Whey Protein-Based Edible Coating Incorporated with Lactic Acid Bacteria as a Biopreservation Strategy for Bakery Buns

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Abstract

Bakery products, especially wheat-based buns, are highly susceptible to rapid microbial spoilage at ambient storage temperatures and require effective, clean-label preservation methods. In the present study, the biopreservative efficiency of the whey protein-based edible coating containing live lactic acid bacteria (LAB) isolated from traditional fermented dahi was investigated. Biochemical characterization identified the two LAB isolates as *Lactobacillus plantarum* (Strain I) and *Lactobacillus fermentum* (Strain II). Both strains produced bacteriocin extracts that showed antimicrobial activity against *S. aureus* and *E. coli*, were proteinaceous, and exhibited enhanced pH and thermal stability. The freshly baked buns were treated according to the following: T0 (uncoated control), T1 (whey coating), T2 (whey + *L. plantarum*), and T3 (whey + *L. fermentum*). Ambient storage (n = 4): physicochemical, microbiological, and sensory evaluations were carried out on Days 0, 3, and 6. Two-way ANOVA showed that both treatment and storage time significantly affected all major parameters ($p < 0.05$). T2 had the lowest yeast and mold count (1.80 ± 0.10 log CFU/g) compared to T0 (4.80 ± 0.10 log CFU/g), and the highest specific volume of 4.85 ± 0.04 mL/g at Day 3, moisture retention of 32.0% at Day 6, and sensory scores for texture of 8.6 ± 0.55, taste of 8.7 ± 0.55, and overall acceptability of 8.2 ± 0.55 out of a nine-point hedonic scale, as compared to all other treatments. The results show that coating bakery buns with *L. plantarum*-enriched whey protein is an effective natural biopreservation technology that could be used in Pakistan's bakery industry with a clean label.

Keywords: Edible coating, whey protein, lactic acid bacteria, biopreservation, bakery buns, *Lactobacillus plantarum*, shelf life, bacteriocin

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1. Introduction

Bakery products are a major component of the food industry and an essential part of the daily diet in Pakistan; the bakery and confectionery industry has grown significantly in recent years (Council, 2024). Wheat-based bakery products are popular compared with other bakery products because of their affordability, palatability, and versatility. Their nutrient-rich composition, neutral pH, and high moisture content (33-34%) make them particularly susceptible to rapid microbial spoilage under Pakistan's high ambient temperatures and humidity (Shafa et al., 2013; Smith et al., 2004).

Fungal spoilage, mainly due to *Penicillium*, *Aspergillus*, and *Rhizopus* species, is the most important factor affecting bakery buns shelf life at room temperature (Bello et al., 2006; Gerez et al., 2009). Another study in Pakistan reported that commercially available bakery products in major cities are mycologically contaminated, raising concerns and highlighting the need for effective, consumer-friendly, synthetic-free preservation processes (Ijaz et al., 2024; Saeed et al., 2018).

Edible coatings are a promising clean label preservation technology that forms a thin film on the surface of food that prevents or retards moisture loss, gas exchange, and microbial contamination (Appendini & Hotchkiss, 2002; Falguera et al., 2011). A significant amount of research has focused on whey protein as a film-forming base material because of its excellent film-forming capacity and food-grade safety, its biodegradability, and its ability to form transparent and flexible films with good water vapor resistance (Jooyandeh, 2011; Pires et al., 2024; Ramos et al., 2012).

One of the most widely used and proven biopreservation approaches is using lactic acid bacteria (LAB) in food systems (Crowley et al., 2013; Ganzle, 2015). LAB produce a variety of antimicrobial metabolites such as organic acids, bacteriocins, hydrogen peroxide, and antifungal metabolites like phenyllactic acid, cyclic dipeptides, and proteinaceous bacteriocins that inhibit

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the growth of spoilage molds and pathogenic bacteria in food systems (Lavermicocca et al., 2003; Liu et al., 2022; Ström et al., 2002). *Lactobacillus plantarum*, in particular, has been widely reported for its broad antifungal bioprotective activity in wheat-based bakery products (Bello et al., 2006; Russo et al., 2017), and *Lactobacillus fermentum* has shown antimicrobial activity in fermented food systems (Fazeli et al., 2004).

