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Exploring the best lactic starter culture suitable for the manufacture of cow milk curd incubated at lower temperature

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Abstract

Cow milk curd samples were made using standardized cow milk inoculated with different starter cultures and their combinations. The sensory scores of cow milk curd samples prepared with various combinations of starter cultures by incubating at 37 °C and stored at refrigeration temperature. Dairy starter cultures were obtained from the National Collection of Dairy Cultures (NCDC), Division of Dairy Microbiology, National Dairy Research Institute, Karnal (Haryana). A panel of six 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 extremely). The sensory evaluations of curd samples made using cow's milk curd made with a mix of starter cultures. In terms of flavor, body and texture, colour, and appearance, overall acceptability scores (8.79 ± 0.01 , 8.68 ± 0.01 , 8.81 ± 0.01 , 8.81 ± 0.01) on day 0 for *Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *cremoris* + *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* inoculation at 37°C incubation outperformed the control and other treatments. On day 0, the curd sample made with cow milk curd inoculated with *Lactococcus lactis* subsp. *cremoris* had the lowest overall acceptability scores (7.46 ± 0.01 , 7.05 ± 0.01 , 8.01 ± 0.01 , 7.48 ± 0.01) for flavor, body and texture, color, and appearance.

Keywords: *Lactococcus lactis*, pH, refrigeration, cow milk curd, starter cultures, incubation temperatures

Introduction

Organoleptic analysis uses the panelists' senses (sight, smell, taste, touch) to assess food and its characteristics (texture, flavor, taste, appearance, fragrance, etc.). For many years, this kind of organoleptic analysis has been utilized to either accept or reject food goods. Due to their crucial role in the majority of fermented foods, including dairy products, lactic acid bacteria are currently the focus of a great deal of research. Curd is a fermented milk product popular in India. Curd is prepared on cottage scale as well as industrial scale. After pasteurizing milk, it is cooled to 35–40 °C, and then inoculated with commercial starter culture kept from the previous day, and transferred to earthenware pots; curdling takes place at room temperature overnight. It is immensely important to meet the current market trend of curd among consumers. Most of the curd manufacturers don't take any precautions to preserve the culture's dependability and purity. There are significant differences in the milk's fat, solids not fat, and total solids content when it comes to the milk needed to make curd.

There is an urgent need to modernize the technology for curd's commercial production given the rising demand for the product across the country and the organized dairy industry's growing interest in large-scale manufacturing of curd. Through organoleptic analysis, this research aims to investigate the suitable lactic starter culture for making cow milk curd that is incubated at a lower temperature and stored in a refrigerator.

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

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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 (NCDC), Division of Dairy Microbiology, National Dairy Research Institute, Karnal (Haryana) were used in this research. Cow milk curd were prepared and incubated at 37 °C using 5 per cent fat and 9 per cent SNF combination in cow milk.

The following starter cultures were used on the cow milk standardized to 5 per cent fat and 9 per cent SNF combination using Pearson square method (Pearson and Freeman, 1972).

Table 1: Experimental treatments and combinations of lactic acid bacterial strains with number of replications.

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

Sensory analysis of curd

A panel of well-trained 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 extremely). Just before serving, a single cup (100 ml) of each treatment was randomly selected from the refrigerator and given to each panelist for evaluation. (Divya *et al.*, 2024; Hussain *et al.*, 2016) [5, 9].

Results and Discussion

Cow milk curd samples were prepared using the different starter cultures as shown in Table 1 using cow milk that was standardized to contain 5% fat and 9% Solids Not Fat. Tables 2, 3, 4, and 5 present the sensory scores of cow milk curd samples that were made using different combinations of starter cultures, incubated at 37°C, and then stored at refrigerator temperature (Figure 1-5). The various starter cultures and their combinations as in table 1 were used to prepare cow milk curd samples with cow milk standardized to 5 per cent fat and 9 per cent Solids Not Fat combination.

All curd samples were compared with the control curd sample by sensory evaluation to select the best starter culture combination with extended shelf life. The sensory scores for curd samples prepared using C1Ll+Lc+Ld combination of starter cultures incubated at 37 °C were higher than the control and other treatments in terms of flavour, body and texture, colour and appearance, overall acceptability (8.79±0.01, 8.68±0.01, 8.81±0.01, 8.81±0.01) on day 0 and lowest flavour, body and texture, colour and appearance, overall acceptability.

The curd samples made *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 days 0 and 6 of storage.

