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## Biopreservation of paneer using partially purified crude extract (cell-free supernatant) of lactic acid bacteria: Comparative efficacy of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Lactobacillus plantarum* during refrigerated storage

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### Abstract

The efficacy of partially purified cell-free supernatant (CFS) of two lactic acid bacteria (LAB) as natural biopreservatives for paneer was evaluated during refrigerated storage ( $4 \pm 1^\circ\text{C}$ ). Paneer was surface-treated with CFS at 5.0, 7.5 and 10.0 per cent of *Lactobacillus delbrueckii* subsp. *Bulgaricus* (T<sub>1</sub>-T<sub>3</sub>) and *Lactobacillus plantarum* (T<sub>4</sub>-T<sub>6</sub>) and compared with an untreated control (T<sub>0</sub>). Samples were analysed every three days for total plate count (TPC), pH, titratable acidity (TA) and sensory quality; coliforms and yeast and mould were absent throughout. In the control and lower doses, TPC rose from about 5.48 to over 6.18 log<sub>10</sub> cfu g<sup>-1</sup> and sensory scores fell below the limit of acceptability by the ninth day. Only the 10.0 per cent treatments (T<sub>3</sub> and T<sub>6</sub>) remained acceptable up to the twelfth day, extending shelf life by about three days, recording significantly lower TPC (5.941 log<sub>10</sub> cfu g<sup>-1</sup>), higher pH (4.85-4.88) and lower TA ( $\approx 0.58$  per cent lactic acid) than the control (6.216 log<sub>10</sub> cfu g<sup>-1</sup>, pH 4.67, 1.157 per cent lactic acid) on day 9. The two organisms behaved comparably, *L. Bulgaricus* giving marginally lower counts and *L. plantarum* marginally better late-storage flavour and overall acceptability. Thus, partially purified LAB CFS, particularly at 10.0 per cent, is an effective clean-label biopreservative for paneer.

**Keywords:** Paneer, biopreservation, cell-free supernatant, partially purified crude extract, *Lactobacillus plantarum*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, refrigerated storage, shelf life

### 1. Introduction

Paneer is a popular heat- and acid-coagulated indigenous dairy product of the Indian subcontinent, valued for its bland flavour, firm body, close-knit texture and high nutritive value as a rich source of milk fat and protein. However, its high moisture content (roughly 50-55 per cent), near-neutral pH and rich nutrient profile make it highly perishable and an ideal substrate for microbial growth. Even under refrigeration its keeping quality is generally limited to about six to nine days, after which surface sliminess, off-flavour, discoloration and acid development render it unacceptable (Kumar *et al.*, 2016).

Conventional shelf-life extension methods for paneer, such as chemical preservatives, modified-atmosphere and vacuum packaging, hurdle technology, antimicrobial packaging and thermal or non-thermal processing, either raise consumer and regulatory concerns or demand substantial investment. This has driven interest in “clean-label” biological preservation that relies on natural antimicrobial systems and leaves no synthetic residues.

Biopreservation, the use of microorganisms or their metabolites to control spoilage and pathogens, is a promising and consumer-friendly alternative. Lactic acid bacteria (LAB) are especially attractive owing to their safe history in fermented foods and their production of diverse antimicrobial metabolites such as organic acids, hydrogen peroxide, diacetyl, carbon dioxide and, importantly, ribosomally synthesised peptides known as bacteriocins (Silva *et al.*, 2018; Garin-Murguialday *et al.*, 2024) [9, 11, 8]. Rather than adding live cultures, the cell-free supernatant (CFS) containing these secreted metabolites can be partially purified and applied

directly to a food surface, delivering antimicrobial activity without altering the product's own fermentation (Bang *et al.*, 2020; Wang *et al.*, 2023).

Both *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Lactobacillus plantarum* (now *Lactiplantibacillus plantarum*) are well-documented producers of antimicrobial compounds effective against food-borne spoilage and pathogenic bacteria (Evivie *et al.*, 2020; Barcenilla *et al.*, 2022; Putri *et al.*, 2024) [3, 12, 6, 4, 10]. Although their CFS has been studied in meat, seafood and produce, little is known about its application to fresh acid-coagulated cheeses such as paneer, or how the two organisms compare under identical conditions. The present study therefore evaluated partially purified CFS of *L. Bulgaricus* and *L. plantarum*, each at 5.0, 7.5 and 10.0 per cent, on the microbiological, physico-chemical and sensory quality of paneer during refrigerated storage, and compared their relative biopreservative efficacy.

## 2. Materials and Methods

### 2.1. Production and partial purification of the crude extract

Pure cultures of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Lactobacillus plantarum* were individually propagated in de Man, Rogosa and Sharpe (MRS) broth and incubated at 37 °C for 24-48 h. The fermented broth was centrifuged (10,000 × g, 15 min, 4 °C) to sediment the cells, and the resulting supernatant was recovered as the crude cell-free supernatant (CFS). The CFS was filter-sterilised (0.22 µm), then pH of CFS was adjusted to 6.5 using NaOH. Then CFS was partially purified by ammonium-sulphate precipitation of the antimicrobial fraction followed by dialysis prior to application.

### 2.2. Preparation and treatment of paneer

Paneer was manufactured from standardised buffalo milk by the conventional heat-acid coagulation method, pressed, and cut into uniform blocks. The treatments were assigned as follows: T<sub>0</sub>, untreated control; T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, paneer treated with 5.0, 7.5 and 10.0 per cent partially purified CFS of *L. Bulgaricus*, respectively; and T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub>, paneer treated with 5.0, 7.5 and 10.0 per cent partially purified CFS of *L. plantarum*, respectively. The extract was applied uniformly to the paneer surface using parchment paper coating, and the treated blocks were packaged and stored under refrigeration at 4 ± 1 °C. Samples were drawn at 0, 3, 6, 9 and 12 days for analysis.