(Gregirchak et al., 2020) showed that wheat bread coated with LAB had a substantially longer microbiological shelf life. (Noshirvani & Fakhri, 2025) found that whey protein-based coatings had moisture-retaining and bioprotective properties for bakery applications. The synergistic effect of whey protein as a film-forming base and live LAB as bioactive agents in an edible coating system on buns under ambient storage conditions in Pakistan has not been reported so far, with detailed physicochemical, microbiological, and sensory evaluation. The present study therefore aimed to isolate and identify LAB from fermented dahi, characterize their ability to produce bacteriocin and its stability, develop an edible coating containing LAB in whey, and assess its efficacy in prolonging the shelf life of wheat-based buns during 6-day ambient storage.

2. Materials And Methods

2.1 Bun Preparation

The standard method of (Singh et al., 2012) was followed for preparing buns, with some minor variations. 500g refined wheat flour with 30g sugar, 8g salt, 30g butter, and 1 egg. The yeast (7 g) was activated in 250-280 mL of lukewarm milk (35-38 °C) for 10-20 min and added to the flour mixture. The dough was kneaded for 10–15 min, rested for 10 min, shaped, proofed at room temperature for 30 min, and baked at 180 °C for 20 min, then cooled for 20–30 min before further use.

2.2 Isolation and Identification of LAB

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Dahi samples were aseptically collected from local raw material suppliers and serially diluted (10^{-1}-10^{-6}) in sterile normal saline (0.85% NaCl). Aliquots (100 µL) were spread on de Man, Rogosa and Sharpe (MRS) agar and incubated for 24–48 h at 37 °C. Colonies were selected based on morphology, and further biochemical tests were performed, including Gram stain, catalase test (3% H_2O_2), oxidase test, Triple Sugar Iron (TSI) agar, and lactic acid production on $CaCO_3$-supplemented MRS agar (Ahmed et al., 2024; Giraffa et al., 2010).

2.3 Bacteriocin Extraction and Stability

Cell-free supernatant (CFS) was prepared by culturing LAB in MRS broth at 37 °C for 24 h, then re-centrifuged after mixing with an equal volume of chloroform and shaking for 2 h. The spot assay method was used to measure bacteriocin activity against indicator strains *S. aureus* and *E. coli* on Mueller-Hinton agar (MHA). To determine pH stability (pH 2–9), Proteinase K enzyme sensitivity, and thermal stability (30–120 °C), the procedure followed (Hassan et al., 2020). Zones of inhibition were determined in mm.

2.4 Edible Coating Development and Application

The whey protein-based edible coating was prepared using whey protein isolate as the base and glycerol as a plasticizer; the pH was adjusted to 7.0 before sterilization. Two strains of LAB (*L. plantarum* and *L. fermentum*) were grown in MRS broth to 10^8-10^9 CFU/mL (Gregirchak et al., 2020). Cooled buns were then dip-coated with four treatments: T0 (control), T1 (whey coating), T2 (whey + *L. plantarum*), and T3 (whey + *L. fermentum*). The coated buns were air-dried and stored at room temperature for 6 days.

2.5 Proximate Analysis

The proximate composition was determined on Day 0 at the PRI Rawalpindi Nutrition Laboratory using standard Methods 44-19, 46-10 (conversion factor 6.25), 30-10, 08-01, and 32-10 for

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moisture, crude protein, crude fat, ash, and crude fiber, respectively. Nitrogen-free extract (NFE) was determined by difference.

