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Storage-Induced Changes in Physicochemical Attributes and Sensorial Quality of Milk Fermented Using EPS- and Non-EPS-Producing Lactic Acid Bacteria

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ABSTRACT: The present investigation was undertaken to evaluate variations in the physicochemical attributes and sensory quality of milk fermented with exopolysaccharide (EPS)-producing and non-EPS-producing strains of lactic acid bacteria (LAB). Dahi was prepared following a standardized method and assessed at 0, 7, 14, and 21 days of refrigerated storage maintained at 5 ± 2°C. The findings revealed statistically significant differences sensory parameters between dahi manufactured using EPS-producing cultures and those produced with non-EPS starters. From a sensory perspective, dahi formulated with the EPS-producing culture combination of *Lactobacillus helveticus* MTCC 5463 and *Streptococcus thermophilus* MTCC 5460 achieved the highest overall acceptability scores. Throughout the storage period, notable alterations were observed in the sensory and physicochemical attributed of the dahi samples; however, the overall trend of these changes was largely consistent regardless of whether EPS or non-EPS starter cultures were employed. Microbiological evaluation indicated that total viable counts remained within acceptable limits for both categories of dahi throughout 21 days of refrigerated storage at 5 ± 2 °C.

Keywords: EPS, Storage, Sensory, Lactic Acid Bacteria, *Dahi.*

INTRODUCTION

Traditional Indian fermented dairy products describes *dahi* (curd) as the most widely consumed fermented milk product in India and discusses its role in traditional diets and probiotic value, embedding it within local dietary practices (Bora and Ahmed 2025). The transformation of milk into *dahi* serves as a crucial intermediary step in the production of traditional high-fat dairy products such as butter and ghee, and it has been estimated that a substantial proportion of India's milk output is processed into *dahi*. Despite evolving lifestyles and changes in dietary practices, *dahi* continues to remain an enduring, indigenous fermented milk product in the Indian diet (Khurana and Kanawajia 2007).

Bacterial exopolysaccharides (EPS) are synthesized and released into the surrounding medium by lactic acid bacteria either through secretion or via enzymes anchored to the cell surface. These high-molecular-weight, long-chain polymers dissolve or disperse in water, imparting thickening and gelling properties that make them valuable functional ingredients in food formulation. EPS generated by LAB are extensively employed to enhance the texture and mouthfeel of yoghurt and other fermented milk products such as *dahi*, improving their overall rheological characteristics (Hernández-Figueroa *et al.*, 2025).

Earlier research has demonstrated that EPS interact with milk proteins to improve texture and structure: they bind water, increase gel firmness, and reduce syneresis, effects that are particularly beneficial in **low-fat** dairy formulations where texture loss is common (Behare *et al.*, 2009; Angelin and Kavitha 2020) thus replacing or reducing the need for added stabilizers or hydrocolloids. Recent research continues to support the role of EPS in fermented dairy quality. Screening of multiple LAB strains has identified those with superior EPS production, which correlates with reduced syneresis, enhanced viscosity, and smoother mouthfeel in **non-fat set yogurts**, highlighting technological and sensory benefits (Sanalibaba and Çakmak 2016; Ramos *et al.*, 2023). Reviews summarizing the structure–function relationships of LAB EPS also emphasize their role in stabilizing casein micelle networks and improving emulsion and gel properties across dairy matrices. They further note that EPS can serve multiple functions—including thickeners, stabilizers, and emulsifiers—depending on environmental conditions and molecular characteristics such as sugar composition and branching (Korcz and Varga 2021).

Together, these studies underscore the multifaceted role of LAB EPS in enhancing the techno-functional properties of fermented dairy foods like yoghurt and dahi while meeting consumer demands for clean-label, additive-free products. There is a scarcity of comprehensive research on the physicochemical attributes and sensory quality of *dahi*. The inclusion of cultures that produce exopolysaccharides (EPS) has the potential to improve product quality and lower production costs. Currently, commercially available starter cultures specifically developed for Indian *dahi* are lacking. Consequently, this research was undertaken to evaluate combinations of cultures that could yield *dahi* with consistent, high-quality attributes.