### 2.3. Microbiological analysis

Total plate count (TPC), coliform count and yeast and mould count were determined by the standard pour-plate technique

using the appropriate selective and non-selective media, with incubation under the conditions prescribed for each group (FSSAI, 2012). Microbial counts were expressed as log<sub>10</sub> colony-forming units per gram (log<sub>10</sub> cfu g<sup>-1</sup>).

### 2.4. Physico-chemical analysis

The pH of paneer was recorded using a calibrated digital pH meter on an aqueous slurry of the sample. Titratable acidity (TA) was estimated by titration against standard alkali and expressed as per cent lactic acid, according to the official methods of analysis (AOAC, 2016) [1].

### 2.5. Sensory evaluation

Sensory quality was assessed by a trained panel using a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely) for appearance and colour, body and texture, flavour, aftertaste and overall acceptability. A mean score of about 6.0 was taken as the lower limit of acceptability.

### 2.6. Statistical analysis

The experiment was conducted in triplicate and data are presented as mean ± standard error. Data were subjected to analysis of variance (ANOVA) and treatment means were compared using Duncan's Multiple Range Test (DMRT) at *p* < 0.05. In the tables, lowercase superscripts denote differences among treatments within a storage interval and uppercase superscripts denote differences across storage intervals within a treatment.

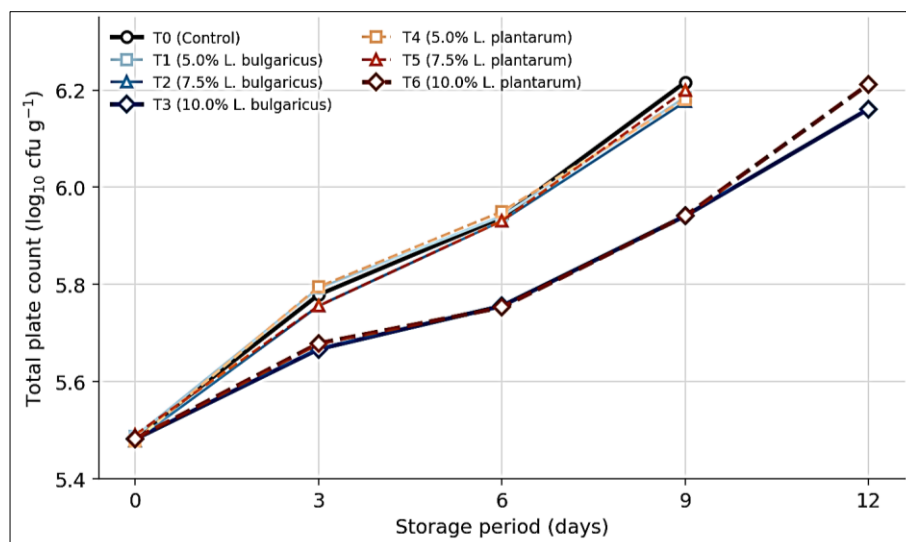
## 3. Results

### 3.1. Total plate count

The changes in total plate count during storage are presented in Table 1 and Fig. 1. The initial TPC on day 0 did not differ significantly among treatments (*F* = 0.1, NS), ranging narrowly from 5.479 to 5.491 log<sub>10</sub> cfu g<sup>-1</sup>, confirming a comparable starting microbial load. Thereafter the count increased in all samples, but the rate of increase was distinctly slower in the 10.0 per cent treatments. In the control (T<sub>0</sub>) the count rose steadily to 6.216 log<sub>10</sub> cfu g<sup>-1</sup> by day 9, beyond which the product was unacceptable. The 5.0 and 7.5 per cent treatments of both organisms (T<sub>1</sub>, T<sub>2</sub>, T<sub>4</sub>, T<sub>5</sub>) behaved similarly to the control, reaching 6.176-6.199 log<sub>10</sub> cfu g<sup>-1</sup> on day 9. Only the 10.0 per cent treatments T<sub>3</sub> (*L. Bulgaricus*) and T<sub>6</sub> (*L. plantarum*) suppressed microbial growth sufficiently to remain within acceptable limits up to day 12, recording 5.941 log<sub>10</sub> cfu g<sup>-1</sup> on day 9 (significantly lower than all other treatments) and 6.161 and 6.212 log<sub>10</sub> cfu g<sup>-1</sup>, respectively, on day 12. The highly significant treatment *F*-values from day 6 onward (251.6 and 60.6 for the two blocks) confirm the dose-dependent antibacterial effect of the extract.

**Table 1:** Effect of biopreservatives on the total plate count (log<sub>10</sub> cfu g<sup>-1</sup>) of paneer during refrigerated storage

