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Cutting-Edge Cheese Crafting: Exploring the Benefits of Postbiotics Coating in Pasteurized Cheese Production

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Abstract

Background and Objective: Cheese is one of the major dairy products with high nutritional value, but its susceptibility to microbial growth and early spoilage remains a challenge for the dairy industry. While chemical additives are widely applied to control microbial contamination, increasing awareness of the potential hazards of synthetic preservatives has led to a growing demand for natural alternatives. This study was designed to evaluate the antimicrobial potential of postbiotics derived from *lactiplantibacillus plantarum* and *Lacticaseibacillus casei* against common spoilage and pathogenic microorganisms in cheese, as well as to investigate their impact on the microbiological and chemical properties of cheese during storage.

Material and Methods: Postbiotics were extracted from cultures of *L. plantarum* and *L. casei* and their antimicrobial activities were tested using standard microbiological assays against Gram-positive and Gram-negative bacteria. To assess practical application, the postbiotics were applied as coatings on cheese samples either alone or in combination with whey protein concentrate (WPC). Microbiological counts, chemical parameters, and sensory evaluation were performed throughout storage. Data were analyzed to determine the comparative effectiveness of treatments.

Results and Conclusion: The findings showed that the postbiotic derived from *L. plantarum* demonstrated stronger antimicrobial effects, particularly against Gram-positive bacteria, compared to that from *L. casei*. However, when combined with WPC, the antimicrobial activity of both postbiotics declined. Despite this limitation, postbiotics applied alone significantly reduced microbial counts during storage without altering the main chemical properties of the cheese. Sensory evaluation confirmed the overall acceptability of postbiotic and postbiotic-WPC treated samples. In conclusion, postbiotics can serve as promising natural antimicrobial agents in cheese preservation, though further optimization is required to enhance their activity when combined with protein-based carriers such as WPC.

Keywords: Antimicrobial activity, Cell-free supernatant, Cheese, Lactic acid bacteria, Postbiotic

What is "already known" on this topic:	➢ Postbiotics produced by lactic acid bacteria are known to exert antimicrobial effects through metabolites such as organic acids and bacteriocins. ➢ Although whey protein–based coatings are applied to extend the shelf life of dairy products, their use as carriers of postbiotics in cheese has been scarcely investigated.
What this article adds:	➢ Postbiotics from lactiplantibacillus plantarum and Lacticaseibacillus casei exhibited strong antimicrobial effects against key foodborne pathogens. ➢ L. plantarum postbiotics showed higher activity, particularly against Gram-positive bacteria, with lower MIC and MBC values. ➢ Incorporation of postbiotics into whey protein concentrate coatings reduced antimicrobial efficacy due to matrix interactions.

- Postbiotic treatments suppressed microbial growth while maintaining the chemical properties and sensory quality of cheese.
- These findings highlight postbiotic-based coatings as natural, consumer-friendly alternatives to chemical preservatives in dairy products.

1. Introduction

The food industry consistently faces substantial challenges from pathogenic and spoilage microorganisms, which are major drivers of foodborne diseases (FBDs), product quality degradation, and significant economic losses [1]. Globally, FBDs remain a pressing public health issue, with more than 600 million cases and nearly 420,000 deaths reported annually due to contaminated food and water [2,3]. These statistics have reinforced the urgency of strengthening food safety measures and minimizing contamination throughout production, processing, and storage stages [4,5]. At the same time, consumer concerns over the potential health risks associated with chemical preservatives, alongside the increasing demand for minimally processed foods, have fueled interest in natural preservation strategies [6,7]. Such approaches not only lessen dependence on artificial additives but also align with the growing trend toward clean-label products.

In this regard, biological protection has emerged as a promising strategy, harnessing beneficial microorganisms and their antimicrobial metabolites to suppress the proliferation of spoilage and pathogenic organisms [4]. Probiotics, especially lactic acid bacteria (LAB), have drawn considerable attention for their ability to generate bioactive substances such as organic acids, hydrogen peroxide, diacetyl, and bacteriocins [8]. Among these, LAB species such as Lactobacillus and Bifidobacterium are generally recognized as safe (GRAS) and have been widely employed for decades in the fermentation of diverse foods, including dairy products, cereals, and vegetables [9]. More recently, growing attention has been directed toward postbiotics, defined as non-viable microbial cells, cellular components, or metabolites that provide functional health benefits [10]. Compared with probiotics, postbiotics present notable practical advantages: they are independent of cell viability, exhibit greater stability under processing and storage conditions, and pose a lower risk of antibiotic resistance or incompatibility with food matrices. Additionally, postbiotics retain antimicrobial activity across a wide range of pH and temperature conditions, are capable of disrupting pathogenic biofilms, and can neutralize harmful contaminants such as pesticides and mycotoxins. In food systems, they have been investigated both as direct additives and as functional components of active packaging technologies, thereby overcoming limitations associated with the use of live microbial cultures [4].

Cheese provides a particularly critical application, as it is highly vulnerable to microbial contamination during both processing and storage. Among potential threats, *Listeria monocytogenes* is of significant concern due to its ability to withstand stress conditions and its high fatality rate in human infections [11,12]. Epidemiological evidence has repeatedly linked outbreaks of listeriosis to cheeses manufactured from raw or inadequately pasteurized milk [13], underscoring the urgent demand for innovative, safe, and effective antimicrobial approaches in dairy preservation.

Recent investigations have demonstrated that postbiotics and bacteriocin-like compounds derived from lactic acid bacteria (LAB) are capable of inhibiting pathogenic bacteria across diverse food matrices, including meat, seafood, and dairy products [14–19].

In parallel, whey protein, a major by-product of cheese production, has attracted considerable interest as a functional material for edible coatings and packaging, owing to its excellent barrier properties and strong film-forming capacity [11]. Incorporating antimicrobial postbiotics into whey protein systems may therefore offer dual advantages: enhancing microbial safety and prolonging shelf life while preserving desirable sensory characteristics.

Although evidence supporting postbiotics as natural preservatives is growing, relatively few studies have systematically assessed their performance in real cheese systems, particularly when combined with whey protein–based coatings. This gap in knowledge constrains a comprehensive understanding of their effectiveness under practical conditions.