MATERIAL AND METHODS

Raw cow's milk used for preparing spreadable dahi was sourced from the Livestock Farm in Anand, Gujarat, India. The milk comprised approximately 3.5 % fat and 12 % total solids, with an acidity of 0.126 % lactic acid and a pH of 6.65. Starter culture combinations in this study—*Lactobacillus helveticus* (V3), *Lactobacillus rhamnosus* (NS6), *Streptococcus thermophilus* (MD8), and *S. thermophilus* (MD2)—were obtained from the culture repository of the Dairy Microbiology Department at SMC College of Dairy Science in Anand. Before use, cultures were grown in sterilized skim milk with 10 % total solids for 16 hours and kept at 5 ± 2 °C. Additional commercially available cultures in direct-to-vessel (DVS) form, originally supplied by SACCO, were kept frozen at –20 °C. Skimmed milk powder (brand: Sagar) was also purchased locally for use in the production process.

In the present study, four distinct starter culture formulations were employed, each differing in bacterial composition and inoculation ratio. Culture formulation A consisted of a mixed starter containing three strains of Streptococcus thermophilus (ST 505, ST 503, and ST 820) combined with three strains of Streptococcus lactis (SL 216, SL 69, and SL 225), with the two bacterial groups incorporated in a proportion of 75:25. Similarly, formulation B comprised the same three Streptococcus thermophilus strains (ST 505, ST 503, and ST 820) blended with an alternative set of Streptococcus lactis strains (SL 119, SL 195, and SL 232), maintaining an identical 75:25 ratio between the two groups. Culture formulation C was prepared using an equal (50:50) combination of strain V3, identified as Lactobacillus helveticus MTCC 5463, and strain MD2, identified as Streptococcus thermophilus. Formulation D consisted of an equal (50:50) mixture of strain NS6 (Lactobacillus rhamnosus) and strain MD8 (Streptococcus thermophilus). Among these starter cultures, strains V3 and NS6 were characterized by their ability to produce exopolysaccharides.

***Dahi* production:** During the study *Dahi* was made as per the flow diagram shown in Fig. 1.

Receiving raw cow milk
⇩
Filtration
⇩
Warming up to 45°C
⇩
Addition of skimmed milk powder to adjust total solids to 14%
⇩
Warm till temperature reaches to 65°C
⇩
Homogenization (1^{st} stage = 1500 psi, 2^{nd} stage = 500 psi)
⇩
Heat treatment (90°C / 10 min)
⇩
Cooling to 39°C
⇩
Inoculation of culture (2 %)
⇩
Filling in cups or plastic container and covered with aluminium foil
⇩
Incubation (39°C) until pH reaches 4.6
⇩
Shifting in refrigerator (5 ± 2°C)
⇩
Cooling (5 ± 2°C) and Storage of *Dahi* (5 ± 2°C)

Fig. 1. Flow Chart for preparation of *Dahi*.

Physico-chemical Evaluation: Dahi samples stored at 5 °C were examined at 1, 7, 14, and 21 days of storage for their physico-chemical attributes. Titratable acidity, expressed as a percentage of lactic acid, was measured following standard AOAC procedures. The pH of each dahi sample was recorded using a portable electronic pH meter (CYBERSCAN Series 600, EUTECH Instruments, Singapore) (Sikombe *et al.,* 2025).

Sensorial Evaluation: A nine-point facial hedonic scale was employed for sensory assessment, where 1 indicated "dislike extremely," 5 meant "neither liked nor disliked," and 9 represented "liked extremely." Each sample was encoded with a unique code and presented in a randomized sequence to panelists. A trained expert panel composed of seven judges conducted the sensory evaluation, rating the products for colour and appearance, flavour, body and texture, and overall acceptability.