| Treatment      | Total plate count (log <sub>10</sub> cfu g <sup>-1</sup> ) |                           |                           |                           |                          | F value   |
|----------------|------------------------------------------------------------|---------------------------|---------------------------|---------------------------|--------------------------|-----------|
|                | Storage days                                               |                           |                           |                           |                          |           |
|                | 0 <sup>th</sup> day                                        | 3 <sup>rd</sup> day       | 6 <sup>th</sup> day       | 9 <sup>th</sup> day       | 12 <sup>th</sup> day     |           |
| T <sub>0</sub> | 5.485±0.017 <sup>aD</sup>                                  | 5.779±0.015 <sup>aC</sup> | 5.938±0.004 <sup>aB</sup> | 6.216±0.013 <sup>aA</sup> | -                        | 533.9***  |
| T <sub>1</sub> | 5.488±0.015 <sup>aD</sup>                                  | 5.792±0.011 <sup>aC</sup> | 5.941±0.005 <sup>aB</sup> | 6.185±0.006 <sup>bA</sup> | -                        | 835.1***  |
| T <sub>2</sub> | 5.479±0.009 <sup>aD</sup>                                  | 5.756±0.008 <sup>aC</sup> | 5.931±0.005 <sup>aB</sup> | 6.176±0.007 <sup>bA</sup> | -                        | 1573.7*** |
| T <sub>3</sub> | 5.482±0.012 <sup>aE</sup>                                  | 5.667±0.010 <sup>bD</sup> | 5.756±0.008 <sup>bC</sup> | 5.941±0.005 <sup>cB</sup> | 6.161±0.011 <sup>A</sup> | 746.0***  |
| <i>F</i> value | 0.1NS                                                      | 24.9***                   | 251.6***                  | 230.6***                  | -                        |           |
| T <sub>0</sub> | 5.485±0.017 <sup>aD</sup>                                  | 5.779±0.015 <sup>aC</sup> | 5.938±0.004 <sup>aB</sup> | 6.216±0.013 <sup>aA</sup> | -                        | 533.9***  |
| T <sub>4</sub> | 5.479±0.017 <sup>aD</sup>                                  | 5.795±0.010 <sup>aC</sup> | 5.950±0.003 <sup>aB</sup> | 6.181±0.005 <sup>bA</sup> | -                        | 820.2***  |
| T <sub>5</sub> | 5.491±0.017 <sup>aD</sup>                                  | 5.756±0.008 <sup>bC</sup> | 5.931±0.005 <sup>aB</sup> | 6.199±0.011 <sup>aA</sup> | -                        | 710.6***  |
| T <sub>6</sub> | 5.483±0.017 <sup>aE</sup>                                  | 5.679±0.006 <sup>cD</sup> | 5.753±0.023 <sup>bC</sup> | 5.941±0.005 <sup>cB</sup> | 6.212±0.016 <sup>A</sup> | 337.0***  |
| <i>F</i> value | 0.1NS                                                      | 24.9***                   | 60.6***                   | 197.7***                  | -                        |           |



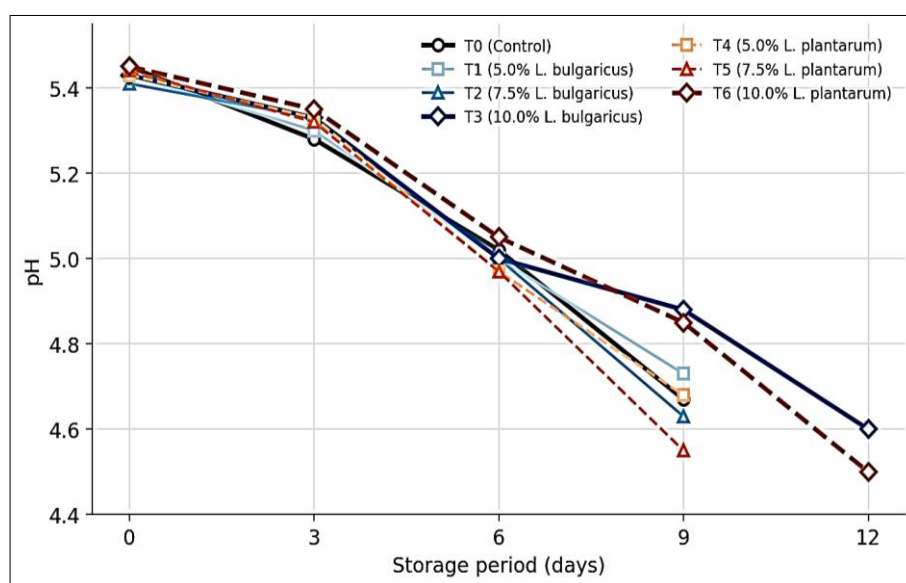
**Fig 1:** Total plate count ( $\log_{10}$  cfu  $\text{g}^{-1}$ ) of paneer for treatments T0-T6 over 12 days of refrigerated storage.

**3.2. pH:** The pH of paneer declined progressively in all treatments during storage as a consequence of microbial acid production (Table 2, Fig. 2). Initial pH values were statistically similar (5.41-5.45;  $F = 0.1-0.2$ , NS). The decline was steepest in the control and lower-dose treatments, which fell to 4.55-4.73 by day 9. The 10.0 per cent treatments

retained a significantly higher pH on day 9 (4.88 for T<sub>3</sub> and 4.85 for T<sub>6</sub>), reflecting slower acidification and delayed spoilage, and continued to be measurable on day 12 (4.60 for T<sub>3</sub> and 4.50 for T<sub>6</sub>). The treatment effect became significant only from day 9 ( $F = 11.4$  and  $6.8$ ), underlining that the extract chiefly retarded the later, spoilage-driven fall in pH.

**Table 2.** Effect of biopreservatives on the pH of paneer during refrigerated storage.

| Treatment      | pH                       |                          |                          |                          |                         | F value  |
|----------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|----------|
|                | Storage days             |                          |                          |                          |                         |          |
|                | 0 <sup>th</sup> day      | 3 <sup>rd</sup> day      | 6 <sup>th</sup> day      | 9 <sup>th</sup> day      | 12 <sup>th</sup> day    |          |
| T <sub>0</sub> | 5.45±0.034 <sup>aA</sup> | 5.28±0.031 <sup>aB</sup> | 5.02±0.040 <sup>aC</sup> | 4.67±0.042 <sup>bD</sup> | -                       | 84.2***  |
| T <sub>1</sub> | 5.43±0.032 <sup>aA</sup> | 5.30±0.026 <sup>aB</sup> | 5.00±0.045 <sup>aC</sup> | 4.73±0.033 <sup>bD</sup> | -                       | 81.7***  |
| T <sub>2</sub> | 5.41±0.031 <sup>aA</sup> | 5.33±0.021 <sup>aA</sup> | 5.00±0.045 <sup>aB</sup> | 4.63±0.033 <sup>cC</sup> | -                       | 112.1*** |
| T <sub>3</sub> | 5.43±0.036 <sup>aA</sup> | 5.33±0.021 <sup>aB</sup> | 5.00±0.045 <sup>aC</sup> | 4.88±0.017 <sup>aD</sup> | 4.60±0.037 <sup>E</sup> | 105.3*** |
| <i>F</i> value | 0.2NS                    | 1.0NS                    | 0.1NS                    | 11.4***                  | -                       |          |
| T <sub>0</sub> | 5.45±0.034 <sup>aA</sup> | 5.28±0.031 <sup>aB</sup> | 5.02±0.040 <sup>aC</sup> | 4.67±0.042 <sup>bD</sup> | -                       | 84.2***  |
| T <sub>4</sub> | 5.43±0.032 <sup>aA</sup> | 5.33±0.021 <sup>aA</sup> | 4.97±0.033 <sup>aB</sup> | 4.68±0.060 <sup>bC</sup> | -                       | 76.9***  |
| T <sub>5</sub> | 5.44±0.033 <sup>aA</sup> | 5.32±0.017 <sup>aB</sup> | 4.97±0.042 <sup>aC</sup> | 4.55±0.050 <sup>bD</sup> | -                       | 113.4*** |
| T <sub>6</sub> | 5.45±0.033 <sup>aA</sup> | 5.35±0.034 <sup>aA</sup> | 5.05±0.050 <sup>aB</sup> | 4.85±0.034 <sup>aC</sup> | 4.50±0.037 <sup>D</sup> | 101.8*** |
| <i>F</i> value | 0.1NS                    | 1.2NS                    | 0.9NS                    | 6.8**                    | -                       |          |