The present study aims to bridge this gap by examining the antimicrobial activity of postbiotics derived from *lactiplantibacillus plantarum* and *Lacticaseibacillus casei* against key dairy pathogens, while also evaluating the microbial and chemical quality of cheese coated with whey protein concentrate (WPC) enriched with these postbiotics. This integrated strategy offers a novel, effective, and sustainable approach to improving food safety and preservation in dairy products.

2. Materials and Methods

2.1 Study design

This descriptive-analytical study was conducted between 22 November 2022 and 15 September 2023. The study protocol was reviewed and approved by the Medical Ethics Committee of Gonabad University of Medical Sciences (IR.GMU.REC.1401.073).

2.2 Bacterial strains

Three *lactiplantibacillus plantarum* strains were isolated from traditional Iranian cheeses [20]. In addition, one *Lacticaseibacillus casei* strain (1608 PTCC, IBRC of Iran), *Listeria monocytogenes* (7644 ATCC), *Escherichia coli* (1338 PTCC), and *Staphylococcus aureus* (1431 PTCC) were obtained from the Laboratory of Specialization in Nutrition, Mashhad University of Medical Sciences.

2.3 Postbiotic preparation

Each lactic acid bacterial strain was cultured separately in MRS broth medium and incubated under anaerobic conditions at 37 °C for 24 hours. The cultures were then centrifuged at 6000 rpm for 10 minutes at 4 °C. The resulting cell-free supernatants were filtered through a 0.4 µm membrane filter and subsequently freeze-dried (freezing temperature −83 °C, pump pressure 0.0026 mBar, storage temperature −60 °C) for use in subsequent experiments [21].

2.4 Chemical analysis of cell-free supernatants of Lactobacillus sp.

The chemical compounds of postbiotics were identified following the method described by Ryan et al. (2009), with minor modifications. For derivatization, 1 mL of the supernatant was mixed with 10 mL of absolute ethanol and 15 drops of sulfuric acid (97%), and the mixture was stirred at 80 °C for one hour. After cooling, 20 mL of distilled water was added, and extraction was performed five times with 50 mL of dichloromethane, collecting the lower phase each time. The pooled extracts were combined with 50 g of sodium sulfate and passed through filter paper. The solvent was then removed using a vacuum evaporator at 50 °C, and the remaining residue was injected into the gas chromatography–mass spectrometry (GC–MS) system.

The chemical composition of the derivatized postbiotics was analyzed using a GC instrument (Agilent HP-6890, Agilent Technologies, Palo Alto, CA, USA) operated with Agilent GC/MS Mass Hunter Acquisition software. The GC system was equipped with an Agilent HP-5ms column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed to increase from 110 °C to 240 °C at a rate of 4 °C/min with no hold time. A 10 µL sample was injected with a 5:1 split ratio [22].

2.5 Antimicrobial Activity In-vitro

2.5.1 Agar-well diffusion

Three pathogens (*L. monocytogenes*, *E. coli*, and *S. aureus*) were inoculated separately at a concentration of 5 log10 CFU/mL onto the surface of Muller Hinton Agar (MHA) plates. Wells with an 8 mm diameter were then created in the agar, and 100 µL of postbiotic suspensions at concentrations of 5%, 10%, and 20% were added to the wells. Plates were incubated aerobically at 37 °C for 24–48 hours. Nisin (625 IU/mL) and sterile distilled water served as the positive and negative controls, respectively. Antimicrobial activity was expressed as the mean diameter (mm) of inhibition zones, measured as the clear areas surrounding the wells. Each assay was performed in triplicate [23].

2.5.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by the microdilution method

MIC and MBC values were determined according to the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines. After 24 hours of aerobic incubation at 37 °C, the wells were examined for turbidity. To determine the MBC, 10 µL samples from wells without turbidity (corresponding to MIC and higher concentrations) were streaked onto MHA plates in triplicate and incubated under the same conditions [24].

2.5.3 Anti-listeria activity of coatings containing postbiotics

To prepare the coating solution, 50 mL of deionized water was heated to 90 °C in a bain-marie, and 4.34 g of whey protein concentrate (WPC; protein 81.2%, lactose 7.4%, fat 6%, moisture 5%, ash 4%, pH 6.1; Alinda, Greece) was fully dissolved. The solution was maintained at this temperature for 45–60 minutes, during which 2.71 g of glycerol and 0.081 g of Tween 80 were added. After cooling, cell-free supernatants (CFS) were incorporated at concentrations corresponding to MIC and MBC [25]. The anti-listeria activity of coatings containing postbiotics was then evaluated using the agar-well diffusion method. CFS without coating, at equivalent concentrations, was included as a positive control [26].

2.6 Inoculation and coating of cheese samples

A pasteurized traditional cheese, commercially available in local markets, was selected for this study. The cheeses analyzed were pasteurized varieties inoculated with a fungal starter culture. Four treatment groups were prepared to evaluate microbial and chemical characteristics over storage on days 0, 1, 2, 4, 6, 8, and 10 (Table 1).

Table 1. The prepared treatments.

Number	Treatmeants
(1)	Cheese+*L. monocytogenes*
(2)	Cheese+ *L.monocytogenes*+CFS
(3)	Cheese+ *L.monocytogenes*+WPC
(4)	Cheese+*L.monocytogenes*+CFS+WPC

Cheese pieces of approximately 10 g (3 × 3 × 1 cm) were inoculated with *L. monocytogenes* at a level of 1×10^5 CFU/g by spreading 1 mL of an appropriately diluted suspension onto the surface [18]. Samples were then allowed to stabilize to ensure bacterial adherence. The treatments were as follows:

1. Inoculated cheese without further treatment,
2. Cheese immersed in CFS solution at the designated concentration,
3. Cheese coated with WPC solution without CFS, and
4. Cheese coated with WPC solution containing CFS (immersion for 4–5 minutes).

All samples were stored at 4 °C until further analysis.

2.7 Microbial analyses in-situ

L. monocytogenes, total viable microorganisms, molds, and yeasts were enumerated using Palcam Agar, PCA, and SDA media, respectively. Palcam and PCA plates were incubated at 37 °C for 48 hours, while SDA plates were incubated at 25 °C for 3–5 days [26]. The enumeration of molds and yeasts was carried out according to the Iranian National Standard No. 2406: *Microbiology of milk and milk products — Specifications and test methods* [27].