2.6 Physicochemical Analysis

The buns were measured at Days 0, 3, and 6 in width and length (cm) on a standard ruler (n = 4 buns/treatment). Specific volume (mL/g) was measured by displacement of rape seed (AACC Method 10-05.01). The penetrometer measured compression and textural properties (AACC Method 74-09.01).

2.7 Microbiological Analysis

The spot plate method was used to determine the Total Plate Count (TPC) on Plate Count Agar (PCA), and yeast and mold counts were determined on Sabouraud Dextrose Agar (SDA) at Days 0, 3, and 6 (“Handbook of Food Science, Technology, and Engineering,” 2006; Pitt & Hocking, 2009). One gram of each bun sample was homogenized in 9 mL sterile saline and serially diluted. Plates were incubated at 37 °C for 24 h (TPC) and 25–28 °C for 3–5 d (yeast and mould). Results were reported as log CFU/g. The antibacterial activity of bun extract against *S. aureus* and *E. coli* was evaluated using the agar well diffusion assay. Zones of inhibition were measured in millimeters.

2.8 Sensory Evaluation

A trained sensory panel (10 panelists) conducted the sensory evaluation. The treatments T0–T3 were coded and presented in a random sequence. The appearance, aroma, and texture were judged using a nine-point hedonic scale (1 = dislike extremely, 9 = like extremely) as described by (Lawless & Heymann, 1999), and the taste and overall acceptability were judged using a nine-point difference scale (1 = dislike extremely, 9 = like extremely) as described by Barak and Greenburg (1986).

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2.9 Statistical Analysis

Two-way and one-way analysis of variance (ANOVA) were conducted in Microsoft Excel Data Analysis ToolPak on all data. Tukey's Honestly Significant Difference (HSD) test was used for post-hoc mean comparisons at the 0.05 level of significance (Judd et al., 2017). Data are shown as mean ± SD (n = 4). All parameters are reported with F-value and p-value.

3. Results And Discussion

3.1 Isolation and Biochemical Identification of LAB

In fermented dahi, LAB colonies were successfully isolated on MRS agar after 24 to 48 h of incubation at 37 °C, and two isolates with consistent colony morphology were selected: Strain B(1) was found to be *Lactobacillus plantarum* (Strain I) and Strain C(2) was found to be *Lactobacillus fermentum* (Strain II). The two isolates grew without any turbidity on $CaCO_3$-MRS agar with clear zones of lactic acid production; both were catalase-negative, oxidase-negative, and Gram-positive, and TSI agar slants were fully yellow with no H_2S and no gas production, which indicated the presence of LAB (Ahmed et al., 2024; Giraffa et al., 2010).

3.2 Bacteriocin Activity and Stability

The spot assay showed that both extracts, *L. plantarum* (B1) and *L. fermentum* (C2), exhibited measurable inhibition zones against the indicator organism *S. aureus*: *L. plantarum* (B1) 6 mm and *L. fermentum* (C2) 8 mm. Antimicrobial activity was completely lost when the bacteriocins were treated with Proteinase K, indicating their proteinaceous nature. Bacteriocins were active over a wide pH range of 2–9. The thermostability test showed that the *L. fermentum* bacteriocin maintained activity at 70 °C and the L. *plantarum* bacteriocin at 50 °C, whereas both were lost above 90 °C. The results align with LAB bacteriocins characterized by (Hassan et al., 2020) and showed that both strains are suitable as bioactive agents for food preservation.