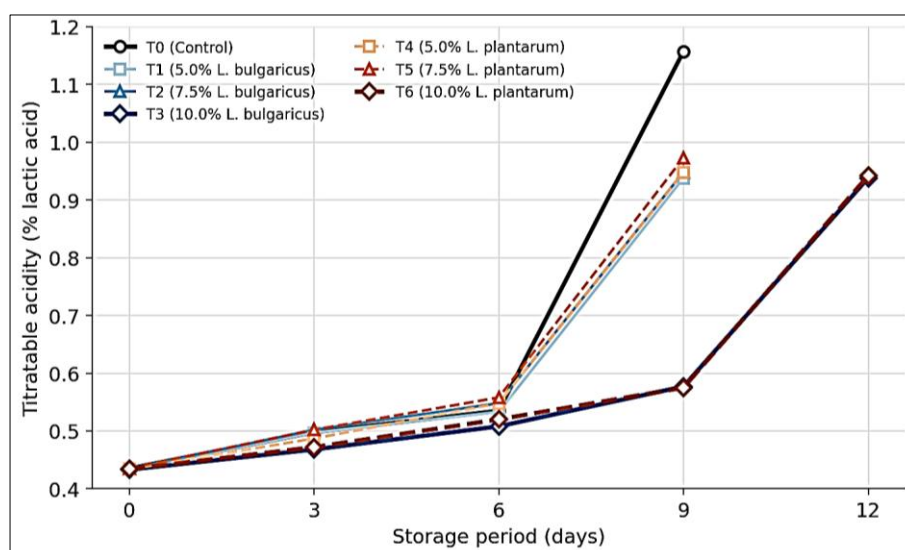
**Fig 2:** Change in pH of paneer for treatments T0-T6 during refrigerated storage.

**3.3. Titratable acidity:** Titratable acidity, expressed as per cent lactic acid, increased throughout storage in a manner mirroring the pH decline (Table 3, Fig. 3). The most striking finding was the sharp rise in acidity of the control on day 9 to 1.157 per cent lactic acid, a clear index of advanced spoilage, whereas the 10.0 per cent treatments held acidity to only 0.577 (T<sub>3</sub>) and 0.575 (T<sub>6</sub>) per cent on the same day. The 5.0 and 7.5 per cent treatments were intermediate (0.938-0.973

per cent on day 9). On day 12, T<sub>3</sub> and T<sub>6</sub> reached 0.938 and 0.942 per cent lactic acid, values comparable to those the control had already attained three days earlier. The very high treatment F-values on day 9 (634.8 and 336.8) confirm that the 10.0 per cent extract strongly suppressed acid development, consistent with its inhibition of the acid-producing spoilage microflora.

**Table 3:** Effect of biopreservatives on the titratable acidity (% lactic acid) of paneer during refrigerated storage.

| Treatment      | Titratable Acidity (%)     |                            |                            |                            |                           | F value   |
|----------------|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|-----------|
|                | Storage days               |                            |                            |                            |                           |           |
|                | 0 <sup>th</sup> day        | 3 <sup>rd</sup> day        | 6 <sup>th</sup> day        | 9 <sup>th</sup> day        | 12 <sup>th</sup> day      |           |
| T <sub>0</sub> | 0.435±0.0085 <sup>aD</sup> | 0.498±0.0060 <sup>aC</sup> | 0.535±0.0161 <sup>aB</sup> | 1.157±0.0105 <sup>aA</sup> | -                         | 947.4***  |
| T <sub>1</sub> | 0.434±0.0081 <sup>aD</sup> | 0.497±0.0061 <sup>aC</sup> | 0.533±0.0120 <sup>aB</sup> | 0.938±0.0101 <sup>bA</sup> | -                         | 599.7***  |
| T <sub>2</sub> | 0.434±0.0080 <sup>aD</sup> | 0.502±0.0054 <sup>aC</sup> | 0.548±0.0098 <sup>aB</sup> | 0.948±0.0122 <sup>bA</sup> | -                         | 633.9***  |
| T <sub>3</sub> | 0.433±0.0082 <sup>aE</sup> | 0.468±0.0060 <sup>bD</sup> | 0.508±0.0031 <sup>bC</sup> | 0.577±0.0021 <sup>cB</sup> | 0.938±0.0065 <sup>A</sup> | 1311.8*** |
| <i>F</i> value | 0.0 <sup>NS</sup>          | 7.1**                      | 2.2NS                      | 634.8***                   | -                         |           |
| T <sub>0</sub> | 0.435±0.0085 <sup>aD</sup> | 0.498±0.0060 <sup>aC</sup> | 0.535±0.0161 <sup>aB</sup> | 1.157±0.0105 <sup>aA</sup> | -                         | 947.4***  |
| T <sub>4</sub> | 0.434±0.0082 <sup>aD</sup> | 0.487±0.0021 <sup>bC</sup> | 0.548±0.0098 <sup>aB</sup> | 0.948±0.0070 <sup>bA</sup> | -                         | 1009.5*** |
| T <sub>5</sub> | 0.435±0.0083 <sup>aD</sup> | 0.502±0.0054 <sup>aC</sup> | 0.558±0.0091 <sup>aB</sup> | 0.973±0.0233 <sup>bA</sup> | -                         | 325.3***  |
| T <sub>6</sub> | 0.434±0.0081 <sup>aE</sup> | 0.472±0.0048 <sup>cD</sup> | 0.520±0.0052 <sup>bC</sup> | 0.575±0.0022 <sup>cB</sup> | 0.942±0.0048 <sup>A</sup> | 1456.4*** |
| <i>F</i> value | 0.0 <sup>NS</sup>          | 7.8**                      | 2.3NS                      | 336.8***                   | -                         |           |