In addition to having a lengthy setting time and a poor coagulation tendency, the cow milk curd made with C1Lnl, C1Lnm, C1Lnl + C1Lnm, C1Lp, C1Bb, and C1Lpp starter

cultures was not assessed for potential contamination from non-starter organisms and was not the subject of additional organoleptic investigations. (Holland and Liu, 2011) [8].

In terms of flavor, body and texture, color and appearance, and overall acceptability, the sensory scores for curd samples made with a C1Ll+Lc+Ld combination of starter cultures incubated at 37 °C were higher than those for the control and other treatments (8.79±0.01, 8.68±0.01, 8.81±0.01, 8.81±0.01) on day 0. The curd sample made with C1Lc starting culture on day 0 had the lowest overall acceptance scores (7.46±0.01, 7.05±0.01, 8.01±0.01, 7.48±0.01) in terms of flavor, body and texture, color, and appearance.

Compared to the control and other treatments, the curd samples made with C1Ll+Lc+Ld (Table 13-16) starter culture combinations and incubated at 37 °C on day 6 of storage at 5.00± 0.5 °C had higher flavor, body, and texture scores (7.00±0.01, 6.59±0.01, 7.02±0.01, 7.02±0.01). The findings of this research align with the conclusions drawn by Farsi *et al.* (2017), who discovered that citrate-positive strains of *Lactobacillus lactis* subsp. *lactis* biovar. *Diacetylactis* or *Leuconostoc* strains that generate low levels of CO₂ are sometimes incorporated into cheese to create diacetyl, an essential flavoring component. The scores for flavour, body, texture, and overall acceptability were found to be lower than those of the control and other treatments for the curd sample made with the C1St starter culture (3.98±0.01, 3.57±0.01, 4.01±0.01) on the sixth day of storage at 5.00± 0.5 °C.

These findings are consistent with the observations made by Marshall *et al.* (1985), which indicate that *Streptococcus salivarius* subspecies *thermophilus* is not suitable for milk fermentations, as the fermentation process using this organism leads to products characterized by poor flavour, aroma, and texture.

Significant ($p<0.01$) difference between treatment and control cow milk curd samples was observed. The *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* sample received a higher flavour, body texture and overall acceptability scores than the other curd samples incubated at

37 °C on 0 day. The same sample was acceptable up to 6 days under refrigerated storage. This is in agreement with Rasane *et al.* (2025) who documented in dairy food fermentation industry, lactic acid bacteria mainly *Lactococcus lactis* acts as a functional starter culture and also as a vital microorganism due to its peculiar characteristics such as food flavoring, acidification, organoleptic and in the production of various fermented dairy products.

Body and texture of curd containing *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* starter culture was characterized by firm homogenous gel and it was free from syneresis and possess typical white, smooth and shiny surface. These results are in agreements with the findings of Joon *et al.* (2017) [10].

Conclusion

Dahi produced on domestic levels has undefined cultures, thus, difference in texture and taste is often observed. With standardization of culture and production conditions a defined product of consistent quality (sensory and textural) can be achieved. Thus exploring the best lactic starter culture suitable for the preparation of cow milk curd incubated at lower temperature and stored under refrigerated conditions through organoleptic analysis revealed that the flavour, body and texture, overall acceptability scores were higher for the curd samples prepared using *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* combined cultures starter culture combinations and incubated at 37 °C on day 6 of storage at refrigeration temperature.

Table 2: Flavour scores of cow milk curd samples with various starter cultures incubated at 37°C during refrigerated storage

Storage period in Days	Starter cultures							
	C1C	C1LI	C1Lc	C1Ld	C1LI+Ld	C1Lc+Ld	C1LI+Lc+Ld	C1St
0	8.64 ^{ba} ±0.10	8.68 ^{ba} ±0.10	7.46 ^{ca} ±0.10	8.68 ^{ba} ±0.10	8.79 ^{aa} ±0.10	7.48 ^{da} ±0.10	8.79 ^{aa} ±0.10	7.48 ^{ca} ±0.10
2	7.96 ^{db} ±0.10	7.96 ^{db} ±0.10	6.98 ^{eb} ±0.10	8.29 ^{ba} ±0.10	8.19 ^{ca} ±0.10	6.98 ^{eb} ±0.10	8.38 ^{aa} ±0.10	6.98 ^{eb} ±0.10
4	6.46 ^c ±0.10	5.97 ^{dc} ±0.10	5.98 ^{dc} ±0.10	6.99 ^{bb} ±0.10	7.00 ^{bb} ±0.10	5.99 ^{dc} ±0.10	7.49 ^{ab} ±0.10	6.48 ^{cb} ±0.10
6	4.97 ^{bd} ±0.10	4.96 ^{cd} ±0.10	4.98 ^{cd} ±0.10	4.99 ^{cc} ±0.10	4.98 ^{cc} ±0.10	4.99 ^{cd} ±0.10	7.00 ^{ab} ±0.10	3.98 ^{dc} ±0.10
8	3.96 ^{be} ±0.10	3.96 ^{be} ±0.10	3.98 ^{be} ±0.13	3.99 ^{bd} ±0.10	4.00 ^{bd} ±0.13	4.00 ^{be} ±0.13	5.20 ^{ac} ±0.13	3.70 ^{cc} ±0.10
10	2.98 ^{bf} ±0.10	3.02 ^{af} ±0.10	2.98 ^{bf} ±0.10	2.99 ^{be} ±0.10	3.01 ^{ae} ±0.10	2.99 ^{cf} ±0.10	3.02 ^{ad} ±0.10	3.00 ^{ad} ±0.10