Microbiological Analysis: The lactic acid bacterial count in the inoculated set of dahi samples was measured following the standard method. Yeast and mold counts were determined in accordance with Indian Standard IS 5403. During storage, lactic count, yeast and mold count, and coliform count were analysed at 7-day intervals up to 21 days of storage.

Statistical Analysis: The data derived from physicochemical, rheological, microbiological, and sensory assessments of the samples were analyzed using a Factorial Completely Randomised Design (FCRD).

RESULTS AND DISCUSSION

The shelf life of *Dahi* depends on the absence of unacceptable physical, chemical, microbiological, or sensory attributes that render the product unsuitable for consumption. Maintaining these quality parameters throughout storage presents a significant challenge. It is well documented that storage induces alterations in pH and acidity, which are strongly correlated with changes in sensory characteristics and rheological behavior of fermented dairy products. This study aims to evaluate how physicochemical properties and sensory attributes of milk fermented with varied bacterial strains evolve during storage. Additionally, ensuring a high viable cell count at the end of the product's shelf life is critical for preserving its functional properties, making the survival of starter cultures during storage an essential quality criterion.

A. Sensory evaluation

High-quality dahi should exhibit an appealing taste that combines a clean acidic note with a subtle aromatic profile and must be free from any off-flavours. In well-set dahi, the upper surface ought to be smooth and glossy, and the interior, when cut, should be uniform without trapped gas bubbles. However, when stored under refrigerated conditions, various physical, chemical, microbiological, and biochemical transformations occur that alter these sensory attributes of the product.

Dahi samples were evaluated for multiple sensory characteristics, including (i) flavor, (ii) body and texture, (iii) acidity, (iv) color and appearance, and (v) overall acceptability, by a panel of trained judges. The flavour ratings of dahi produced with cultures A and C were significantly greater ($p < 0.05$). Samples fermented with EPS-producing cultures C and D exhibited a richer mouthfeel than those made with cultures A and B. Sensory scores were highest at the start of storage, but as storage progressed and acidity increased, flavour scores declined. Previous research on yoghurt has shown that flavour is influenced by storage; flavour may initially improve during cooling and early storage, likely because of the formation of specific aroma compounds, but then diminishes over time (Khurana and Kanawajia 2007; Zang *et al.,* 2025).

The sensory quality of dahi exhibited a progressive decline during refrigerated storage across all starter cultures evaluated. With respect to flavour, freshly prepared samples recorded high sensory scores on day 0, which gradually decreased by days 7, 14, and 21 of storage. Among the cultures, samples fermented with culture C consistently demonstrated marginally higher flavour scores at all storage intervals, whereas those prepared using culture D showed the most pronounced decline by the end of the storage period. Statistical analysis indicated that both storage duration and culture type significantly influenced flavour perception, with a significant interaction effect between these two factors.The results are in accordance with Behare *et al.* (2009); Angelin and Kavitha (2020); Hernández-Figueroa *et al.* (2025).

In contrast, the body and texture scores of dahi remained relatively stable throughout the storage period. Although a slight reduction was observed with advancing storage time, the differences among cultures were minimal, and all samples maintained high scores even after 21 days of refrigeration. Statistical evaluation revealed that neither storage period nor starter culture exerted a significant interactive effect on body and texture attributes. The colour and appearance of dahi showed a gradual but consistent decrease in sensory scores as storage progressed. Fresh samples exhibited superior visual appeal, which declined moderately by day 14 and further by day 21. Differences among cultures were relatively small, and although storage time significantly affected colour and appearance scores, the interaction between storage period and culture was not statistically significant.

Overall acceptability scores followed a pattern similar to that observed for flavour attributes. Fresh dahi samples received high acceptance ratings, which declined steadily during storage. Samples prepared with culture C retained comparatively higher acceptability scores across all storage intervals, whereas those fermented with culture D exhibited the lowest scores by the end of the storage period. Statistical analysis confirmed that both storage duration and culture type had a significant effect on overall acceptability, with a significant interaction between these factors.

