38^{bA}±0.10	8.28^{cB}±0.10	7.07^{eB}±0.10	8.47^{aA}±0.10	$7.07^{eB\ A}$±0.10
4	6.55^{cC}±0.10	6.05^{dC}±0.10	6.07^{dC}±0.10	7.08^{bB}±0.10	7.09^{bC}±0.10	6.08^{dC}±0.10	7.58^{aB}±0.10	6.57^{cC}±0.10
6	4.97^{bD}±0.10	4.96^{cD}±0.10	4.98^{cD}±0.10	4.97^{cC}±0.10	4.96^{cD}±0.10	4.98^{cD}±0.10	6.92^{aC}±0.10	3.89^{dD}±0.10
8	3.92^{bE}±0.10	3.93^{bE}±0.10	3.92^{bE}±0.13	3.93^{bD}±0.10	3.99^{bE}±0.13	3.99^{bE}±0.13	5.17^{aD}±0.13	2.96^{cE}±0.10
10	2.94^{bF}±0.10	3.00^{aF}±0.10	2.95^{bF}±0.10	2.96^{bE}±0.10	3.00^{aF}±0.10	2.94^{bF}±0.10	3.00^{aE}±0.10	3.01^{aE}±0.10

Mean ± Standard error values from six trials.
Mean values bearing different superscripts in a column differed significantly (p<0.01)
* indicates curd sample with long setting time/poor coagulation effect

Table 4: Body and texture scores of buffalo milk curd with various starter cultures incubated at 37 °C during refrigerated storage

Storage period in Days	Starter cultures							
	B1C	B1Ll	B1Lc*	B1Ld	B1Ll+Ld	B1Lc+Ld	B1Ll+Lc+Ld	B1St
0	8.68^{cA}±0.10	8.69^{cA}±0.10	7.13^{dA}±0.10	8.70^{bA}±0.10	8.72^{aA}±0.10	7.15^{dA}±0.10	8.76^{aA}±0.10	7.15^{dA}±0.10
2	7.63^{dB} ±0.10	7.63^{dB}±0.10	6.65^{eB}±0.10	7.96^{bB}±0.10	7.86^{cB}±0.10	6.65^{eB}±0.10	8.05^{aB}±0.10	6.65^{eB}±0.10
4	6.13^{cC}±0.10	5.54^{dC}±0.10	5.55^{dC}±0.10	6.66^{bC}±0.10	6.67^{bC}±0.10	5.67^{dC}±0.10	7.16^{aC}±0.10	6.15^{cC}±0.10
6	4.54^{dD}±0.10	4.63^{cD}±0.10	4.65^{bD}±0.10	4.66^{aD}±0.10	4.65^{bD}±0.10	4.66^{aD}±0.10	6.67^{aD}±0.10	3.65^{eD}±0.10
8	3.63^{bE}±0.10	3.63^{bE}±0.10	3.65^{bE}±0.13	3.66^{bE}±0.10	3.67^{bE}±0.13	3.67^{bE}±0.13	4.87^{aE}±0.13	2.87^{cE}±0.10
10	3.05^{bF}±0.10	2.69^{cF}±0.10	2.65^{cF}±0.10	2.66^{cF}±0.10	2.68^{cF}±0.10	2.66^{cF}±0.10	3.72^{aF}±0.10	2.69^{cE}±0.10

Mean ± Standard error values from six trials.
Mean values bearing different superscripts in a column differed significantly (p<0.01)
* indicates curd sample with long setting time/poor coagulation effect

Table 5: Colour and appearance scores of buffalo milk curd with various starter cultures incubated at 37°C during refrigerated storage

Storage period in Days	Starter cultures							
	B1C	B1Ll	B1Lc*	B1Ld	B1Ll+Ld	B1Lc+Ld	B1Ll+Lc+Ld	B1St
0	8.75^{cA}±0.10	8.77^{bA}±0.10	8.03^{dA}±0.10	8.78^{bA}±0.10	8.83^{aA}±0.10	8.14^{dA}±0.10	8.84^{aA}±0.10	8.07^{dA}±0.10
2	8.48^{dB} ±0.10	8.68^{cB}±0.10	7.99^{eA}±0.10	8.66^{cA}±0.10	8.77^{bA}±0.10	8.08^{eA}±0.10	8.77^{aA}±0.10	8.02^{eA}±0.10
4	7.52^{bC}±0.10	7.02^{dC}±0.10	7.05^{dB}±0.10	7.03^{dB}±0.10	7.04^{dB}±0.10	7.03^{dB}±0.10	7.53^{aB}±0.10	7.22^{cB}±0.10
6	6.87^{bD}±0.10	5.74^{cD}±0.10	5.81^{dC}±0.10	5.80^{dC}±0.10	5.79^{dC}±0.10	5.85^{dC}±0.10	7.05^{aB}±0.10	6.16^{cC}±0.10
8	5.28^{cE}±0.10	5.25^{cD}±0.10	5.31^{cC}±0.13	5.24^{cD}±0.10	5.26^{cD}±0.13	5.25^{cD}±0.13	6.84^{aC}±0.13	5.83^{bD}±0.10
10	5.14^{cF}±0.10	3.17^{cE}±0.10	5.21^{cD}±0.10	5.15^{cD}±0.10	5.08^{cD}±0.10	5.11^{cD}±0.10	6.06^{aD}±0.10	5.54^{bD}±0.10