Mean ± Standard error values from six trials.

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

Table 3: Body and texture scores of cow milk curd samples with various starter cultures incubated at 37°C during refrigerated storage

Storage period in Days	Starter cultures							
	C1C	C1LI	C1Lc	C1Ld	C1LI+Ld	C1Lc+Ld	C1LI+Lc+Ld	C1St
0	8.68 ^{aA} ±0.22	8.21 ^{bA} ±0.10	7.05 ^{cA} ±0.10	8.27 ^{bA} ±0.10	8.38 ^{aA} ±0.10	7.07 ^{cA} ±0.10	8.68 ^{aA} ±0.10	7.07 ^{cA} ±0.10
2	7.55 ^{dB} ±0.10	7.55 ^{dB} ±0.10	6.57 ^{eb} ±0.10	7.88 ^{bb} ±0.10	7.78 ^{cb} ±0.10	6.57 ^{eb} ±0.10	7.97 ^{aB} ±0.10	6.57 ^{eb} ±0.10
4	6.05 ^c ±0.10	5.46 ^{dc} ±0.10	5.47 ^{dc} ±0.10	6.58 ^{bc} ±0.10	6.59 ^{bc} ±0.10	5.59 ^{dc} ±0.10	7.08 ^{aC} ±0.10	6.07 ^{cc} ±0.10
6	4.46 ^{dD} ±0.10	4.55 ^{cd} ±0.10	4.57 ^{bd} ±0.10	4.58 ^{bd} ±0.10	4.57 ^{bd} ±0.10	4.58 ^{bd} ±0.10	6.59 ^{aD} ±0.10	3.57 ^{ed} ±0.10
8	3.55 ^{bE} ±0.10	3.55 ^{bE} ±0.10	3.57 ^{bE} ±0.13	3.58 ^{bE} ±0.10	3.59 ^{bE} ±0.13	3.59 ^{bE} ±0.13	4.79 ^{aE} ±0.13	2.58 ^{cE} ±0.10
10	2.57 ^{bF} ±0.10	2.61 ^{aF} ±0.10	2.57 ^{bF} ±0.10	2.58 ^{bF} ±0.10	2.60 ^{aF} ±0.10	2.58 ^{bF} ±0.10	2.61 ^{aF} ±0.10	2.61 ^{aF} ±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: Colour and appearance scores of cow milk curd with various starter cultures incubated at 37°C during refrigerated storage