All sensory evaluations were conducted in triplicate, and the observed trends clearly demonstrate that storage duration played a critical role in influencing the sensory quality of dahi, while the choice of starter culture contributed to variations in flavour and overall acceptability during refrigerated storage.

Colour and appearance constitute key determinants of the sensory quality of *Dahi*. Only minor changes in colour and appearance scores were noted during storage under refrigeration. Similar observations were reported by Nahar *et al.* (2009), who documented that *Dahi* remained acceptable with respect to colour and appearance for up to 21 days when stored at refrigerated temperature. The overall acceptability scores of the *Dahi* samples varied between 5.49 and 8.27, and statistically significant differences were observed among the samples ($p < 0.05$). A progressive decline in acceptability scores was evident with increasing storage duration. Among the samples, Sample A recorded the highest mean score (7.24), whereas Sample D exhibited the lowest mean score (6.88). In a related study, cow milk *Dahi* formulated with 12% total solids and fermented using three thermophilic starter cultures showed an initial overall acceptability score of 15.78 (out of 20), which decreased moderately to 13.89 after 12 days of refrigerated storage (Dave *et al.*, 1993; Sanalibaba and Çakmak 2016).

B. Chemical Changes during Storage

The physicochemical characteristics of the Dahi samples differed significantly as a function of the additives used ($p < 0.05$). Among the cultures, culture D exhibited the lowest mean pH value (3.74), whereas the highest pH (3.85) was recorded for culture A on the 21st day of storage. Evaluation of the overall pH trend during storage indicated a progressive decline, with mean pH values of 4.64 on day 1, 4.17 on day 7, 3.97 on day 14, and 3.79 on day 21. Cultures C and D demonstrated greater acidity compared to cultures A and B.

Such changes are expected, as lactic acid bacteria remain metabolically active during storage, albeit at a reduced rate, leading to continued acid production and a consequent decrease in pH. The observed reduction in pH with advancing storage period is consistent with the findings of Kondratenko *et al.* (1978), who also reported a decline in pH of Dahi during storage. Earlier investigation by Dave *et al.* (1993) documented that freshly prepared Dahi typically exhibits pH values ranging from 4.75 to 4.91, which subsequently decrease to values between 4.10 and 4.67 by the end of the storage period (12–18 days).

During refrigerated storage, dahi exhibited a progressive decline in pH across all culture treatments (A–D). On the day of preparation (day 0), the pH values were comparable among the different cultures, ranging narrowly from 4.62 ± 0.01 to 4.66 ± 0.01. By the 7th day of storage, a noticeable reduction in pH was observed, with values decreasing to 4.24 ± 0.01, 4.23 ± 0.01, 4.13 ± 0.05, and 4.08 ± 0.01 for cultures A, B, C, and D, respectively. This declining trend continued on day 14, when pH values further dropped to approximately 4.02 ± 0.01 in culture A, 4.00 ± 0.01 in culture B, 3.95 ± 0.01 in culture C, and 3.93 ± 0.02 in culture D. At the end of the storage period (day 21), the lowest pH values were recorded, varying between 3.74 ± 0.03 and 3.85 ± 0.01 among the different cultures. Statistical analysis indicated that both the storage period and culture type had a significant influence on pH, with a significant interaction effect between these factors ($P \leq 0.05$).

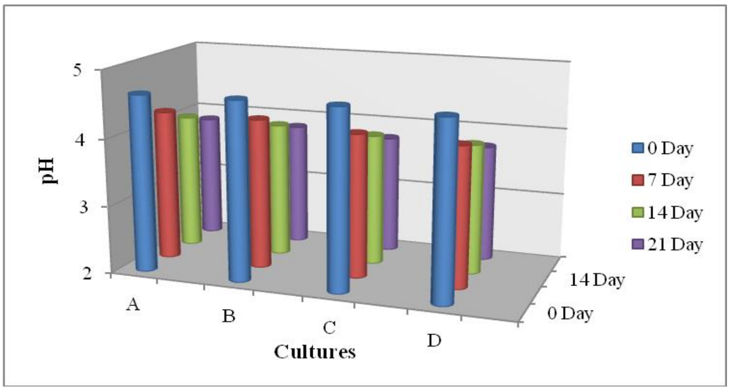

Fig. 2. Changes in pH value of Dahi samples during storage.