**Fig 3:** Development of titratable acidity (% lactic acid) in paneer for treatments

### 3.4. Sensory quality

The sensory attributes like appearance and colour, body and texture, flavour, aftertaste and overall acceptability, all declined with storage, but the rate of deterioration was markedly slower in the 10.0 per cent treatments (Tables 4-8, Fig. 4). For every attribute, the control and the lower-dose treatments fell to about 5.8-6.3 by day 9, at or below the limit of acceptability, and could not be evaluated on day 12. In contrast, T<sub>3</sub> and T<sub>6</sub> retained scores of roughly 7.1-7.75 on day 9 and remained acceptable ( $\approx$  6.05-6.35) up to day 12. Appearance and colour (Table 4) were significantly better preserved by the 10.0 per cent treatments, with T<sub>3</sub> scoring 7.65 and T<sub>6</sub> scoring 7.50 on day 9 against 6.20-6.25 for the

control. Body and texture (Table 5) followed the same pattern, T<sub>3</sub> and T<sub>6</sub> scoring 7.10 and 7.70 respectively on day 9. Flavour (Table 6), the attribute most sensitive to spoilage showed the clearest separation: the control and lower doses dropped to about 5.80-6.00 by day 9, while T<sub>3</sub> and T<sub>6</sub> retained 7.60 and 7.75. Aftertaste (Table 7) and overall acceptability (Table 8) reinforced these trends, with T<sub>6</sub> (*L. plantarum*, 10.0 per cent) recording the highest late-storage overall acceptability (7.62 on day 9 and 6.18 on day 12) and T<sub>3</sub> (*L. Bulgaricus*, 10.0 per cent) closely matching it (7.36 and 6.20). The highly significant treatment F-values from day 6 onward (up to 280 for appearance) confirm that the biopreservative markedly slowed sensory deterioration.

**Table 4:** Effect of biopreservatives on the sensory appearance and colour of paneer during refrigerated storage.

| Treatment      | Sensory attributes - Appearance and colour |                         |                         |                         |                      | F value    |
|----------------|--------------------------------------------|-------------------------|-------------------------|-------------------------|----------------------|------------|
|                | Storage days                               |                         |                         |                         |                      |            |
|                | 0 <sup>th</sup> day                        | 3 <sup>rd</sup> day     | 6 <sup>th</sup> day     | 9 <sup>th</sup> day     | 12 <sup>th</sup> day |            |
| T <sub>0</sub> | 8.80±0.03 <sup>aA</sup>                    | 8.10±0.06 <sup>bB</sup> | 7.35±0.05 <sup>cB</sup> | 6.25±0.08 <sup>dB</sup> | -                    | 1066.42*** |
| T <sub>1</sub> | 8.85±0.05 <sup>aA</sup>                    | 8.10±0.04 <sup>bB</sup> | 7.33±0.07 <sup>cB</sup> | 6.25±0.06 <sup>dB</sup> | -                    | 1175.77*** |



|                |                         |                         |                         |                         |                        |           |
|----------------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------|-----------|
| T <sub>2</sub> | 8.75±0.04 <sup>aA</sup> | 8.05±0.06 <sup>bB</sup> | 7.32±0.05 <sup>cB</sup> | 6.22±0.08 <sup>dB</sup> | -                      | 994.87*** |
| T <sub>3</sub> | 8.80±0.06 <sup>aA</sup> | 8.40±0.05 <sup>bA</sup> | 8.00±0.08 <sup>cA</sup> | 7.65±0.07 <sup>dA</sup> | 6.30±0.09 <sup>c</sup> | 539.41*** |
| <i>F value</i> | 2.33NS                  | 27.21***                | 81.91***                | 280.13***               | -                      |           |
| T <sub>0</sub> | 8.70±0.08 <sup>aA</sup> | 7.95±0.04 <sup>bB</sup> | 7.40±0.05 <sup>cB</sup> | 6.20±0.03 <sup>dB</sup> | -                      | 389.11*** |
| T <sub>4</sub> | 8.95±0.06 <sup>aA</sup> | 8.20±0.09 <sup>bA</sup> | 7.30±0.05 <sup>cB</sup> | 5.95±0.09 <sup>dB</sup> | -                      | 298.65*** |
| T <sub>5</sub> | 8.60±0.07 <sup>aB</sup> | 8.20±0.09 <sup>bA</sup> | 7.20±0.04 <sup>cC</sup> | 6.15±0.09 <sup>dB</sup> | -                      | 211.86*** |
| T <sub>6</sub> | 8.85±0.05 <sup>aA</sup> | 8.25±0.06 <sup>bA</sup> | 8.05±0.05 <sup>bA</sup> | 7.50±0.08 <sup>cA</sup> | 6.15±0.07 <sup>d</sup> | 262.06*** |
| <i>F value</i> | 5.56**                  | 3.43*                   | 64.74***                | 85.39***                | -                      |           |