Mean ± Standard error values from six trials.
Mean values bearing different superscripts in a column differed significantly (p<0.01)

Table 6: Overall acceptability scores of buffalo milk curd with various starter cultures incubated at 37 °C during refrigerated storage

Storage period in Days	Starter cultures							
	B1C	B1Ll	B1Lc*	B1Ld	B1Ll+Ld	B1Lc+Ld	B1Ll+Lc+Ld	B1St
0	8.72^{cA}±0.10	8.83^{bA}±0.10	7.50^{dA}±0.10	8.82^{bA}±0.10	8.85^{aA}±0.10	7.52^{dA}±0.10	8.87^{aA}±0.10	7.51^{dA}±0.10
2	8.02^{dB} ±0.10	8.02^{dB}±0.10	7.00^{eA}±0.10	8.33^{bB}±0.10	8.23^{cB}±0.10	7.03^{eA}±0.10	8.43^{aA}±0.13	6.96^{fB}±0.10
4	6.52^{cC}±0.10	6.03^{dC}±0.10	6.01^{dB}±0.10	7.03^{bC}±0.10	7.05^{bC}±0.10	6.03^{dB}±0.10	7.54^{aB}±0.10	6.54^{cC}±0.10
6	6.04^{bD}±0.10	5.03^{cD}±0.10	5.02^{cC}±0.10	5.02^{cD}±0.10	5.03^{cD}±0.10	5.04^{cC}±0.10	7.04^{aC}±0.10	4.04^{dD}±0.13
8	4.02^{bE}±0.10	4.04^{bE}±0.10	4.02^{bD}±0.13	4.03^{bE}±0.10	4.05^{bE}±0.13	4.04^{bD}±0.13	5.26^{aD}±0.13	3.02^{cE}±0.10
10	3.01^{bF}±0.10	3.04^{aF}±0.10	3.01^{bE}±0.10	3.01^{bF}±0.10	3.03^{aF}±0.10	3.01^{bE}±0.10	3.04^{aE}±0.10	3.02^{bF}±0.10

Mean ± Standard error values from six trials.

Mean values bearing different superscripts in a column differed significantly (p<0.01)