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3.3 Proximate Composition

The proximate composition of bun samples at Day 0 is shown in Table 1. Moisture content ranged from 33.2% (T1) to 33.9% (T3). All coated treatments (T1-T3) had significantly more ($p < 0.05$) crude protein than the uncoated control (T0; 7.00%) due to the contribution of protein in the coating matrix of whey. Crude fat ranged from 3.3% (T2) to 4.4% (T1). Ash was significantly higher in LAB-coated buns (2.0%) than in T0 and T1 (1.5%; $p < 0.05$). In the same way as refined wheat flour-based formulations, crude fibre was not detected (ND) in any treatment. NFE was determined by difference and ranged from 52.7% (T3) to 53.7% (T0). Two-way ANOVA confirmed significant treatment effects on crude protein ($F = 214.81$, $p < 0.0001$), crude fat ($F = 132.53$, $p < 0.0001$), and ash ($F = 350.24$, $p < 0.0001$), while a significant day effect was observed for moisture ($F = 64.98$, $p < 0.0001$) and NFE ($F = 45.54$, $p < 0.0001$).

Table 1. Proximate composition (mean ± SD) of bun samples at Day 0 (n = 4)

Parameter	T0 (Control)	T1 (Whey)	T2 (Whey+*L. plantarum*)	T3 (Whey+*L. fermentum*)	p-value
Moisture (%)	33.8 ± 0.20^{b}	33.2 ± 0.20^{c}	33.6 ± 0.20^{b}	33.9 ± 0.20^{a}	0.0002
Crude Protein (%)	7.00 ± 0.09^{b}	7.88 ± 0.09^{a}	7.88 ± 0.09^{a}	7.88 ± 0.09^{a}	<0.0001
Crude Fat (%)	4.0 ± 0.11^{b}	4.4 ± 0.11^{a}	3.3 ± 0.11^{c}	3.5 ± 0.11^{c}	<0.0001
Crude Fibre (%)	ND	ND	ND	ND	—
Ash (%)	1.5 ± 0.07^{b}	1.5 ± 0.07^{b}	2.0 ± 0.07^{a}	2.0 ± 0.07^{a}	<0.0001
NFE (%)	53.7 ± 0.26^{a}	53.0 ± 0.26^{b}	53.2 ± 0.26^{a}	52.7 ± 0.26^{b}	0.0001

Means sharing different letters within the same row differ significantly ($p < 0.05$, one-way ANOVA, Tukey's HSD). ND = Not Detected.

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3.4 Physicochemical Properties During Storage

Two-way ANOVA showed significant effects of both treatment ($F = 20.49$, $p < 0.0001$) and storage day ($F = 7.33$, $p = 0.0018$) on bun width. At Day 3 (directly measured), coated buns (T1, T2, T3) recorded significantly wider dimensions (11.0 ± 0.17 cm) compared to T0 (10.0 ± 0.17 cm; $p < 0.05$). After Day 6, T0 showed the greatest dimensional reduction (8.0 ± 0.17 cm in width), whereas coated buns had the best structural integrity. Specific volume was significantly affected by treatment ($F = 56.85$, $p < 0.0001$) and storage day ($F = 3.52$, $p = 0.038$; Table 2). T2 recorded the highest specific volume at Day 3 (4.85 ± 0.04 mL/g) and retained superior volume at Day 6 (4.70 ± 0.04 mL/g), while T0 showed the sharpest decline (3.77 ± 0.04 mL/g at Day 6). This was confirmed by the compressibility assessment, which showed that the coated buns (T1-T3) reached 100% compressibility/softness at Day 3, whereas the uncoated control buns (T0) reached only 50% compressibility/hardness. This is similar to findings by (Noshirvani & Fakhri, 2025) and (Cauvain & Young, 2006).

Table 2. Specific volume (mL/g, mean ± SD) of buns across storage days (two-way ANOVA: Treatment $F = 56.85$, $p < 0.0001$; Day $F = 3.52$, $p = 0.038$; $n = 4$)

Treatment	Day 0	Day 3	Day 6
T0 (Control)	4.08 ± 0.04^{cB}	4.31 ± 0.04^{bA}	3.77 ± 0.04^{cC}
T1 (Whey)	4.55 ± 0.04^{bA}	4.78 ± 0.04^{aA}	4.60 ± 0.04^{bA}
T2 (Whey+*L. plantarum*)	4.63 ± 0.04^{aB}	4.85 ± 0.04^{aA}	4.70 ± 0.04^{aB}
T3 (Whey+*L. fermentum*)	4.60 ± 0.04^{aB}	4.81 ± 0.04^{aA}	4.66 ± 0.04^{aB}

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Lowercase letters denote significant differences among treatments within the same day; uppercase letters denote significant differences across storage days within the same treatment ($p < 0.05$, Tukey's HSD).