**Table 5:** Effect of biopreservatives on the sensory body and texture of paneer during refrigerated storage.

| Treatment      | Sensory attributes - Body and Texture |                         |                         |                         |                        | F value    |
|----------------|---------------------------------------|-------------------------|-------------------------|-------------------------|------------------------|------------|
|                | Storage days                          |                         |                         |                         |                        |            |
|                | 0 <sup>th</sup> day                   | 3 <sup>rd</sup> day     | 6 <sup>th</sup> day     | 9 <sup>th</sup> day     | 12 <sup>th</sup> day   |            |
| T <sub>0</sub> | 8.70±0.05 <sup>aA</sup>               | 8.20±0.04 <sup>bA</sup> | 7.39±0.07 <sup>cA</sup> | 6.29±0.06 <sup>dB</sup> | -                      | 1054.63*** |
| T <sub>1</sub> | 8.70±0.04 <sup>aA</sup>               | 8.10±0.06 <sup>bA</sup> | 7.30±0.05 <sup>cA</sup> | 6.20±0.08 <sup>dB</sup> | -                      | 995.04***  |
| T <sub>2</sub> | 8.65±0.06 <sup>aA</sup>               | 7.15±0.05 <sup>cB</sup> | 7.31±0.07 <sup>bA</sup> | 6.21±0.06 <sup>dB</sup> | -                      | 830.03***  |
| T <sub>3</sub> | 8.75±0.04 <sup>aA</sup>               | 8.15±0.07 <sup>bA</sup> | 7.30±0.05 <sup>cA</sup> | 7.10±0.09 <sup>dA</sup> | 6.25±0.06 <sup>c</sup> | 678.80***  |
| <i>F value</i> | 2.15NS                                | 239.68***               | 1.54NS                  | 104.74***               | -                      |            |
| T <sub>0</sub> | 8.65±0.05 <sup>aB</sup>               | 7.90±0.08 <sup>bB</sup> | 7.20±0.07 <sup>cB</sup> | 6.15±0.06 <sup>dB</sup> | -                      | 259.96***  |
| T <sub>4</sub> | 8.80±0.08 <sup>aA</sup>               | 7.85±0.08 <sup>bB</sup> | 7.20±0.07 <sup>cB</sup> | 5.95±0.05 <sup>dB</sup> | -                      | 283.50***  |
| T <sub>5</sub> | 8.50±0.05 <sup>aB</sup>               | 8.00±0.07 <sup>bB</sup> | 7.35±0.03 <sup>cB</sup> | 6.15±0.04 <sup>dB</sup> | -                      | 416.84***  |
| T <sub>6</sub> | 8.90±0.03 <sup>aA</sup>               | 8.30±0.05 <sup>bA</sup> | 8.00±0.05 <sup>cA</sup> | 7.70±0.09 <sup>dA</sup> | 6.35±0.05 <sup>c</sup> | 272.73***  |
| <i>F value</i> | 9.96***                               | 8.04**                  | 44.13***                | 167.67***               | -                      |            |

**Table 6:** Effect of biopreservatives on the sensory flavour of paneer during refrigerated storage.

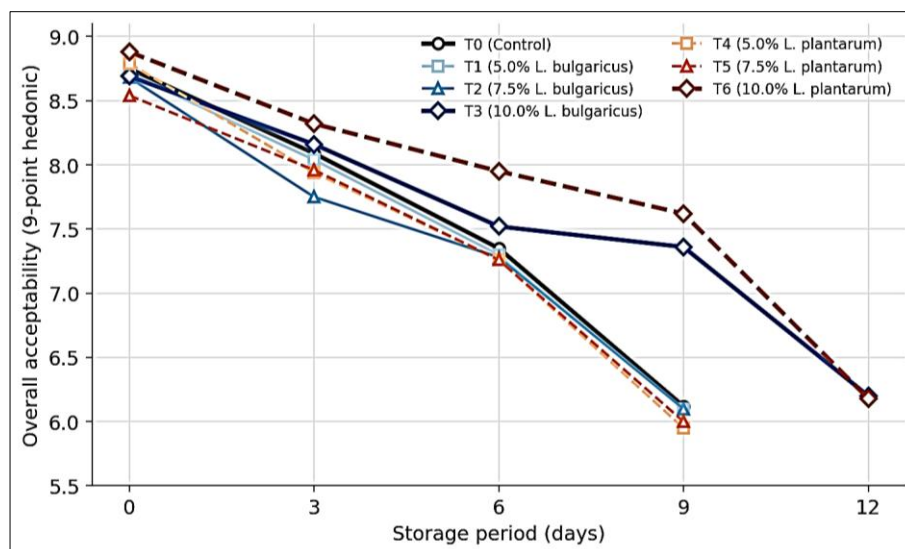
| Treatment      | Sensory attributes - Flavour |                         |                         |                         |                        | F value    |
|----------------|------------------------------|-------------------------|-------------------------|-------------------------|------------------------|------------|
|                | Storage days                 |                         |                         |                         |                        |            |
|                | 0 <sup>th</sup> day          | 3 <sup>rd</sup> day     | 6 <sup>th</sup> day     | 9 <sup>th</sup> day     | 12 <sup>th</sup> day   |            |
| T <sub>0</sub> | 8.75±0.04 <sup>aA</sup>      | 8.05±0.06 <sup>bA</sup> | 7.30±0.05 <sup>cA</sup> | 5.95±0.09 <sup>dB</sup> | -                      | 1090.35*** |
| T <sub>1</sub> | 8.65±0.06 <sup>aA</sup>      | 8.01±0.05 <sup>bA</sup> | 7.30±0.08 <sup>cA</sup> | 5.95±0.07 <sup>dB</sup> | -                      | 924.84***  |
| T <sub>2</sub> | 8.75±0.03 <sup>aA</sup>      | 7.95±0.07 <sup>bA</sup> | 7.30±0.06 <sup>cA</sup> | 5.95±0.09 <sup>dB</sup> | -                      | 961.57***  |
| T <sub>3</sub> | 8.69±0.05 <sup>aA</sup>      | 8.00±0.04 <sup>bA</sup> | 7.31±0.07 <sup>dA</sup> | 7.60±0.06 <sup>cA</sup> | 6.15±0.08 <sup>c</sup> | 695.17***  |
| <i>F value</i> | 3.35NS                       | 1.61NS                  | 0.02NS                  | 330.67***               | -                      |            |
| T <sub>0</sub> | 8.90±0.05 <sup>aA</sup>      | 7.95±0.09 <sup>bB</sup> | 7.35±0.04 <sup>cB</sup> | 5.80±0.03 <sup>dB</sup> | -                      | 516.54***  |
| T <sub>4</sub> | 8.65±0.09 <sup>aA</sup>      | 7.85±0.06 <sup>bB</sup> | 7.35±0.09 <sup>cB</sup> | 6.00±0.07 <sup>dB</sup> | -                      | 200.37***  |
| T <sub>5</sub> | 8.55±0.08 <sup>aB</sup>      | 7.90±0.06 <sup>bB</sup> | 7.35±0.08 <sup>cB</sup> | 5.80±0.06 <sup>dB</sup> | -                      | 275.67***  |
| T <sub>6</sub> | 8.85±0.04 <sup>aA</sup>      | 8.35±0.05 <sup>bA</sup> | 8.05±0.06 <sup>cA</sup> | 7.75±0.08 <sup>dA</sup> | 6.05±0.06 <sup>c</sup> | 320.06***  |
| <i>F value</i> | 5.87**                       | 11.75***                | 24.87***                | 226.74***               | -                      |            |