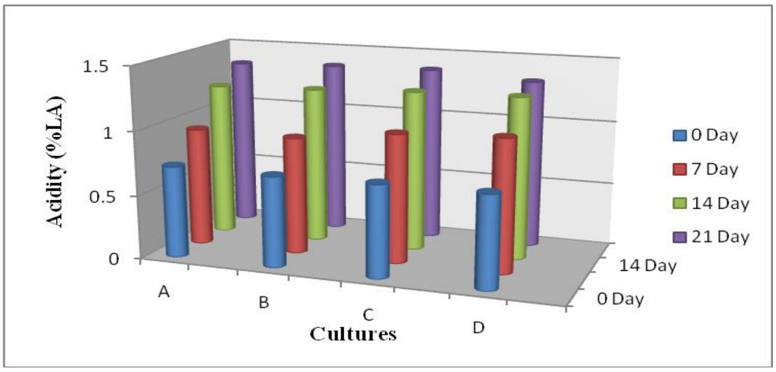

Fig. 3. Changes in acidity value of *Dahi* samples during storage.

A corresponding increase in titratable acidity, expressed as percent lactic acid, was noted throughout the storage duration for all dahi samples. Initially, acidity levels were nearly identical across cultures, measuring approximately 0.71–0.72% on day 0. After 7 days of storage, acidity increased to values ranging from 0.92 ± 0.01 to 1.04 ± 0.01, with cultures C and D showing slightly higher acidity compared to cultures A and B. By day 14, acidity levels rose further, reaching 1.21 ± 0.01, 1.23 ± 0.01, 1.26 ± 0.01, and 1.27 ± 0.01 for cultures A, B, C, and D, respectively. At the end of 21 days, acidity values attained their maximum levels, varying between 1.32 ± 0.04 and 1.37 ± 0.01 across all cultures. The effects of storage period and culture type on acidity were found to be statistically significant, and a significant interaction between these two factors was also observed ($P \leq 0.05$).

The statistically significant elevation in acidity across all cultures during storage demonstrates their continued metabolic activity and sustained acid production. This accumulation of acidity was identified as the primary factor responsible for the termination of product shelf life, which was also reflected by a decline in sensory evaluation scores. The observed mean acidity values of Dahi are consistent with earlier reports by Ghosh and Rajorhia (1987); Sarkar *et al.* (1996); Korcz and Varga (2021), who documented a steady increase in acidity with prolonged storage duration. Similarly, Cardoso *et al.* (1991) reported that the acidity of buffalo milk Dahi increased from 0.8% at day zero to 1.3% after 20 days of storage. Dave *et al.* (1993) observed that the acidity of freshly prepared Dahi ranged between 1.015 and 1.181% LA and increased gradually during refrigerated storage to values between 1.06 and 1.398% LA. He reported that the acidity of fresh Dahi varied from 0.866 to 0.952% LA, which further increased during refrigerated storage (18 days) to a range of 0.914–1.181% LA.