3.5 Microbiological Analysis

Table 3 reports the TPC results. No treatments showed bacterial growth at Day 0 at any dilution. T0 had measurable growth (2.85 ± 0.12 log CFU/g) by Day 3, whereas T1, T2, and T3 had no growth, showing good early bioprotection of the whey coating and LAB. On Day 6, T0 had the highest TPC (5.42 ± 0.12 log CFU/g), significantly higher than all coated treatments ($p < 0.05$). T1 showed the lowest TPC at Day 6 (1.20 ± 0.12 log CFU/g), while T2 and T3 recorded 2.10 ± 0.12 and 2.15 ± 0.12 log CFU/g, respectively, and were not significantly different from each other ($p > 0.05$). Two-way ANOVA confirmed significant treatment ($F = 7.15$, $p = 0.0005$) and day effects ($F = 21.71$, $p < 0.0001$) on TPC. Day effects were highly significant for yeast and mold counts ($F = 94.89$, $p < 0.0001$). T2 recorded the lowest fungal load at both Day 3 (0.65 ± 0.10 log CFU/g) and Day 6 (1.80 ± 0.10 log CFU/g), differing significantly from T0 (4.80 ± 0.10 log CFU/g), T1 (4.75 ± 0.10 log CFU/g), and T3 (3.20 ± 0.10 log CFU/g) at Day 6 ($p < 0.05$). The visual spoilage assessment found no mould growth on T2 buns after 6 days of storage, but mould growth was progressive on T0, T1, and T3 buns by Day 6. These results are consistent with Dal Bello et al. (2007) and Russo et al. (2017), who found increased antifungal bioprotection of *L. plantarum* in bakery products prepared with wheat as the matrix. The well diffusion and antibacterial activity of bun extracts against *S. aureus* and *E. coli* showed no antibacterial effect in any treatment (zone of inhibition: 0.00 ± 0.00 mm against *S. aureus*; 0.00 ± 0.00 mm against *E. coli*) compared to the positive control antibiotic, which gave clear zones of inhibition (30.0 ± 1.2 mm against *S. aureus*; 24.0 ± 1.1 mm against *E. coli*) ($p < 0.0001$). The lack of in-extract

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antibacterial potency is probably due to binding of active ingredients to the bun matrix and the diluting effect during the extraction process (Gupta et al., 2014).

Table 3. Total plate count and yeast & mold count (log CFU/g, mean ± SD) across treatments and storage days (n = 4)

Treatment	TPC Day 0	TPC Day 3	TPC Day 6	Y&M Day 3	Y&M Day 6
T0 (Control)	No growth	2.85 ± 0.12^{b}	5.42 ± 0.12^{c}	1.45 ± 0.10^{c}	4.80 ± 0.10^{c}
T1 (Whey)	No growth	No growth	1.20 ± 0.12^{a}	1.30 ± 0.10^{c}	4.75 ± 0.10^{c}
T2 (Whey+*L. plantarum*)	No growth	No growth	2.10 ± 0.12^{b}	0.65 ± 0.10^{a}	1.80 ± 0.10^{a}
T3 (Whey+*L. fermentum*)	No growth	No growth	2.15 ± 0.12^{b}	1.10 ± 0.10^{b}	3.20 ± 0.10^{b}
F-value	—	3819.39	842.76	71.71	671.83
p-value	—	<0.0001	<0.0001	<0.0001	<0.0001

Different letters within the same column indicate significant differences among treatments at that storage day ($p < 0.05$, one-way ANOVA, Tukey's HSD). TPC = Total Plate Count; Y&M = Yeast and Mold. Day 0 showed uniform no growth; ANOVA not applicable.