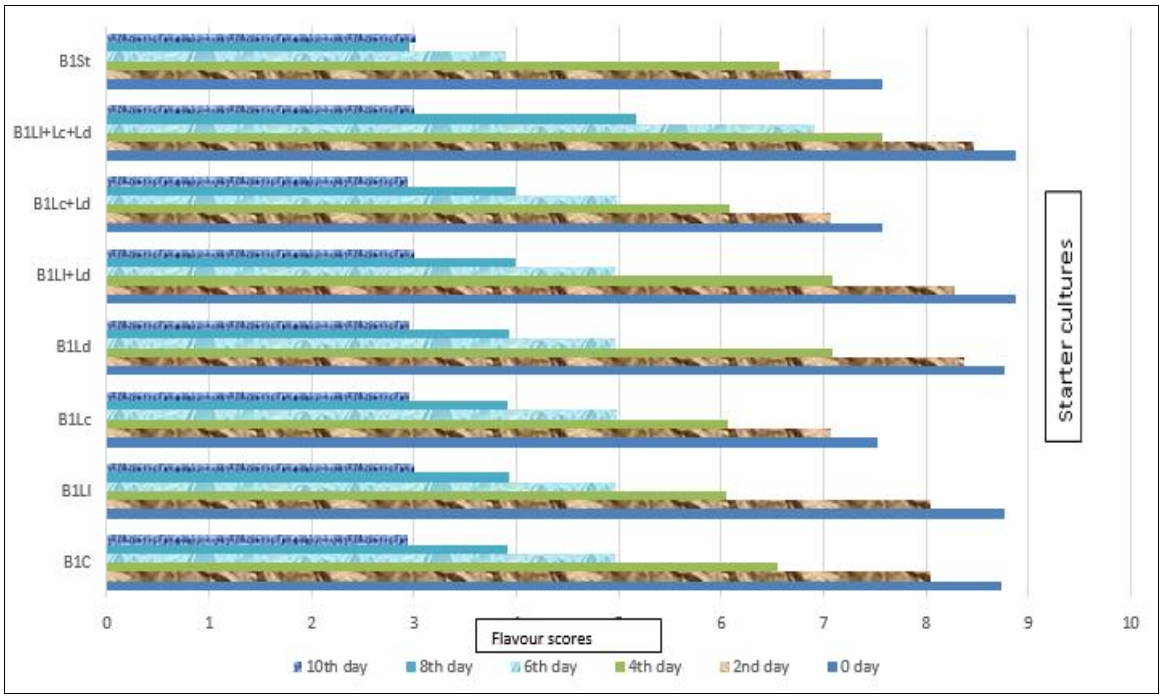

Fig 1: Flavour scores of Buffalo milk curd samples with various starter cultures incubated at 37°C during refrigerated storage

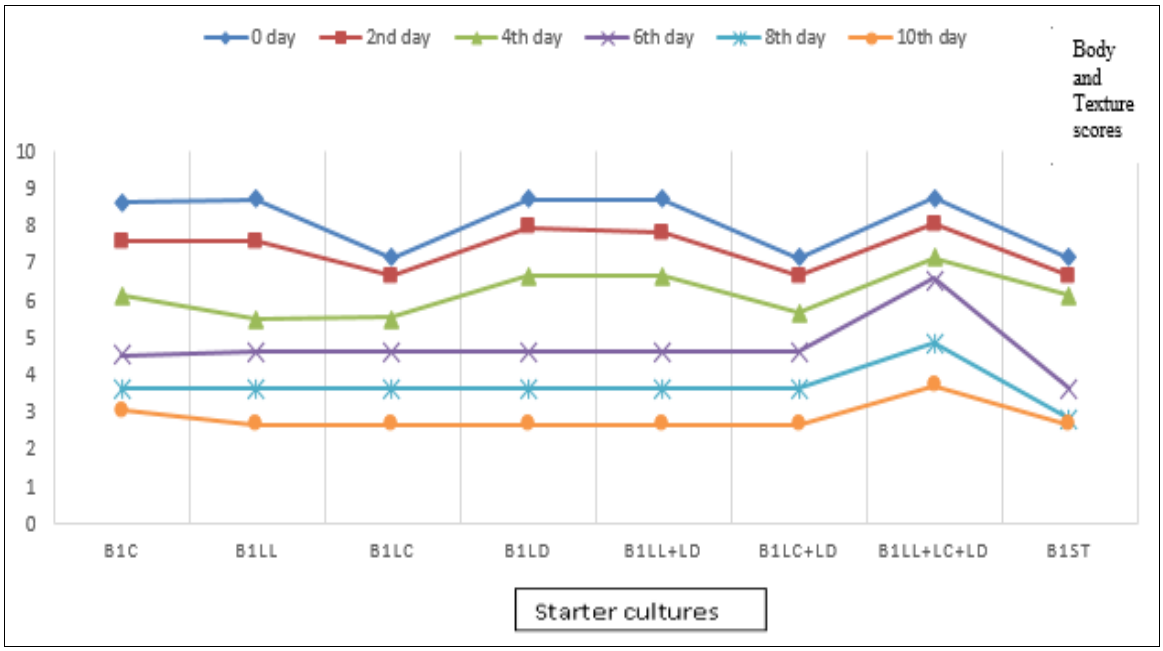

Fig 2: Body and texture scores of buffalo milk curd samples with various starter cultures incubated at 37°C during refrigerated storage