2.8 Chemical analyses

The pH of cheese samples was measured using a pre-calibrated pH meter, and moisture content was determined by the gravimetric method on the designated sampling days [17,21].

2.9 Sensory analyses

Cheese slices coated with cell-free supernatants, free of *L. monocytogenes*, were evaluated for taste, color, aroma, texture, and overall acceptability by a panel of 10 semi-trained assessors using a 5-point hedonic scale [15]. All participants were adults above the legal age and voluntarily provided written informed consent in compliance with ethical standards for human subject research. Evaluations were conducted under identical environmental and temporal conditions to ensure consistency.

2.10 Statistical analyses

Statistical analyses were performed using SPSS software version 26. Mean values from three independent replicates were compared between two groups using the independent t-test, while comparisons among more than two independent groups were carried out using one-way ANOVA. Changes in data trends over the 10-day storage period were analyzed using one-way repeated measures ANOVA. A p-value of <0.05 was considered statistically significant.

3. Results and Discussion

3.1 Antimicrobial activity of postbiotics

Postbiotics derived from both *Lactobacillus* species demonstrated inhibitory effects against the three tested pathogens, with higher postbiotic concentrations corresponding to stronger antimicrobial activity (Fig. 1).

Fig. 1. Antimicrobial activity of postbiotics against L. monocytogenes evaluated by the agar-well diffusion method.

The greatest inhibition was observed with the postbiotic from *L. plantarum* at a 20% concentration against *L. monocytogenes*, producing an inhibition zone of 30.67 ± 0.57 mm. In contrast, the weakest inhibition was observed with the postbiotic from *L. casei* at a 5% concentration against *S. aureus*, yielding an inhibition zone of 8.63 ± 0.55 mm. Across all concentrations, the postbiotic of *L. plantarum* exhibited significantly stronger inhibitory activity against *L. monocytogenes* and *S. aureus* compared to that of *L. casei* ($p < 0.05$) (Tables 2–4). For *E. coli*, no significant differences were detected between the two postbiotics except at the 10% concentration (Table 3). These findings suggest that postbiotics from *L. plantarum* are more effective against Gram-positive pathogens than those from *L. casei*.

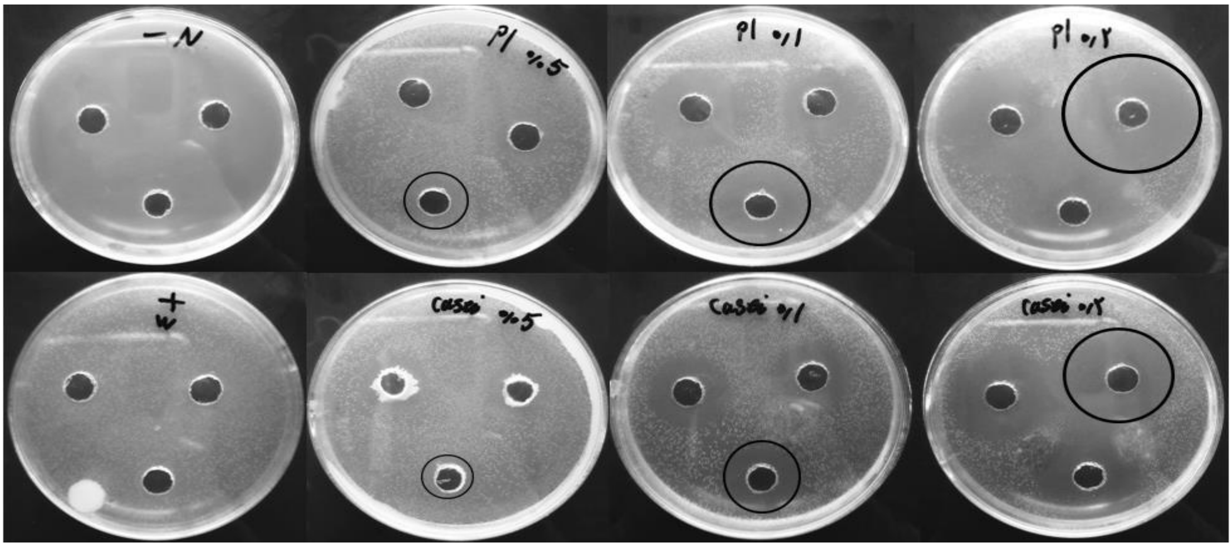

Fig.1. Evaluation test of the antimicrobial ability of postbiotics against *L. monocytogenes* pathogen by Agar-well diffusion method.

Table 2. Diameter of inhibitory of postbiotics against *L. monocytogenes*.

Postbiotic	5%	10%	20%
L. plantarum	12.83±0.28	21.50±0.50	30.67±0.57
L. casei	10.66±0.57	18.73±0.64	26.33±0.57
*P-Value**	0.004*	0.004*	0.001*

All values are in millimeters and are expressed as mean ± standard deviation in three replicates. * Indicates a significant difference between the size of the two inhibition zones (p<0.05)

Table 3. Diameter of inhibitory of postbiotics against *E. coli*.

Postbiotic	5%	10%	20%
L. plantarum	0.57±10.66	0.55±13.36	0.51±17.56
L. casei	0.28±9.66	0.57±11.66	0.55±16.36
*P-Value**	0.055	0.021*	0.051

All values are in millimeters and are expressed as mean ± standard deviation in three replicates. * Indicates a significant difference between the size of the two inhibition zones (p<0.05)

Table 4. Diameter of inhibitory of postbiotics against *S. aureus*.