C. Microbiological changes (Lactic count)

The changes in lactic acid bacterial counts of dahi during refrigerated storage were evaluated across four different starter cultures over a period of 21 days. On the day of preparation (day 0), the lactic count was highest in dahi prepared with culture A (9.14 ± 0.01 log cfu/g) and culture B (9.11 ± 0.01 log cfu/g), followed by samples fermented with culture C (8.93 ± 0.02 log cfu/g) and culture D (8.91 ± 0.02 log cfu/g). After 7 days of storage, a gradual decline in viable counts was observed in all samples, with values ranging from 8.65 ± 0.02 to 8.82 ± 0.02 log cfu/g, depending on the culture used. By the 14th day, lactic counts further decreased, with dahi prepared using culture C maintaining relatively higher counts (8.58 ± 0.01 log cfu/g), while samples prepared with cultures A and B recorded slightly lower values (8.38 ± 0.01 and 8.37 ± 0.01 log cfu/g, respectively). At the end of the storage period (21 days), the lowest lactic counts were recorded in dahi prepared with culture B (7.83 ± 0.01 log cfu/g), whereas samples fermented with culture C retained comparatively higher viability (8.12 ± 0.02 log cfu/g). Statistical analysis indicated that the effects of storage period and starter culture were significant ($P \leq 0.05$), and a significant interaction between storage period and culture was also observed.

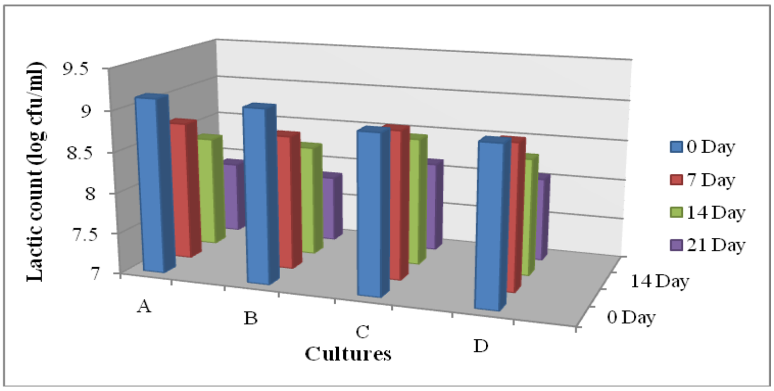

Fig. 4. Changes in lactic count of Dahi sample during storage.

Iniguez *et al.* (2001); Ramos *et al.* (2023) similarly observed that lactic acid bacteria populations in fermented milk produced from a blend of buffalo and cow milk consistently remained above 10^7 cfu/g across all samples during refrigerated storage at 4°C for a period of 10 days. Mani-López *et al*. (2014) reported a decline in *Streptococcus thermophilus* counts ranging between 1.8 and 3.5 log units over the storage duration. Nighswonger *et al.* (1996) demonstrated that the population of *Lactobacillus acidophilus* strain La-5 showed a statistically significant reduction ($P < 0.05$) after 28 days of storage in buttermilk. Throughout the 21-day storage period, coliform bacteria were not detected in any of the dahi samples. Moreover, the mean yeast and mold count across all samples during storage remained below 10 log cfu/g for the entire study duration.

CONCLUSIONS

The sensory and physicochemical characteristics of dahi are markedly influenced by the nature of the starter culture employed, particularly its acidification rate and exopolysaccharide (EPS)-producing ability. Dahi manufactured using EPS-producing starter cultures exhibited significant differences in sensory perception when compared with products prepared using non-EPS-producing cultures. Among the treatments, dahi produced with an EPS-producing starter combination of *Lactobacillus helveticus* MTCC 5463 and *Streptococcus thermophilus* MTCC 5460 achieved the highest sensory evaluation scores. Throughout storage, significant alterations were observed in the sensory and physicochemical attributes of all dahi samples; however, the trend of these changes remained largely consistent regardless of whether EPS or non-EPS starter cultures were used. Given that consumer acceptance of fermented dairy products such as dahi is predominantly governed by sensory preferences, it may be inferred that both EPS-producing and non-EPS-producing starter cultures are suitable for producing dahi with acceptable quality attributes.

FUTURE SCOPE

Future research may focus on optimizing the concentration and combinations of EPS-producing cultures to enhance textural attributes and consumer acceptability while maintaining product stability during extended storage. Detailed characterization of the type, structure, and functional properties of exopolysaccharides produced by different starter cultures could provide deeper insights into their role in improving rheological behavior and mouthfeel of dahi.

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Conflict of Interest. None.

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