Storage period in Days	Starter cultures							
	C1C	C1LI	C1Lc	C1Ld	C1LI+Ld	C1Lc+Ld	C1LI+Lc+Ld	C1St
0	8.78 ^{abA} ±0.10	8.70 ^{bA} ±0.10	8.01 ^{dA} ±0.10	8.70 ^{bA} ±0.10	8.81 ^{aA} ±0.10	8.12 ^{cA} ±0.10	8.81 ^{aA} ±0.10	8.05 ^{dA} ±0.10
2	8.45 ^{cA} ±0.10	8.66 ^{bA} ±0.10	7.97 ^{eA} ±0.10	8.64 ^{bA} ±0.10	8.75 ^{aA} ±0.10	8.06 ^{dA} ±0.10	8.75 ^{aA} ±0.10	8.00 ^{deA} ±0.10
4	7.50 ^{aB} ±0.10	7.01 ^{cB} ±0.10	7.03 ^{cB} ±0.10	7.01 ^{cB} ±0.10	7.02 ^{cB} ±0.10	7.01 ^{cB} ±0.10	7.51 ^{aB} ±0.10	7.20 ^{bb} ±0.10
6	6.85 ^{bC} ±0.10	5.72 ^{cC} ±0.10	5.79 ^{ec} ±0.10	5.78 ^{cC} ±0.10	5.77 ^{cC} ±0.10	5.83 ^{dc} ±0.10	7.02 ^{aC} ±0.10	6.14 ^{cC} ±0.10
8	5.26 ^{cd} ±0.10	5.23 ^{cC} ±0.10	5.29 ^{cC} ±0.13	5.22 ^{cd} ±0.10	5.24 ^{cd} ±0.13	5.23 ^{cd} ±0.13	6.82 ^{aC} ±0.13	5.81 ^{bd} ±0.10
10	5.12 ^{cd} ±0.10	5.15 ^{cC} ±0.10	5.19 ^{cC} ±0.10	5.13 ^{cd} ±0.10	5.06 ^{cd} ±0.10	5.09 ^{cd} ±0.10	6.04 ^{aD} ±0.10	5.52 ^{bd} ±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: Overall acceptability scores of cow milk curd with various starter cultures incubated at 37°C during refrigerated storage

Storage period in Days	Starter cultures							
	C1C	C1LI	C1Lc	C1Ld	C1LI+Ld	C1Lc+Ld	C1LI+Lc+Ld	C1St
0	8.76 ^{abA} ±0.15	8.70 ^{bA} ±0.10	7.48 ^{cA} ±0.10	8.70 ^{bA} ±0.10	8.81 ^{aA} ±0.10	7.50 ^{dA} ±0.10	8.81 ^{aA} ±0.10	7.50 ^{cA} ±0.10
2	8.00 ^{dB} ±0.10	8.00 ^{dB} ±0.10	7.02 ^{eA} ±0.10	8.31 ^{bA} ±0.10	8.21 ^{cB} ±0.10	7.01 ^{eA} ±0.10	8.41 ^{aA} ±0.10	7.04 ^{eA} ±0.10
4	6.50 ^c ±0.10	6.01 ^{dc} ±0.10	6.03 ^{db} ±0.10	7.01 ^{bb} ±0.10	7.02 ^{bc} ±0.10	6.01 ^{db} ±0.10	7.51 ^{aB} ±0.10	6.50 ^{cb} ±0.10
6	6.01 ^{bc} ±0.10	5.01 ^{cd} ±0.10	5.02 ^{cC} ±0.10	5.01 ^{cC} ±0.10	5.01 ^{cd} ±0.10	5.01 ^{cC} ±0.10	7.02 ^{aC} ±0.10	4.01 ^{dc} ±0.10
8	4.00 ^{bd} ±0.10	4.01 ^{bE} ±0.10	4.02 ^{bd} ±0.13	4.01 ^{bd} ±0.10	4.02 ^{bE} ±0.13	4.02 ^{bd} ±0.13	5.22 ^{ad} ±0.13	3.23 ^{cd} ±0.10
10	3.05 ^{aE} ±0.10	3.04 ^{aF} ±0.10	3.06 ^{aE} ±0.10	3.05 ^{aE} ±0.10	3.07 ^{aF} ±0.10	3.06 ^{aE} ±0.10	3.07 ^{aE} ±0.10	3.06 ^{ad} ±0.10

Mean ± Standard error values from six trials.

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

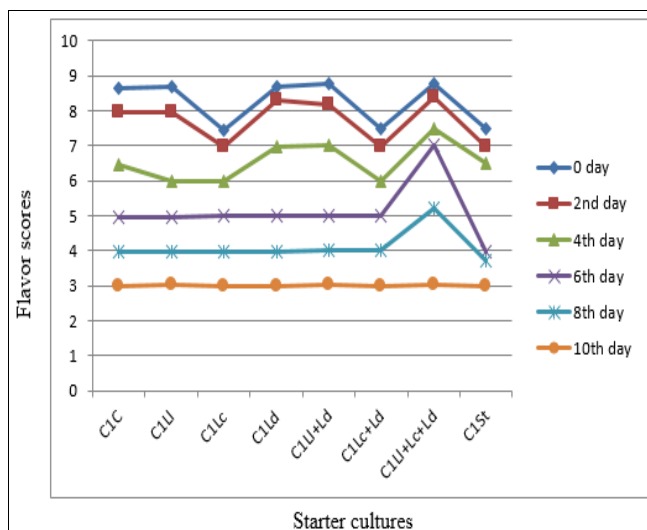


Fig 1: Flavour scores of cow milk curd samples with various starter cultures incubated at 37°C during refrigerated storage