Postbiotic	5%	10%	20%
L. plantarum	10.43±0.37	15.67±0.57	20±1.00
L. casei	8.63±0.55	12±1.00	17.67±0.57
*P-Value**	0.010*	0.005*	0.025*

All values are in millimeters and are expressed as mean ± standard deviation in three replicates. * Indicates a significant difference between the size of the two inhibition zones (p<0.05)

Overall, the results indicate that *L. plantarum* postbiotics exert stronger antimicrobial effects against Gram-positive bacteria, with the most consistent reductions achieved through the "CFS only" treatment rather than the CFS–WPC combination. The maximum reduction compared to control (~0.80 log CFU/g for *L. monocytogenes* at day 2) declined over subsequent storage days, while pH, moisture, and sensory acceptability remained unaffected. This strain- and target-dependent pattern is consistent with previous evidence showing that LAB-derived cell-free supernatants inhibit Gram-positive pathogens primarily through organic acids and bacteriocin-like metabolites, mechanisms that involve pH reduction and disruption of microbial membranes [4,6,9,28–30]. Arena et al. also reported strong anti-pathogen activity of *L. plantarum* supernatants, with acidification identified as a major contributing factor [31,32]. The reduced inhibitory effect observed when CFS was incorporated into a whey protein carrier is consistent with the well-documented "matrix effects" described in the literature. Previous studies have shown that interactions between proteins and bioactive metabolites, along with the barrier properties of protein films, can delay the release and reduce the bioavailability of antimicrobial compounds [11,26,33–36]. Similar patterns of initial but transient inhibition, followed by partial recovery of pathogen populations, have been reported in fresh cheese, meat, and fish products treated with CFS- or bacteriocin-based films [15–17,19].

In agreement with these findings, the present study demonstrated that chemical attributes (pH and moisture) and sensory acceptance were not adversely affected, supporting the feasibility of integrating postbiotics into dairy preservation systems. However, further optimization of carrier composition and release kinetics is required to maximize antimicrobial effectiveness [37].

The MIC and MBC values of *L. plantarum* postbiotics against the three tested pathogens were determined as 31.25 mg/mL and 62.5 mg/mL, respectively, for Gram-positive bacteria, and 125 mg/mL for *E. coli*. These results highlight a greater inhibitory effect against Gram-positive bacteria at lower concentrations (Table 5).

Table 5. The results of the MIC and MBC tests of postbiotics obtained from *L. plantarum* and *casei* against the three tested pathogens in mg/ml

Postbiotics	*L. Plantarum*		*L. Casei*	
	MIC	MBC	MIC	MBC
L. monocytogenes	31.25	62.50	62.50	62.50
E. coli	31.25	125	31.25	62.50
S. aureus	31.25	62.50	31.25	62.50

The postbiotic of *L. casei* exhibited a comparable inhibitory effect against *L. monocytogenes* to that of *L. plantarum*, except at the 5% concentration, where a difference was observed in comparison with *E. coli* (Table 6). No significant differences were detected in the inhibition of *S. aureus* and *E. coli* (p > 0.05) (Table 7).

The MIC of *L. casei* postbiotics against *L. monocytogenes* was higher (62.5 mg/mL) than that observed for *L. plantarum*, although both species showed identical MBC values. For *E. coli*, the MBC of *L. casei* postbiotics was lower than that of *L. plantarum*. In contrast, for *S. aureus*, the MIC and MBC values were the same for both postbiotics.

Table 6. Comparison of the significant difference between the postbiotic inhibition size of *L. plantarum* against the three tested pathogens.

Concentration	*L.monocytogenes/E.coli*	*L.monocytogenes /S.aureus*	*E.coli/S.aureus*
	P-Value	*P-Value*	*P-Value*
5%	0.002*	0.001*	0.793
10%	0.000*	0.000*	0.005*
20%	0.000*	0.000*	0.015*

* Indicates a significant difference between the size of the two inhibition zones (p<0.05)

Table 7. Comparison of the significant difference between the postbiotic inhibition size of *L. casei* against the three tested pathogens.

Concentration	*L.monocytogenes*/*E.coli*	*L.monocytogenes* /*S.aureus*	*E.coli*/*S.aureus*
	P-Value	*P-Value*	*P-Value*
5%	0.102	0.005*	0.092
10%	0.000*	0.000*	0.858
20%	0.000*	0.000*	0.070

* Indicates a significant difference between the size of the two inhibition zones ($p<0.05$)

Arrioja et al. (2020) similarly reported that CFS derived from *L. plantarum* exhibited stronger inhibitory activity against most pathogens compared with CFS from *L. casei* [33]. Arena et al. (2016) further demonstrated variability in inhibition zones and MICs among different *L. plantarum* strains against various pathogens, with generally greater effects observed against Gram-positive bacteria [31]. Consistent with the present findings, Tenea and Barrigas (2018) showed that bacteriocin-containing supernatants from *L. plantarum* (Cys5-4) exhibited variable inhibitory effects against two *E. coli* strains [7]. Koohestani et al. (2018) also reported that CFS from *L. casei* 431 produced an inhibition zone of 13 mm against *S. aureus*, which closely aligns with the current results [23]. In contrast, Yordshahi et al. (2020) documented smaller inhibition zones for *L. plantarum* postbiotics against *L. monocytegenes* compared with those observed in this study [21].

The antimicrobial activity of lactic acid bacteria has been attributed to a range of metabolites, including organic acids, polyamines, proteases, and bacteriocins. The effectiveness of postbiotics depends on multiple factors such as bacterial strain, metabolite composition and concentration, preparation method, and pathogen type. Numerous studies have confirmed that Gram-positive bacteria are generally more susceptible to the antagonistic compounds in postbiotics than Gram-negative bacteria, consistent with the findings of the present work [38].

Results from the agar-well diffusion assays indicated that postbiotics incorporated into WPC coating solutions at MBC concentrations generally exhibited reduced inhibition against *L. monocytogenes* compared to postbiotics applied alone (Table 8). However, the combination of *L. plantarum* postbiotics with WPC coatings produced greater inhibition than *L. casei* postbiotics at equivalent concentrations.

Based on the in-vitro assays, the *L. plantarum* postbiotic at twice the MBC concentration was identified as the most effective formulation and was subsequently selected for testing in the food model.

Table 8. Diameter of inhibitory of postbiotics in combination with WPC against *L. monocytogenes*.

Postbiotic	62.5 mg/ml	125 mg/ml
L. plantarum	1.00±14.00	1.00±24.00
L. casei	1.00±11.00	1.52±18.33
*P-Value**	0.021*	0.006*

All values are in millimeters and are expressed as mean ± standard deviation in three replicates. * Indicates a significant difference between the size of the two inhibition zones ($p<0.05$)

3.2 Identification of chemical compounds of extracted CFS

The chemical compounds identified in the postbiotics derived from *L. plantarum* and *L. casei* are presented in their respective chromatograms (Figs. 2 and 3).