**Table 7:** Effect of biopreservatives on the sensory aftertaste of paneer during refrigerated storage.

| Treatment      | Sensory attributes - After taste |                         |                         |                         |                        | F value   |
|----------------|----------------------------------|-------------------------|-------------------------|-------------------------|------------------------|-----------|
|                | Storage days                     |                         |                         |                         |                        |           |
|                | 0 <sup>th</sup> day              | 3 <sup>rd</sup> day     | 6 <sup>th</sup> day     | 9 <sup>th</sup> day     | 12 <sup>th</sup> day   |           |
| T <sub>0</sub> | 8.75±0.06 <sup>aA</sup>          | 8.00±0.05 <sup>bA</sup> | 7.35±0.08 <sup>cA</sup> | 6.00±0.07 <sup>dB</sup> | -                      | 938.51*** |
| T <sub>1</sub> | 8.65±0.03 <sup>aA</sup>          | 7.95±0.07 <sup>bB</sup> | 7.25±0.06 <sup>cB</sup> | 6.00±0.09 <sup>dB</sup> | -                      | 875.86*** |
| T <sub>2</sub> | 8.55±0.05 <sup>aB</sup>          | 7.85±0.04 <sup>bB</sup> | 7.21±0.08 <sup>cB</sup> | 6.00±0.07 <sup>dB</sup> | -                      | 914.56*** |
| T <sub>3</sub> | 8.51±0.03 <sup>aB</sup>          | 8.10±0.06 <sup>bA</sup> | 7.45±0.05 <sup>cA</sup> | 7.10±0.08 <sup>dA</sup> | 6.10±0.07 <sup>e</sup> | 715.39*** |
| <i>F value</i> | 17.57***                         | 10.32**                 | 7.34*                   | 149.38***               | -                      |           |
| T <sub>0</sub> | 8.70±0.06 <sup>aA</sup>          | 8.05±0.03 <sup>bB</sup> | 7.20±0.06 <sup>cB</sup> | 5.90±0.03 <sup>dB</sup> | -                      | 649.91*** |
| T <sub>4</sub> | 8.75±0.08 <sup>aA</sup>          | 7.85±0.05 <sup>bC</sup> | 7.20±0.06 <sup>cB</sup> | 5.90±0.07 <sup>dB</sup> | -                      | 330.46*** |
| T <sub>5</sub> | 8.50±0.07 <sup>aB</sup>          | 7.75±0.06 <sup>bC</sup> | 7.15±0.07 <sup>cB</sup> | 5.90±0.06 <sup>dB</sup> | -                      | 284.12*** |
| T <sub>6</sub> | 8.90±0.06 <sup>aA</sup>          | 8.40±0.05 <sup>bA</sup> | 7.70±0.09 <sup>cA</sup> | 7.55±0.03 <sup>cA</sup> | 6.15±0.08 <sup>d</sup> | 252.73*** |
| <i>F value</i> | 5.90**                           | 34.65***                | 13.33***                | 264.32***               | -                      |           |

**Table 8:** Effect of biopreservatives on the overall acceptability of paneer during refrigerated storage.

| Treatment      | Sensory attributes - Overall Acceptability |                         |                         |                         |                        | F value    |
|----------------|--------------------------------------------|-------------------------|-------------------------|-------------------------|------------------------|------------|
|                | Storage days                               |                         |                         |                         |                        |            |
|                | 0 <sup>th</sup> day                        | 3 <sup>rd</sup> day     | 6 <sup>th</sup> day     | 9 <sup>th</sup> day     | 12 <sup>th</sup> day   |            |
| T <sub>0</sub> | 8.75±0.04 <sup>aA</sup>                    | 8.09±0.05 <sup>bA</sup> | 7.35±0.06 <sup>cB</sup> | 6.12±0.08 <sup>dB</sup> | -                      | 1081.84*** |
| T <sub>1</sub> | 8.71±0.04 <sup>aA</sup>                    | 8.04±0.06 <sup>bA</sup> | 7.30±0.06 <sup>cB</sup> | 6.10±0.08 <sup>dB</sup> | -                      | 986.86***  |
| T <sub>2</sub> | 8.68±0.04 <sup>aA</sup>                    | 7.75±0.06 <sup>bB</sup> | 7.28±0.06 <sup>cB</sup> | 6.10±0.08 <sup>dB</sup> | -                      | 909.02***  |
| T <sub>3</sub> | 8.69±0.05 <sup>aA</sup>                    | 8.16±0.06 <sup>bA</sup> | 7.52±0.06 <sup>cA</sup> | 7.36±0.08 <sup>cA</sup> | 6.20±0.08 <sup>d</sup> | 587.45***  |
| <i>F value</i> | 1.58NS                                     | 29.29***                | 9.91**                  | 184.13***               | -                      |            |
| T <sub>0</sub> | 8.74±0.06 <sup>aA</sup>                    | 7.96±0.06 <sup>bB</sup> | 7.29±0.06 <sup>cB</sup> | 6.01±0.04 <sup>dB</sup> | -                      | 431.55***  |
| T <sub>4</sub> | 8.79±0.08 <sup>aA</sup>                    | 7.94±0.07 <sup>bB</sup> | 7.26±0.07 <sup>cB</sup> | 5.95±0.07 <sup>dB</sup> | -                      | 272.79***  |
| T <sub>5</sub> | 8.54±0.07 <sup>aB</sup>                    | 7.96±0.07 <sup>bB</sup> | 7.26±0.06 <sup>cB</sup> | 6.00±0.06 <sup>dB</sup> | -                      | 281.29***  |
| T <sub>6</sub> | 8.88±0.04 <sup>aA</sup>                    | 8.32±0.05 <sup>bA</sup> | 7.95±0.06 <sup>cA</sup> | 7.62±0.07 <sup>dA</sup> | 6.18±0.06 <sup>c</sup> | 317.56***  |
| <i>F value</i> | 5.02**                                     | 8.48***                 | 29.50***                | 178.04***               | -                      |            |