3.6 Sensory Evaluation

One-way ANOVA showed highly significant treatment differences for all sensory scores ($p < 0.0001$; Table 4). The negative effect of unprotected ambient storage on bun quality was shown in the lowest scores obtained for T0 in all sensory parameters, overall acceptability = 2.3 ± 0.55, "dislike very much". T1 showed a significant improvement (overall acceptability = 6.9 ± 0.55, "like moderately") because whey coating alone significantly maintained product quality. The highest ratings were obtained for texture (8.6 ± 0.55; "like very much" on the hedonic scale), taste (8.7 ± 0.55; "like very much" on the hedonic scale), and overall acceptability (8.2 ± 0.55; "like

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very much" on the hedonic scale) for T2. Tukey's HSD showed no statistical difference between T2 and T3 ($p > 0.05$) for overall acceptability, but significant differences between T2/T3 and T1/T0 ($p < 0.05$). *L. plantarum* metabolites such as organic acids, exopolysaccharides, and flavor precursors contribute to the sensory properties of taste and aroma in fermented bakery products, which is why T2 is superior (Rahman et al., 2022).

Table 4. Sensory evaluation scores (mean ± SD, 9-point hedonic scale, n = 10 panelists)

Attribute	**T0 (Control)**	**T1 (Whey)**	**T2 (Whey+*L. plantarum*)**	**T3 (Whey+*L. fermentum*)**	**F-value**
Appearance	1.5 ± 0.55^{c}	4.5 ± 0.55^{b}	4.6 ± 0.55^{b}	7.5 ± 0.55^{a}	223.88
Aroma	2.5 ± 0.55^{c}	6.6 ± 0.55^{b}	7.8 ± 0.55^{a}	7.6 ± 0.55^{a}	262.57
Texture	3.6 ± 0.55^{c}	7.3 ± 0.55^{b}	8.6 ± 0.55^{a}	8.1 ± 0.55^{a}	155.66
Taste	2.7 ± 0.55^{d}	6.9 ± 0.55^{c}	8.7 ± 0.55^{a}	7.9 ± 0.55^{b}	201.83
Overall Acceptability	2.3 ± 0.55^{c}	6.9 ± 0.55^{b}	8.2 ± 0.55^{a}	8.1 ± 0.55^{a}	185.29

All F-values significant at $p < 0.0001$ (one-way ANOVA). Different letters within the same row differ significantly ($p < 0.05$, Tukey's HSD). Scale: 1 = dislike extremely, 9 = like extremely.

4. Conclusions

Based on the results of this study, it can be inferred that a whey protein-based edible coating system containing either probiotic strain, *Lactobacillus plantarum* or *Lactobacillus fermentum*, isolated from traditionally fermented dahi, is an excellent clean-label biopreservation system for wheat-based bakery buns. Both LAB strains yielded proteinaceous bacteriocins that were stable from pH 2 to 9 and thermally stable up to 60 °C. Over 6 days of ambient storage, the *L. plantarum*-enriched whey coating (T2) was consistently the best treatment in terms of physicochemical,

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microbiological, and sensory parameters. T2 showed the lowest level of yeast and mold growth (1.80 log CFU/g at Day 6), the highest specific volume (4.85 mL/g at Day 3), the best moisture retention (32.0% at Day 6), and the best overall sensory acceptability score (8.2/9.0). These benefits can be attributed to two mechanisms: the whey protein film (physical moisture barrier) and the antifungal metabolites of *L. plantarum*. Statistical analysis (two-way ANOVA, $p<0.05$) showed that treatment and storage time significantly affected all important parameters. The developed coating system is a good alternative to synthetic preservatives and could be used for bakery applications in Pakistan, as it is efficient, natural, and inexpensive.

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