Sezen Özcelik et al. (2016) reported that LAB strains are particularly efficient producers of succinic acid, especially when cultivated in MRS broth. Succinic acid serves as a key intermediate in the Krebs cycle and a common fermentation byproduct, reflecting the strong metabolic capacity of LABs. The quantity and composition of organic acids produced by LABs vary considerably across strains and culture media, with pH and temperature exerting significant influence [28]. Similarly, Iqbal Hossain et al. (2021) identified nine distinct organic acids, including succinic acid, in several LAB strains such as *L. plantarum*, highlighting the diverse metabolite production potential of these bacteria [29]. In the present study, the derivatization technique applied proved particularly effective in detecting compounds such as esters and alkanes.

Shehata et al. demonstrated that LABs synthesize antifungal metabolites that differ across strains, with organic acids and hydrogen peroxide serving as the primary contributors to antifungal activity. Their study identified compounds such as pentadecane and 2,4-di-tert-butylphenol, both known to inhibit foodborne pathogens and fungi. Additionally, antimicrobial compounds including 6-octadecenoic acid methyl ester and hexadecanoic acid methyl ester—also detected in the current work—were previously reported. Deepthi et al. further showed that LABs generate a wide variety of antifungal carboxylic acid esters, whose effectiveness depends on strain variability and fatty acid chain length. For example, 10-octadecenoic acid methyl ester, an unsaturated fatty acid ester with 19 carbon atoms, has been described as an effective antifungal metabolite. In agreement with Shehata et al., our findings also identified nonadecane, a compound without known antimicrobial activity [39,40].

Benzoic acid was detected in small quantities in both postbiotics. This compound, obtained from *L. plantarum*, has previously been reported by Siedler et al. as possessing antimicrobial properties [41].

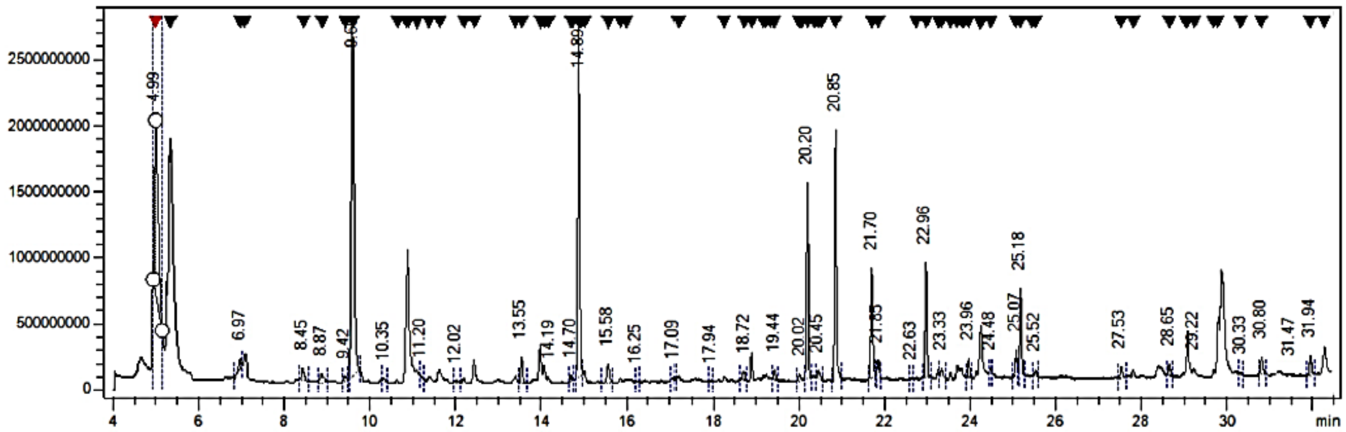

Fig.2. Postbiotic chromatogram obtained from *L. plantarum*

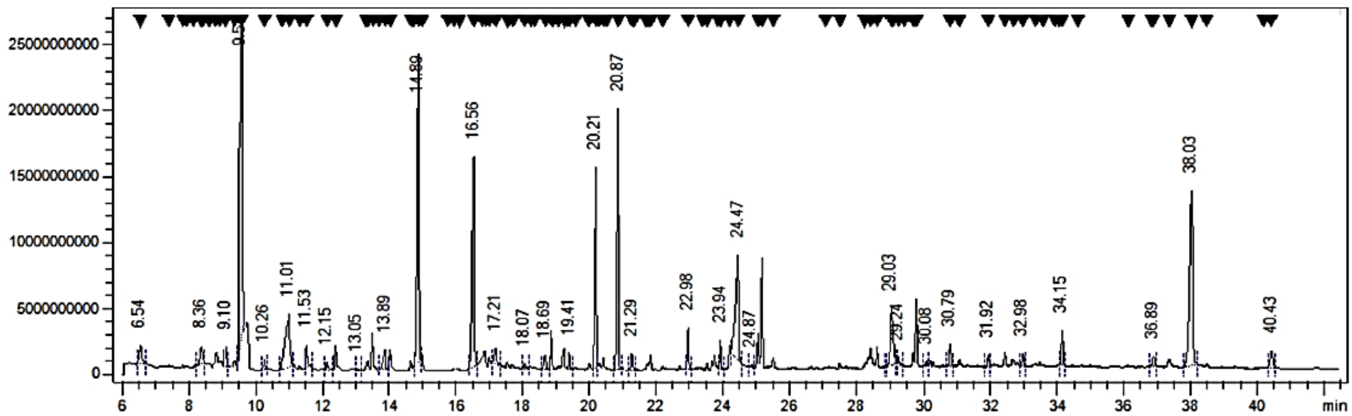

Fig.3. Postbiotic chromatogram obtained from *L. casei*

In addition, several compounds not previously described for their antimicrobial effects were identified in the chemical analysis of the postbiotics, including phthalic acid, eicosane, chloroacetic acid, benzene propanoic acid, propenoic acid, triethyl citrate, isopropyl myristate, butanedioic acid, and tetradecanoic acid.

3.3 Microbiological analyses

The growth of *L. monocytogenes* was monitored in the control samples (treatment one), which demonstrated a continuous increase throughout 10 days of storage at 4 °C, with an overall rise of 1.1 log CFU/g (Fig. 4).

Fig. 4. Growth of L. monocytogenes across four food treatments during 10 days of storage at 4 °C.