**Fig 4:** Overall acceptability (9-point hedonic) of paneer for treatments T<sub>0</sub>-T<sub>6</sub> during refrigerated storage.

### 3.5. Coliform and yeast and mould counts

Coliforms and yeast and mould were not detected in any sample, in any treatment, on any day of storage. The absence of these indicator and spoilage groups throughout the study reflects both satisfactory hygienic manufacture and the additional antimicrobial protection conferred by the partially purified LAB extract, and indicates that spoilage was governed by the general aerobic mesophilic flora captured in the total plate count rather than by coliforms or fungi.

**4. Discussion:** The results clearly establish that partially purified cell-free supernatant of lactic acid bacteria acts as an effective biopreservative for paneer, and that the effect is strongly dose-dependent. The antimicrobial activity of LAB supernatants is attributed to the combined action of organic acids, hydrogen peroxide, diacetyl and, most significantly, bacteriocins and bacteriocin-like inhibitory substances secreted during fermentation (Silva *et al.*, 2018; Garin-Murguialday *et al.*, 2024). Because these metabolites are concentrated in the supernatant, applying the CFS at a higher inclusion level (10.0 per cent) delivered a larger antimicrobial dose to the paneer surface, which explains why only T<sub>3</sub> and T<sub>6</sub> restrained the total plate count, moderated the pH decline and suppressed acid development sufficiently to extend keeping quality from about nine to twelve days.

The parallel behaviour of the microbiological, physico-chemical and sensory data strengthens this interpretation. The sharp rise in titratable acidity and the corresponding fall in pH in the control on day 9 coincided with the total plate count exceedingly roughly  $6.2 \log_{10} \text{ cfu g}^{-1}$  and with sensory scores dropping to the limit of acceptability, indicating that acid-producing mesophilic spoilage organisms drove the loss of quality. By curbing this population, the 10.0 per cent extract simultaneously slowed acidification and preserved appearance, texture, flavour and aftertaste. Similar shelf-life extension and quality retention using LAB cell-free supernatants have been reported in ground beef gel (Wang *et al.*, 2023), in meat products more broadly (Barcenilla *et al.*, 2022) and as a surface disinfectant alternative (Bang *et al.*, 2020), supporting the applicability of the approach to a fresh dairy matrix such as paneer.

Comparing the two organisms, the biopreservative efficacy of *L. Bulgaricus* and *L. plantarum* was broadly equivalent at each concentration, and both were maximally effective at

10.0 per cent. Where differences emerged, they were small: the *L. Bulgaricus* extract (T<sub>3</sub>) tended to give marginally lower total plate counts on day 12 ( $6.161$  versus  $6.212 \log_{10} \text{ cfu g}^{-1}$ ) and slightly better-controlled acidity, consistent with the strong antimicrobial and antipathogenic activity reported for this subspecies (Evivie *et al.*, 2020). Conversely, the *L. plantarum* extract (T<sub>6</sub>) delivered marginally higher sensory flavour and overall acceptability scores in the later stages of storage (flavour  $7.75$  versus  $7.60$  and overall acceptability  $7.62$  versus  $7.36$  on day 9), in line with the versatile, broad-spectrum plantaricin-based activity that has made this species a favoured biopreservation candidate (Putri *et al.*, 2024). From a practical standpoint the two organisms can therefore be regarded as comparably suitable, with the choice guided by whether maximal microbial suppression or maximal sensory retention is prioritised [11, 8, 12, 4, 3, 6, 10].

The complete absence of coliforms and of yeast and mould throughout storage is an additional favourable outcome. It confirms good manufacturing hygiene and shows that the extract did not introduce fungal or coliform contamination, while the observed shelf-life extension was achieved without any synthetic chemical preservative, an important advantage for clean-label positioning of the product.

**5. Conclusion:** Partially purified crude extract (cell-free supernatant) of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Lactobacillus plantarum* is an effective, natural, clean-label biopreservative for paneer. Applied at 10.0 per cent, the extract of either organism restrained the total plate count, moderated the decline in pH, suppressed the development of titratable acidity and preserved the sensory quality of paneer, extending its acceptable shelf life under refrigeration from approximately nine to twelve days, while coliforms and yeast and mould remained undetectable throughout. The two organisms were comparably effective, with *L. Bulgaricus* CFS giving marginally superior microbial control and *L. plantarum* CFS giving marginally superior late-storage sensory acceptability. A concentration of 10.0 per cent is recommended as optimal, and the approach offers a practical, residue-free route to enhancing the keeping quality of paneer. Further work on the characterisation of the active antimicrobial fractions, optimisation of the application method and validation against specific food-borne pathogens is warranted.

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