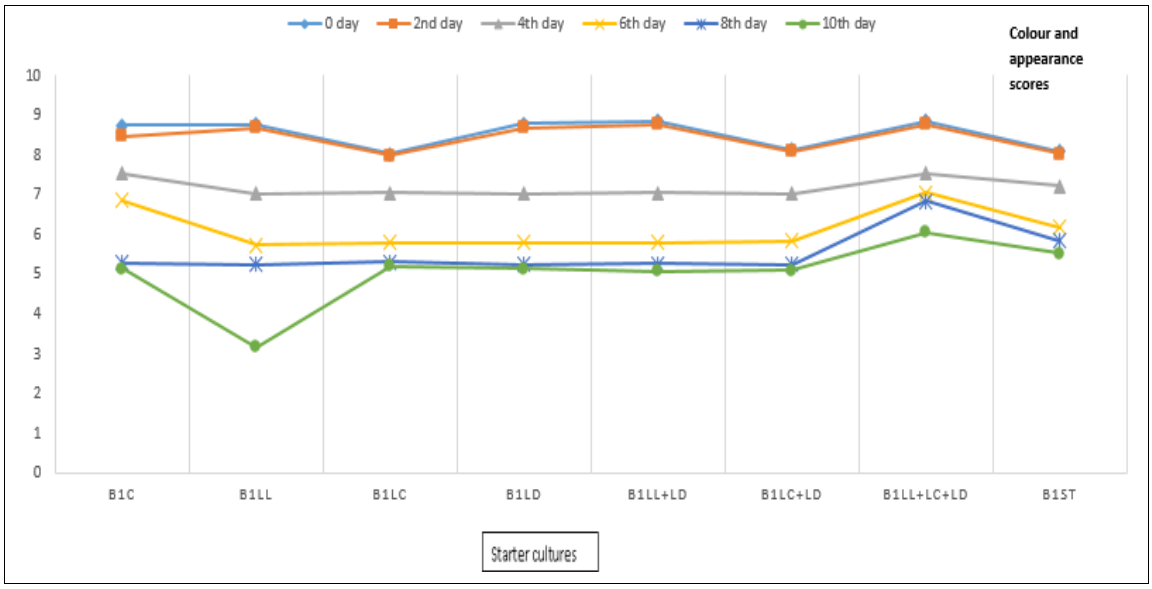

Fig 3: Colour and appearance scores of Buffalo milk curd with various starter cultures incubated at 37 °C during refrigerated storage

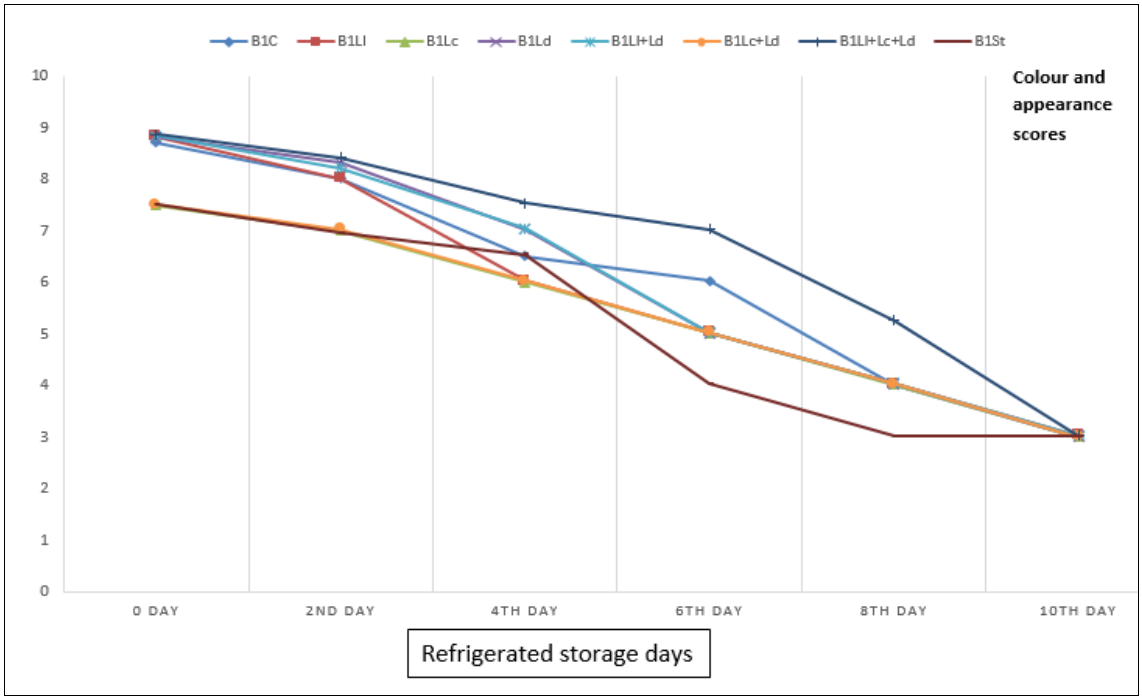

Fig 4: Overall acceptability scores of Buffalo milk curd with various starter cultures incubated at 37 °C during refrigerated storage

Conflict of Interest

The authors declare that there are no conflicts of interest related to the publication of this study.

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