A significant reduction in pathogen counts was observed in the second treatment, which contained CFS, compared with the other three treatments over the 10-day storage period ($p < 0.05$). In contrast, no significant differences were detected in contamination levels between the third and fourth treatments and the control ($p > 0.05$). The largest reduction in *L. monocytogenes* relative to the control was 0.80 log CFU/g, recorded in the second treatment on day two; however, this difference gradually declined over the storage period (Table 9).

The results for total microbial counts (Fig. 5) and mold and yeast counts (Fig. 6) mirrored those observed for *L. monocytogenes*, with only the second treatment showing a significant reduction ($p < 0.05$) compared with the other treatments over the 10-day storage period. Fig. 5. Total microbial counts across four food treatments during 10 days of storage at 4 °C. Fig. 6. Mold and yeast count across four food treatments during 10 days of storage at 4 °C.

These findings are consistent with the study by Iqbal Hossain et al. (2021), which reported differences in the inhibitory effect (MEC) of CFS derived from *L. plantarum* when tested in a culture medium (TSB) compared with a food model (whole milk) against *L. monocytogenes* [29]. Similarly, Hartmann et al. (2011) demonstrated that the efficiency of bacterial fermentations varied depending on the food matrix, with bacteriocin IDE0105 from *L. plantarum* showing weaker activity in milk than in BHI medium. Such discrepancies may be explained by limited solubility or uneven distribution of antimicrobial compounds in food systems, inactivation of antagonistic chemicals by food components or microflora-derived enzymes, or interactions between antimicrobials and food ingredients [34].

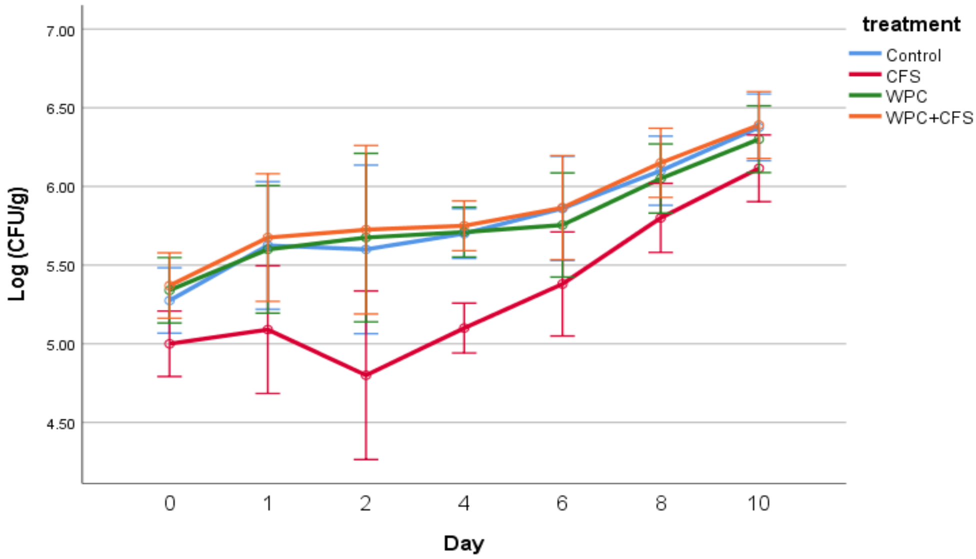

Fig.4. Comparison of the growth of *L. monocytogenes* in four food treatments during 10 days of storage at 4 ˚C.

Table 9. The results of *L. monocytogenes* growth (Log_{10}CFU/g) in four food treatments during 10 days of storage at 4˚C.

Day / T	0	1	2	4	6	8	10
(1)	5.27±0.03 Eab	5.62±0.03 DFEab	5.60±0.28 BGEb	5.70±0.01 CFGb	5.86±0.12 BFb	6.10±0.14 Bab	6.37±0.00 Aa
(2)	5.00±0.00 Ca	5.09±0.29 Ca	4.80±0.07 Ca	5.10±0.14 Ca	5.38±0.11 Ca	5.80±0.14 Ba	6.11±0.16 Aa
(3)	5.34±0.18 CEb	5.60±0.28 CDEab	5.67±0.45 BEb	5.71±0.02 BDb	5.75±0.21 BCab	6.05±0.07 Bab	6.30±0.14 Aa
(4)	5.37±0.09 Eb	5.67±0.03 DGEb	5.72±0.03 BFGEb	5.75±0.07 CDFb	5.86±0.19 BCDEb	6.15±0.07 Bb	6.39±0.01 Aa

(1) Control, (2) CFS, (3) WPC, (4) WPC+CFS. Non-similar uppercase Latin letters in each row indicate significant differences at the 5% level between treatments. Non-similar lowercase Latin letters in each column indicate significant differences at the 5% level between treatments.

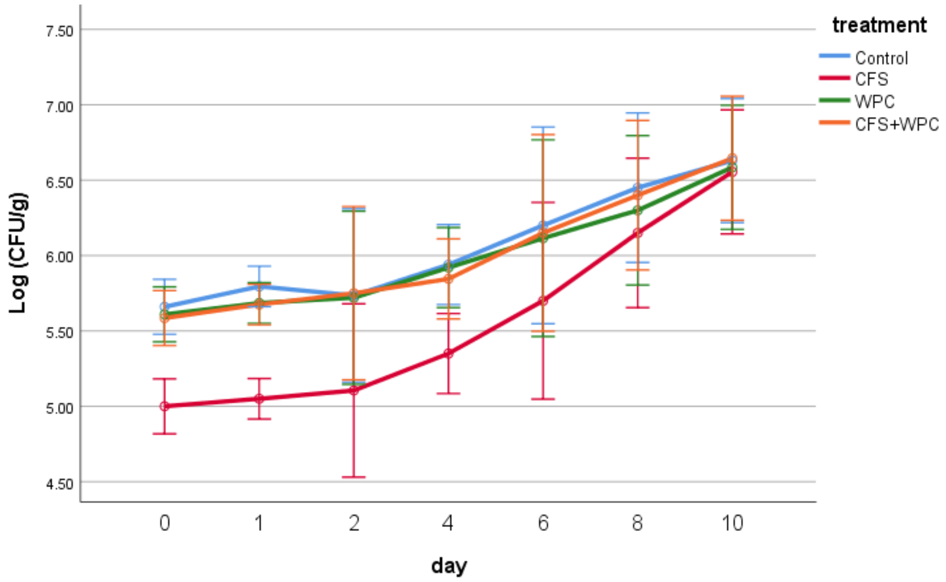

Fig.5. Comparison of the growth of total counting results in four food treatments during 10 days of storage at 4 ˚C.