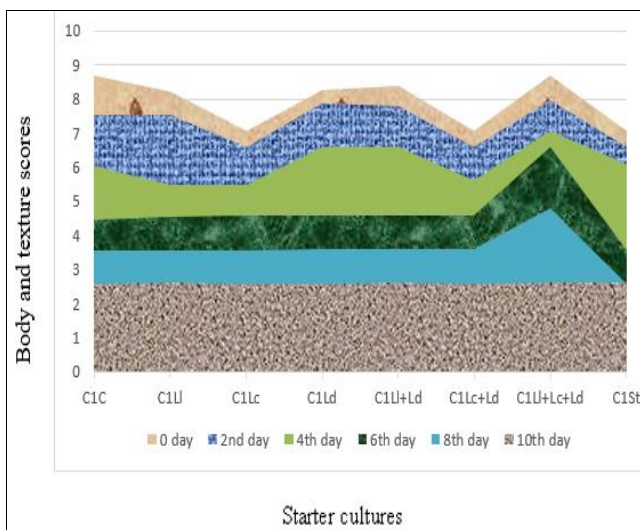


Fig 2: Body and texture scores of cow milk curd samples with various starter cultures incubated at 37°C during refrigerated storage

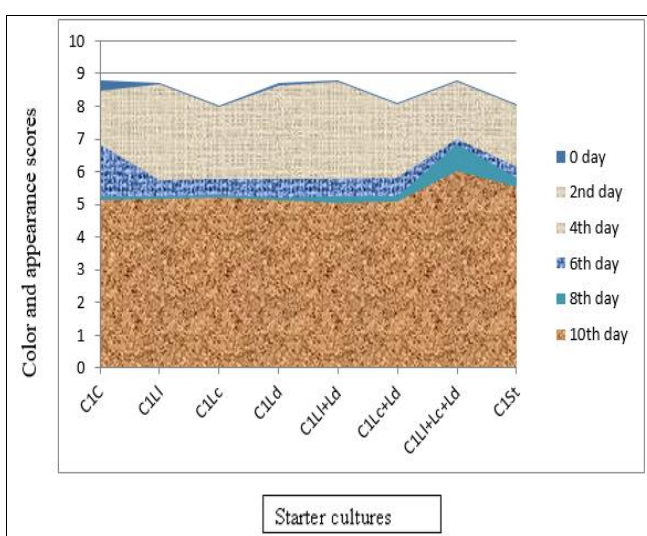


Fig 3: Colour and appearance scores of cow milk curd with various starter cultures incubated at 37°C during refrigerated storage

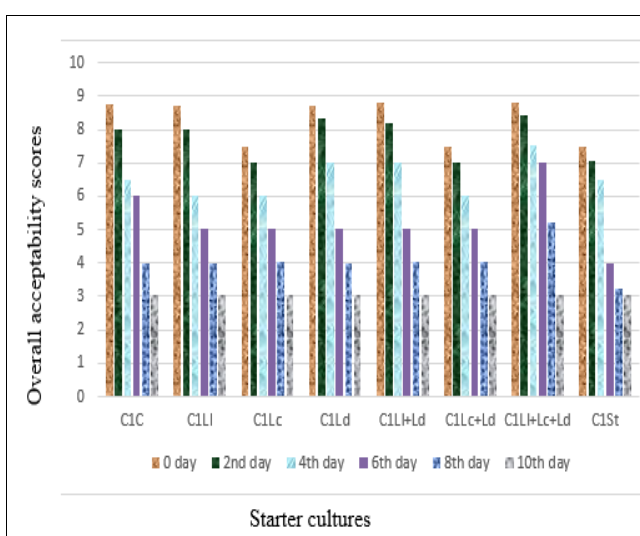


Fig 4: Overall acceptability scores of cow milk curd with various starter cultures incubated at 37°C during refrigerated storage

The flavour, body and texture, overall acceptability scores were lower for the curd sample prepared using starter culture *Lactococcus lactis* subsp. *cremoris* inoculation and incubated at 37 °C on day 0. Cow milk curd prepared by *Streptococcus salivarius* subsp. *thermophilus* inoculation and incubated at 37 °C starter culture on day 6 of storage at 5.00±0.5 °C showed lowest flavor, body and texture, colour and appearance, overall acceptability scores.

Thus, these experimental findings will ensure the selection of suitable starter cultures for the preparation of cow milk curd incubated at lower temperature.

Conflict of Interest: Not available

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