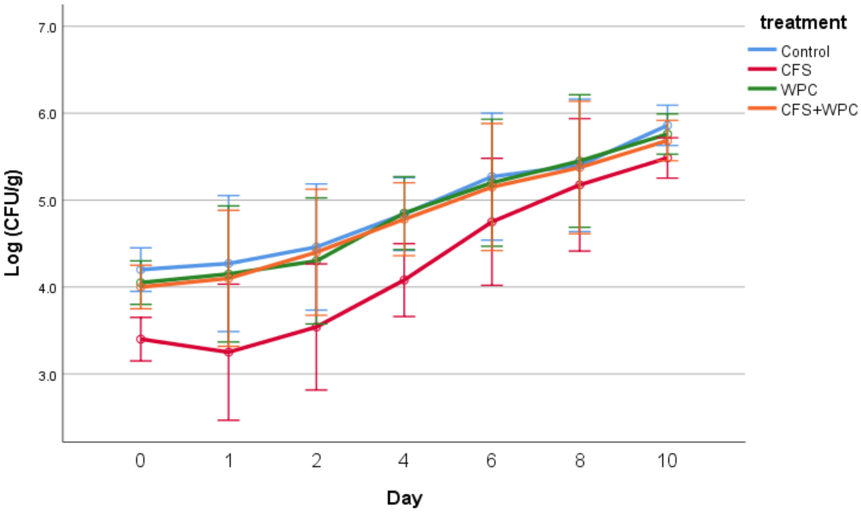

Fig.6. Comparison of the results of counting mold and yeast in four food treatments during 10 days of storage at 4 ˚C.

Comparable to the present study, Jutinico-Shubach et al. (2020) reported a 0.83 log CFU/g reduction in *L. monocytogenes* in cheese samples treated with CFS from *Pediococcus pentosaceus* 147 on day two of storage compared with the control. This closely aligns with the 0.80 log CFU/g reduction observed in our second treatment at the same time point [17]. Similarly, Salvucci et al. (2019) found reductions in *L. monocytogenes* in cheese treated with films containing bacteriocins from *Enterococcus faecium* ES216 [18]. Marques et al. (2017) also observed that *L. monocytogenes* counts in actively packaged cheese samples treated with CFS from *Latilactobacillus curvatus* P99 reached levels comparable to the control by day 10. Moreover, films containing CFS at MIC concentrations (15.6 µL/mL) did not significantly reduce bacterial counts compared with controls, consistent with our findings [26]. Yordshahi et al. (2020) likewise reported the survival of *L. monocytogenes* in all meat samples treated or untreated with active packaging containing postbiotics from *L. plantarum* (ATCC 14917), with a 1.0 log CFU/g increase in control samples by day nine—similar to the 1.1 log CFU/g increase observed in our study [21].

The type of film or coating, as well as the nature of the food, plays a critical role in the release of antimicrobial agents from polymer matrices into food systems [21]. Beristain-Bauza et al. (2017) demonstrated that untreated beef samples exhibited a modest reduction (<0.5 log CFU/g) in *L. monocytogenes* Scott A growth after five days, whereas beef treated with WPI films containing CFS from *Latilactobacillus sakei* (NRRL B-1917) showed a reduction of 1.4 log CFU/g after the same period [19]. Such variability highlights the dependence of antimicrobial efficacy on factors including film or coating type, bacterial strain, food model, and release kinetics of antagonistic compounds.

The study by Beristain-Bauza et al. (2016) suggested that the stronger antimicrobial performance of WPI films may be attributed to the hydrophobic nature of whey proteins, which facilitates compatibility with hydrophilic CFS obtained from *Lacticaseibacillus rhamnosus* (NRRL B-442). This mechanism may help explain the diminished inhibitory effect observed in the present study when CFS was combined with WPC coatings [38].

3.4 Chemical analyses

The pH values of cheese samples from the different treatments showed no significant differences ($p > 0.05$), with levels ranging from 6.50 to 6.56. This observation is consistent with Jutinico-Shubach et al. (2020), who reported comparable pH values in both control and CFS-treated cheese samples derived from *Pediococcus pentosaceus* 147 [17]. Similarly, Salvucci et al. (2019) found no significant differences in moisture content between control and treated cheese samples during storage, further corroborating the findings of the present study [18].

3.5 Sensory analyses

Sensory evaluation revealed no significant differences ($p > 0.05$) in overall acceptability or odor index between the control treatment and the WPC–CFS combination treatment (Fig. 7).

No significant differences were detected between the two CFS treatments and the WPC–CFS combination. However, a significant difference ($p < 0.05$) was observed in the color parameter of the control treatment compared with the other treatments, likely attributable to the MRS culture medium used as the primary substrate for LAB growth and CFS production (Table 10). This finding aligns with Beristain-Bauza et al. (2016), who reported pronounced color differences in WPI films containing CFS from *Lacticaseibacillus rhamnosus* (NRRL B-442) [37].

In contrast, no significant differences were detected in texture or taste across treatments ($p > 0.05$), indicating good sensory acceptance of the treated samples. These results are in line with those of Beristain-Bauza et al. (2017), who observed favorable sensory evaluations in grilled beef treated with WPI films containing CFS from *Latilactobacillus sakei* (NRRL B-1917) [19].

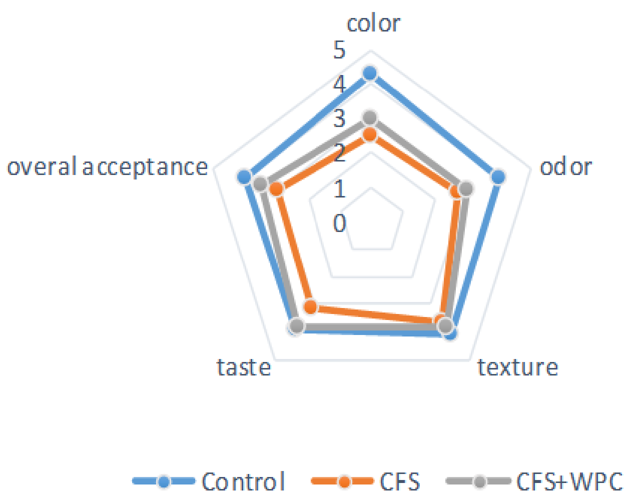

Fig.7. Sensory evaluation of cheese samples.

Table 10. Sensory analysis results of cheese samples.

T	Color	Odor	Texture	Taste	Overal acceptance
Control	4.30±0.48[a]	4.00±0.94[a]	4.10±0.56[a]	3.90±0.87[a]	4.00±0.81[a]
CFS	2.50±0.52[b]	2.70±0.94[b]	3.60±0.96[a]	3.10±1.19[a]	3.00±0.66[b]
CFS+WPC	3.00±0.81[b]	3.00±1.05[ab]	3.80±0.78[a]	3.80±0.78[a]	3.50±0.70[ab]

Non-similar lowercase Latin letters in each column indicate significant differences at the 5% level between treatments.

4. Conclusion

This study demonstrated that postbiotics derived from *L. plantarum* and *L. casei* possess significant antimicrobial activity against major foodborne pathogens when applied as coatings in pasteurized cheese. Among the two, *L. plantarum* exhibited stronger inhibitory effects, particularly against Gram-positive bacteria. However, incorporation of postbiotics into WPC-based coatings reduced their antimicrobial efficacy, likely due to matrix interactions. Importantly, postbiotic treatments successfully suppressed microbial growth during refrigerated storage without affecting the key chemical characteristics of cheese and were well accepted in sensory evaluations.

These findings underscore the potential of postbiotic-based coatings as natural, consumer-friendly alternatives to chemical preservatives in dairy products. Beyond cheese, this strategy could be extended to a broad range of perishable foods where safety, shelf life, and sensory acceptance are of critical importance. Nevertheless, further research is required to optimize carrier systems, enhance stability, and evaluate large-scale industrial applications across diverse food matrices.

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6. Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7. Authors' Contributions

Zeinab Hadadfar: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Supervision; Visualization; Writing – original draft. Asma Afshari: Conceptualization; Investigation; Methodology; Resources; Supervision; Validation; Writing – review and editing. Alireza Mohammadzadeh: Investigation;

Methodology; Resources; Supervision; Validation; Writing – review and editing. Zohreh Abdimoghadam: Funding acquisition; Investigation; Project administration; Resources; Supervision; Validation; Writing – review and editing.
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8. Using Artificial Intelligent Chatbots

The authors declare no artificial intelligent chatbot use.

9. Ethical Consideration

The study protocol was reviewed and approved by the Medical Ethics Committee of Gonabad University of Medical Sciences (IR.GMU.REC.1401.073).

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فنآوری نوین در صنعت پنیر: ارزیابی مزایای کاربرد پوششهای پست‌بیوتیکی در تولید پنیر پاستوریزه

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چکیده

سابقه و هدف: پنیر یکی از محصولات لبنی اصلی با ارزش غذایی بالا است، اما حساسیت آن به رشد میکروبی و فساد زودهنگام همچنان یک چالش برای صنعت لبنیات محسوب می‌شود. در حالی که افزودنی‌های شیمیایی به طور گسترده برای کنترل آلودگی میکروبی به کار می‌روند، افزایش آگاهی از خطرات احتمالی نگهدارنده‌های مصنوعی منجر به افزایش تقاضا برای جایگزین‌های طبیعی شده است. این مطالعه برای ارزیابی پتانسیل ضد میکروبی پست‌بیوتیک‌های مشتق شده از *لاکتی‌پلنتی‌باسیلوس پلانتاروم* و *لاکتی‌کازی‌باسیلوس کازئی* در برابر میکروارگانیسم‌های فسادزا و بیماری‌زای رایج در پنیر، و همچنین بررسی تأثیر آن‌ها بر خصوصیات میکروبیولوژیکی و شیمیایی پنیر در طول نگهداری طراحی شده است.

مواد و روش ها: پست‌بیوتیک‌ها از کشت‌های *L. plantarum* و *L. casei* استخراج شدند و فعالیت‌های ضدمیکروبی آن‌ها با استفاده از سنجش‌های میکروبیولوژیکی استاندارد در برابر باکتری‌های گرم مثبت و گرم منفی آزمایش شد. برای ارزیابی کاربرد عملی، پست‌بیوتیک‌ها به صورت پوشش بر روی نمونه‌های پنیر، چه به تنهایی و چه در ترکیب با کنسانتره پروتئین آب پنیر(WPC) ، اعمال شدند. شمارش میکروبی، پارامترهای شیمیایی و ارزیابی حسی در طول دوره نگهداری انجام شد. داده‌ها برای تعیین اثربخشی مقایسه‌ای تیمارها تجزیه و تحلیل شدند.

یافته‌ها و نتیجه‌گیری: یافته‌ها نشان داد که پست‌بیوتیک مشتق شده از *L. plantarum* در مقایسه با پست‌بیوتیک مشتق شده از *L. casei* ، اثرات ضد میکروبی قوی‌تری، به ویژه در برابر باکتری‌های گرم مثبت، نشان داد. با این حال، هنگامی که با WPC ترکیب شد، فعالیت ضدمیکروبی هر دو پست‌بیوتیک کاهش یافت. علیرغم این محدودیت، پست‌بیوتیک‌های اعمال شده به تنهایی، شمارش میکروبی را در طول نگهداری به طور قابل توجهی کاهش دادند بدون اینکه خواص شیمیایی اصلی پنیر را تغییر دهند. ارزیابی حسی، پذیرش کلی نمونه‌های تیمار شده با پست‌بیوتیک و پست‌بیوتیک WPCرا تأیید کرد. در نتیجه، پست‌بیوتیک‌ها می‌توانند به عنوان عوامل ضد میکروبی طبیعی امیدوارکننده در نگهداری پنیر عمل کنند، اگرچه برای افزایش فعالیت آن‌ها در ترکیب با حامل‌های پروتئینی مانندWPC ، بهینه‌سازی بیشتر لازم است.

واژگان کلیدی: فعالیت ضدمیکروبی، سوپرناتانت بدون سلول، پنیر، باکتری‌های اسید لاکتیک، پست‌بیوتیک